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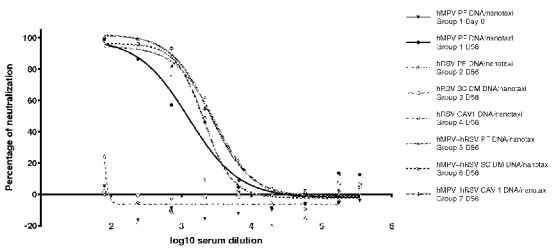
(54) Title: PHARMACEUTICAL COMPOSITIONS FOR TREATMENT OR PREVENTION OF VIRAL INFECTIONS

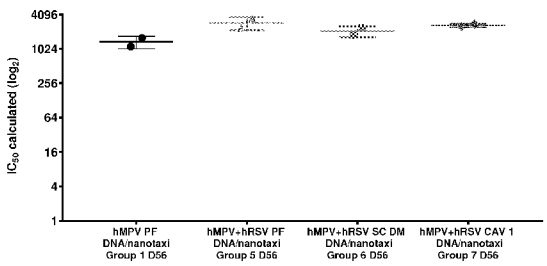
(57) Abstract: Described herein are constructs and pharmaceutical compositions for the treatment or prevention of viral infections.

Figure 13

A.



B.



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PHARMACEUTICAL COMPOSITIONS FOR TREATMENT OR PREVENTION OF VIRAL INFECTIONS

FIELD OF THE INVENTION

The invention relates to constructs and compositions for use as prophylactic or therapeutic treatments against viral infections.

BACKGROUND OF THE INVENTION

Human metapneumovirus (hMPV) is a member of the Paramyxoviridae family, assigned to the genus Metapneumovirus in the subfamily of Pneumovirinae and is an enveloped, negative single-stranded RNA virus. hMPV is closely related to respiratory syncytial virus (RSV), which is the most significant respiratory pathogen of infancy and early childhood. The more recently discovered hMPV is also an important respiratory pathogen and is associated with significant morbidity in infants and other high-risk populations, such as immunocompromised patients and individuals with underlying conditions, including prematurity, asthma, and cardiopulmonary disease (Kahn, *et al.* (2006) Epidemiology of Human Metapneumovirus. Clinical Microbiol. Reviews 19(3):546-557). Hospitalization rates due to hMPV infection are comparable to those due to other common respiratory viruses such as RSV, parainfluenza virus (PIV) or influenza virus and viral co-infection is common (Tregoning and Schwarze (2010) Respiratory Viral Infections in Infants: Causes, Clinical Symptoms, Virology, and Immunology. Clin. Microbiol. Rev. 23(1):74-98).

Isolates of hMPV are separated into two major lineages (A and B) and at least four subgroups (A1, A2, B1 and B2) (van den Hoogen, *et al.* (2004) Antigenic and genetic variability of human metapneumoviruses. Emerg. Inf. Dis. 10:658-666). The hMPV genome consists of a single negative strand of RNA of approximately 13 kb, containing eight genes presumed to encode nine different proteins. Of these, there are three hMPV surface glycoproteins: the attachment glycoprotein (G), which is involved in cell attachment, the fusion glycoprotein (F-glycoprotein or F-protein), which mediates fusion of the host cell and viral membranes and a small hydrophobic protein (SH). Viral coat proteins are prime targets for neutralizing antibodies; however, studies regarding the induction of protective immunity to hMPV have demonstrated that only the highly-conserved F-protein elicited a high-titer neutralizing antibody response (Skiadopoulos, *et al.* (2006) Individual contributions of the human metapneumovirus F, G, and SH surface glycoproteins to the induction of neutralizing antibodies and protective immunity.

Virology (345):492-501). Similarly, the F-protein from the related Respiratory Syncytial virus (RSV) has also been shown to be a main target of neutralizing antibodies (Magro, *et al.*, 2012, Neutralizing antibodies against the preactive form of respiratory syncytial virus fusion protein offer unique possibilities for clinical intervention PNAS 109(8):3089-3094).

For several decades, a vaccine has been sought for RSV. In the 1960s, a trial was conducted with alum-adjuvanted formalin-inactivated human RSV (hRSV). Not only did the vaccine not confer protection, it was associated with dramatically enhanced disease in infants upon natural infection with RSV and resulted in two deaths (Kim, *et al.* (1969) Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. Am. J. Epidemiol. 89:422-434). With regard to the more recently-discovered hMPV, similar deleterious effects of formalin-inactivated virus were observed in rhesus macaques and in the cotton rat model (Swart, *et al.*, 2007, Immunization of macaques with formalin-inactivated human metapneumovirus induces hypersensitivity to hMPV infection. Vaccine 25:8518-8528 and Yim, *et al.*, 2007, Human metapneumovirus: Enhanced pulmonary disease in cotton rats immunized with formalin-inactivated virus vaccine and challenged. Vaccine 25(27):5034-5040, respectively). Natural infection with RSV does not result in enhanced disease upon reinfection; neither does it confer lasting protection (Kim, *et al.*, *supra*). Similarly, natural infection with hMPV provides only transient protection and does not prevent further infections throughout the lifetime of the individual (Lenneke, *et al.* (2013) Human Metapneumovirus in Adults. Viruses 5:87-110). An effective vaccine to hMPV, therefore, must not only improve on natural immunity induced by infection, but must also simultaneously avoid harmful responses induced by the inactivated virus vaccine.

Although the deleterious effect of the inactivated RSV vaccine was initially hypothesized to be due to effects of inactivation on neutralizing epitopes, it has since been shown that inadequate affinity maturation of antibodies to critical epitopes was likely responsible. More specifically, the Th2-bias of the stimulated immune response may have contributed to the production of non-protective pathogenic antibodies in response to the vaccine (Delgado, *et al.* (2009) Lack of antibody affinity maturation due to poor Toll stimulation led to enhanced RSV disease. Nat. Med. 15(1):34-41). In this regard, Delgado *et al.* (*supra*) provided evidence that adjuvants which stimulate toll-like receptors may shift the immune response to inactivated RSV in a favorable direction; that is, by increasing the relative magnitude of the Th1 response. This and other approaches to increasing the Th1/Th2 ratio in response to RSV antigens (and potentially hMPV antigens) may be key to providing a safe and effective vaccine.

Several vaccination strategies for hMPV have been investigated in recent years, such as live-attenuated or live-vectored viruses and recombinant or DNA-encoding viral proteins. The successful development of a vaccine to hMPV presents major challenges, however. One is the need to induce strong and long-lasting immune responses which are greater than those induced by naturally-acquired immunity. Additionally, the vaccine must be sufficiently safe for use in high-risk populations such as immunocompromised patients and infants. Furthermore, induction of cross-reactive immunity against both clinically relevant isolates (A and B) would be highly desirable. In similar fashion, a combination vaccine simultaneously conferring protection against hMPV and RSV would be a valuable contribution to the field.

SUMMARY

The current disclosure provides DNA molecules encoding hMPV F-proteins and variants thereof, particularly hMPV F-proteins in a trimeric post-fusion conformation, vectors comprising such DNA molecules, as well as purified hMPV F-protein polypeptides and variants thereof. The disclosure further provides pharmaceutical compositions comprising the DNA molecules, vectors and/or polypeptides of the invention, particularly pharmaceutical compositions for stimulating an immune response in a subject, particularly an immune response which is protective against or neutralizes hMPV. The disclosure further provides pharmaceutical compositions comprising the hMPV DNA molecules of the invention and DNA molecules encoding RSV antigens as combination vaccines. The DNA molecules, vectors, polypeptides and pharmaceutical compositions as disclosed herein are particularly suitable for use as a medicament, particularly for the prophylactic or therapeutic treatment of viral infections in a subject, especially metapneumovirus and/or respiratory syncytial virus infections.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are not intended to be drawn to scale. The Figures are illustrative only and are not required for enablement of the disclosure. For purposes of clarity, not every component may be labeled in every drawing. In the drawings:

Figure 1 shows the primary structure of the fusion glycoprotein (F-protein) of hMPV. The full-length protein is 539 amino acids long and, during processing, becomes first the F0 form (aa 19-539) following removal of the signal peptide (aa 1-18), then a heterodimer of the F2 and F1 portions (aa 20-102 and 103-539, respectively) linked by disulfide bonds, following proteolytic cleavage of F0. SP: Signal peptide; FP: Fusion peptide; HRA: Heptad repeat A; HRB: Heptad repeat B; TM: Transmembrane region and CT:

Cytoplasmic tail. The black points indicate cysteine residues taking part in intermolecular bonds (indicated by "S-S"), the triangles represent N-glycosylation sites; the arrow shows the proteolytic processing site. Also indicated is the F1 ectodomain, defined herein as the F1 domain without the transmembrane region and the cytoplasmic tail.

Figure 2 schematically depicts the process of fusion of virus and host-cell membranes, which is mediated by paramyxovirus F-protein. (a) The pre-fusion F-protein trimer is depicted inserted into the viral membrane (via the transmembrane region) with a globular head connected to the transmembrane region through the HRB stalk. (b) Upon activation, the short α-helices of HRA refold into a long trimeric coiled-coil, inserting the fusion peptide of each subunit (encircled) into the host-cell membrane and forming the so-called pre-hairpin intermediate. (c) Collapse of this unstable intermediate draws the two membranes together. (d) Assembly of the six-helix-bundle (6-HB), formed by a core of three HRA α-helices surrounded by three antiparallel HRB α-helices completes the merging of the two membranes, resulting in the formation of the fusion pore and the transformation of the F-protein to the post-fusion conformation. The whole process probably requires the coordinated action of more than one F molecule; thus, two F-protein molecules are represented in the last two steps to emphasize the cooperation. (Figure is adapted from Melero and Mas, The Pneumovirinae fusion (F) protein: A common target for vaccines and antivirals (2015) Virus Research (209):128-135.)

Figure 3 shows a schematic representation of a DNA sequence (A) of an expression construct for a post-fusion hMPV F-protein heterodimer of the invention and a processed protein (B) encoded by the expression construct. The molecules depicted represent the subunit post-fusion hMPV F-protein of the invention, showing polypeptides A and B, containing the F1 ectodomain and F2 domain, respectively. The polynucleotide constructs for the DNA vaccine preparations as described herein do not encode a Cleavage Site C or a His6 sequence for purification; these features are present, however, in the construct for *in vitro* production of the protein subunit. Such a protein from an A1 genotype of hMPV optionally contains a G294E substitution in the F1 portion. The expressed heterodimeric protein forms homotrimers during processing, facilitated by the presence of the trimerization domain.

Figure 4 shows representative sequences for insertion into a protein expression plasmid comprising coding sequences of post-fusion hMPV F-protein heterodimers of the invention which are derived from (A) an A1 strain of hMPV (isolate "NL/1/00") and (B) a B1 strain of hMPV (isolate "NL/1/99"). Major domains and restriction sites are indicated. The preferred coding sequences for A1 and B1 subunit post-fusion F-proteins are provided as SEQ ID Nos: 18 and 19, respectively.

Figure 5 Confirmation of expression of post-fusion ($sPoF_{hMPV}$), full-length (FlF_{hMPV}) and soluble (sF_{hMPV}) forms of hMPV F-protein following ICAFectin®441 transfection of Hela cells as shown by staining of permeabilized cells by the DS7 monoclonal antibody in flow cytometry.

Figure 6 A. Purified $sPoF_{hMPV}$A1-MFur and sF_{hMPV}A1-V hMPV F-proteins as visualized by coomassie staining and Western blot with anti-penta-His antibody (Qiagen). B. FACS analysis of expression of sF_{hMPV}A1-V and $sPoF_{hMPV}$A1-MFur following transfection of CHO cells as shown by staining of permeabilized cells by MPE8 and DS7 monoclonal antibodies in flow cytometry.

Figure 7 Neutralization capacity of pooled sera from mice vaccinated with DNA constructs encoding either post-fusion or full-length hMPV F-protein or with purified post-fusion hMPV F-protein. Neutralization of the A1 hMPV strain is shown. IC50 values were calculated from the obtained curves and shown in the table.

Figure 8 Comparison of the immunogenicity of FlF_{hMPV} DNA and $sPoF_{hMPV}$ subunit as tested in flow cytometry with FlF_{hMPV}/ICAFectin®441 transfected Hela cells. Each plot shows binding of serum antibodies from individual vaccinated mice, comparing day 0 (thicker trace) and day 56 responses.

Figure 9 Comparison of the immunogenicity of $sPoF_{hMPV}$ DNA and $sPoF_{hMPV}$A1-Mfur subunit as tested in flow cytometry with $sPoF_{hMPV}$/ICAFectin®441 transfected Hela cells. Each plot shows binding of serum antibodies from individual vaccinated mice, comparing day 0 (thicker trace) and day 56 responses.

Figure 10 Comparison of immunogenicity of DNA vaccines and subunit vaccines in C57Bl/6 mice by IC_{50} on day 56 following three immunizations (A) and IgG1 titers on day 42 following two immunizations (B).

Figure 11 Comparison of immunogenicity of DNA vaccines and subunit vaccines in Balb/c mice by IC_{50} on day 56 following three immunizations (A) and IgG titers including IgG2a, IgG1 and the IgG2a/IgG1 ratio (Th1/Th2) on day 42 following two immunizations (B).

Figure 12 Antibodies stimulated in Balb/c mice at day 42 following vaccination (2x at three week intervals) with post-fusion F-protein DNA (50 µg $sPoF_{hMPV}$ + 0.15% Nanotaxi® 1) bound to both post-fusion trimer ($sPoF_{hMPV}$A1-Mfur) and pre-fusion trimer (sF_{hMPV}A1-K) as well as to native monomer

($sF_{hMPV}A1$-V) in an ELISA assay; whereas antibodies elicited by vaccination (2x at three week intervals) with post-fusion F-protein subunit (10 µg $sPoF_{hMPV}A1$-Mfur + alum) bound preferentially to the post-fusion trimer. The monoclonal antibody DS7, which binds to both pre- and post-fusion forms, served as a control.

Figure 13 RSV antigen encoding vectors added in combination with hMPV post-fusion F-protein DNA vaccine do not influence the production of neutralizing hMPV antibodies. Characterization of anti-hMPV immunogenicity of DNA vaccines encoding hMPV antigen alone, RSV antigen alone, or both hMPV and RSV antigens in C57Bl/6 mice is shown by hMPV neutralization curves (A) and IC_{50} (B) on day 56.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a nucleic acid encoding a heterodimeric protein consisting of a) a polypeptide A comprising an immunogenic F1 ectodomain of the hMPV F-protein; and b) a polypeptide B comprising an immunogenic F2 domain of the hMPV F-protein, wherein the F1 and F2 domains are covalently linked by at least one disulfide bond. In a preferred embodiment, the encoded heterodimer of the invention combines to form a homotrimeric form. The encoded F-protein heterodimers of the invention differ from wild type F-protein heterodimers at least in that they do not possess a transmembrane domain or a cytoplasmic tail.

The wild-type hMPV F-protein is a glycoprotein consisting of a signal peptide, an F2 domain and an F1 domain (see **Figure 1**). After processing, the signal peptide is cleaved off and the F2 and F1 domains are proteolytically cleaved, but are joined covalently by disulfide bonds, forming a heterodimer. The F-protein exists in trimeric form, each trimer consisting of three F-protein heterodimers, and is inserted into the viral envelope via the transmembrane domain with an outward orientation. The hMPV F-protein trimer is processed first as a metastable pre-fusion form, which is capable of initiating fusion of the viral membrane with host-cell membranes. During viral particle fusion with the host cell, the pre-fusion form engages with the host cell membrane and the subsequent transformation of the F-protein to the post-fusion form facilitates the fusion of the two membranes (see **Figure 2**). Additionally, over time, even in the absence of fusion, the pre-fusion form of the F-protein spontaneously undergoes a conformational change to the more stable post-fusion form.

As used herein, in the hMPV F-protein in trimeric post-fusion conformation is defined as a fully-processed hMPV F-protein, consisting of F1 and F2 regions, wherein the two regions have been

proteolytically separated, but are covalently linked by at least one, preferably two, disulfide bonds, and wherein the transmembrane and cytoplasmic domains of the F1 region have been removed, resulting in a soluble protein in a post-fusion configuration. Further, the post-fusion F-protein combines to form a homotrimer comprising three F1/F2 heterodimers. An F1 domain which lacks a transmembrane domain and a cytoplasmic tail is referred to herein as the "F1 ectodomain". It has been previously demonstrated that truncation of the RSV F-protein to remove the transmembrane domain and cytoplasmic tail (leaving only the ectodomain of the F1 region) results in a soluble form of the F-protein which spontaneously folds into a post-fusion configuration (Magro, *et al.* (2012), *supra*). When the F-protein is no longer inserted into the viral envelope via the transmembrane domain, heptad repeats A and B can come together to form a coiled-coil structure known as the six-helix bundle (6-HB; see **Fig. 2**), which is characteristic of the post-fusion form of the F-protein. In one embodiment, one disulfide bond is formed between the F1 and F2 regions of the hMPV post-fusion F-protein. In one embodiment, the one disulfide bond is formed between amino acid residues 60 and 182 of the hMPV post-fusion F-protein. In one embodiment, the one disulfide bond is formed between amino acid residues 28 and 407 of the hMPV post-fusion F-protein. In one embodiment, two disulfide bonds are formed between the F1 and F2 regions of the hMPV post-fusion F-protein. In one embodiment, the two disulfide bonds are formed between amino acid residues 60 and 182 and amino acid residues 28 and 407 of the hMPV post-fusion F-protein.

In a preferred embodiment, the hMPV F-protein in post-fusion conformation as encoded by the nucleic acid of the invention includes the following features (see **Figure 3A**):

-deletion of both the TM domain (TM) and the cytoplasmic tail (CT),

-placement of a TEV protease cleavage site (SEQ ID NO: 3) C-terminally to the heptad repeat B (HRB) ("cleavage site B"),

-placement of a trimerization domain (Fibritin T4 foldon; SEQ ID NO: 6) C-terminally to the TEV protease cleavage site,

-deletion of the first nine amino acids of the fusion peptide (Δ103-111) to avoid aggregation of the heterodimers,

-insertion of an RSV furin cleavage site II (KKRKRR; SEQ ID NO: 2) at the beginning of the fusion peptide (aa 102-107) for processing of F0 by co-expressed furin protease.

Optionally, the F-protein in post-fusion conformation as encoded by the nucleic acid of the invention further comprises:

-a serine endopeptidase factor Xa cleavage site (SEQ ID NO: 4) added C-terminally to the trimerization domain ("cleavage site C"),

-a C-terminal His_6 tag (SEQ ID NO: 5) for purification purposes.

In one aspect, the hMPV F-protein F1 ectodomain and F2 domain encoded by the nucleic acid of the invention are both selected from A1 or B1 strains of hMPV. As used herein, the terms "protein" and "polypeptide" are interchangeable. In a preferred embodiment, the encoded heterodimeric protein comprises an immunogenic F1 ectodomain consisting of: a) amino acids 112 to 489 of the hMPV F-protein from the A1 genotype, especially wherein the sequence contains a G294E mutation; i.e. the amino acid sequence of SEQ ID NO: 10 and b) the immunogenic F2 domain consists of amino acid 20 to 101 of the hMPV F-protein from the A1 genotype; i.e. the amino acid sequence of SEQ ID NO: 11. In a preferred embodiment, the encoded heterodimeric protein comprises an immunogenic F1 ectodomain consisting of a) amino acids 112 to 489 of the hMPV F-protein from the B1 genotype; i.e. the amino acid sequence of SEQ ID NO: 12 and b) the immunogenic F2 domain consists of amino acid 20 to 101 of the hMPV F-protein from the B1 genotype; i.e. amino acid sequence of SEQ ID NO: 13. In a further aspect, the F1 ectodomain from the A1 genotype is the wild-type sequence; i.e., does not contain a G294E mutation (SEQ ID NO: 9).

In one aspect, the heterodimeric protein encoded by the nucleic acid of the invention contains one or more engineered changes for cleavage of the protein during processing or for enhancing or improving the purification or function of the encoded protein. In one aspect, the native cleavage site for proteolytic processing of F0 (RQSR; SEQ ID NO: 1), which is sensitive to trypsin, is replaced by or overlapped with an alternative cleavage site. In one aspect, the alternative cleavage site is a furin cleavage site. In one aspect, the furin cleavage site is derived from the RSV F-protein. In one aspect, the RSV-derived furin cleavage site is the RSV cleavage site II defined by SEQ NO: 2. In one aspect, the furin protease for proteolytic processing of F0 to F2 and F1 is provided by cloning a nucleic acid sequence encoding a furin protease into the same plasmid as the nucleic acid encoding the post-fusion form of the hMPV F-protein. In one aspect, the nucleic acid sequence encoding the furin protease is provided separately in a different plasmid to be co-transfected with the post-fusion F-protein construct. In one aspect, the furin protease is stably expressed by the cell line used for expression of the post-fusion F-protein construct. In one aspect, the furin protease is a human furin protease. In one aspect, the human furin protease is defined by SEQ ID NO: 26. In one aspect, the coding sequence of the human furin protease is defined by SEQ ID NO: 31.

In one aspect, the polypeptide A encoded by the nucleic acid of the invention additionally comprises a trimerization domain C-terminal to the F1 ectodomain. In one aspect, the trimerization domain of the polypeptide A comprises or consists of the fibritin T4 foldon domain (Bhardwaj, *et al.* (2008) Foldon-

guided self-assembly of ultra-stable protein fibers Protein Science (2008), 17:1475-1485). In a preferred aspect, the T4 foldon domain is defined by SEQ ID NO: 6. In one aspect, the polypeptide A encoded by the nucleic acid of the invention additionally comprises another cleavage site B between the F1 ectodomain and the trimerization domain. In a preferred aspect, the additional cleavage site B of polypeptide A is a cleavage site for TEV protease (Tobacco Etch Virus nuclear-inclusion-a endopeptidase). In one embodiment, the TEV protease cleavage site is of the general form EXXYXQ(G/S). In a preferred embodiment, the cleavage sequence for the TEV protease is ENLYFQG as defined by SEQ ID NO: 3.

In one aspect, the polypeptide A encoded by the nucleic acid of the invention additionally comprises a tag at the C-terminal end of the trimerization domain. In a preferred aspect, the tag is a His_6 tag (SEQ ID NO: 5). In one aspect, the polypeptide A encoded by the nucleic acid of the invention additionally comprises another cleavage site C between the trimerization domain and the tag. In a preferred aspect, the additional cleavage site C is a cleavage site of the serine endopeptidase factor Xa. In one aspect, the cleavage site of serine endopeptidase factor Xa is of the general form I(E/D)GR. In a preferred aspect, the serine endopeptidase factor Xa cleavage site is IEGR as defined by SEQ ID NO: 4.

In one aspect, the trimeric configuration of the heterodimeric protein encoded by the nucleic acid according to the current disclosure comprises F-protein heterodimers consisting of a polypeptide A with SEQ ID NO: 14 or a functional variant thereof that is more than 80% identical to the polypeptide with SEQ ID NO: 14, especially more than 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90% identical to the polypeptide with SEQ ID NO: 14, most preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the polypeptide with SEQ ID NO: 14, and a polypeptide B with SEQ ID NO: 15 or a functional variant thereof that is more than 80% identical to the polypeptide with SEQ ID NO: 15, especially more than 81, 82, 83, 84, 85, 86, 87, 88, 89 or 90% identical to the polypeptide with SEQ ID NO: 15, most preferably more than 91, 92, 93, 94, 95, 96, 97, 98, 99% identical to the polypeptide with SEQ ID NO: 15.

In one aspect, the trimeric configuration of the heterodimeric protein encoded by the nucleic acid according to the current disclosure comprises F-protein heterodimers consisting of a polypeptide A with SEQ ID NO: 16 or a functional variant thereof that is more than 80% identical to the polypeptide with SEQ ID NO: 16, especially more than 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90% identical to the polypeptide with SEQ ID NO: 16, most preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the polypeptide with SEQ ID NO: 16 and a polypeptide B with SEQ ID NO:

17 or a functional variant thereof that is more than 80% identical to the polypeptide with SEQ ID NO: 17 especially more than 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90% identical to the polypeptide with SEQ ID NO: 17, most preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the polypeptide with SEQ ID NO: 17.

In one aspect, the trimeric configuration of the heterodimeric protein encoded by the nucleic acid according to the current disclosure comprises F-protein heterodimers consisting of a polypeptide A with SEQ ID NO: 29 or a functional variant thereof that is more than 80% identical to the polypeptide with SEQ ID NO: 29, especially more than 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90% identical to the polypeptide with SEQ ID NO: 29, most preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the polypeptide with SEQ ID NO: 29 and a polypeptide B with SEQ ID NO: 15 or a functional variant thereof that is more than 80% identical to the polypeptide with SEQ ID NO: 15 especially more than 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90% identical to the polypeptide with SEQ ID NO: 15, most preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to the polypeptide with SEQ ID NO: 15.

In one aspect, the current invention provides a vector which comprises the nucleic acid of the invention. In one aspect, the nucleic acid of the invention is comprised in a vector suitable for use in DNA vaccines, providing a vector suitable for inoculation of a subject. In one aspect, said vector suitable for use in DNA vaccines contains an element or elements allowing propagation and selection in a host cell, e.g., *E. coli*. In one aspect, said vector optimized for use in DNA vaccines contains an element or elements that direct expression of the transgene in the target organism, e.g., a mammal such as a human. In one aspect, said vector optimized for use in DNA vaccines is a first generation DNA vaccine vector such as pVAX1 or gWIS. In one aspect, said vector optimized for use in DNA vaccines is a second-generation DNA vaccine vector. In a preferred aspect, the vector optimized for use in DNA vaccines is the pVAX1 vector. In one aspect, the nucleic acid of the invention is comprised in a vector suitable for *in vitro* expression for subsequent (optional) purification of the encoded polypeptide. In one aspect, the vector suitable for *in vitro* expression of the encoded polypeptide is suitable for use in bacteria or in eukaryotic cells such as mammalian cells, avian cells, insect cells or yeast cells.

In further embodiments, the vector is a viral vector, such as a recombinant viral vector. In one embodiment, the viral vector is a Newcastle Disease Virus (NDV). In one aspect, the NDV is a lentigenic strain of NDV, especially a LaSota or Hitchner B1 strain. A lentigenic strain is defined as having relatively lower virulence in birds. In one aspect, the NDV is a moderate to high virulent strain of NDV,

i.e., a mesogenic or velogenic strain, such as, e.g., AF2240. In one aspect, the NDV strain is an oncolytic strain; i.e., a strain with capacity to selectively induce apoptosis in tumors or cancer cells *in vivo* or *in vitro*. In one aspect, the oncolytic strain is a LaSota strain of NDV. In a preferred aspect, the oncolytic strain is the highly virulent AF2240 strain of NDV. In some embodiments, the viral vector is a vaccinia virus vector. In one aspect the vaccinia virus vector is pRB21. In one aspect, the vector is a baculovirus vector. In a preferred embodiment, the vector suitable for *in vitro* expression is pVVS1371 (as defined by SEQ ID NO: 29).

In some embodiments, the host cell used for *in vitro* expression of the encoded polypeptide is an insect cell, such as SF9, SF21 or Tni (*e.g.*, High Fives or Tn 368 cells), or a duck cell line, especially a duck cell line derived from duck retina or embryonic fibroblasts, such as those described in WO2005/042728, especially EB66. In a preferred embodiment, the host cells used for *in vitro* expression of the encoded polypeptide are Chinese Hamster Ovary (CHO) cells.

In one aspect, the nucleic acid of the invention, the vector of the invention or the protein encoded by the nucleic acid of the invention is for use as a medicament. In a further aspect, the nucleic acid of the invention, the vector of the invention or the protein encoded by the nucleic acid of the invention is for use as a prophylactic or therapeutic treatment against a viral infection. In a further aspect, the nucleic acid of the invention, the vector of the invention or the protein encoded by the nucleic acid of the invention is comprised in a pharmaceutical composition. In one aspect, the pharmaceutical composition comprising the nucleic acid of the invention, the vector of the invention or the protein encoded by the nucleic acid of the invention is used as a medicament. In a further aspect, the pharmaceutical composition comprising the nucleic acid of the invention, the vector of the invention or the protein encoded by the nucleic acid of the invention is used as a prophylactic or therapeutic treatment against a viral infection. In a preferred aspect, the pharmaceutical composition for use is a vaccine. In a preferred embodiment, the vaccine of the invention is used for the prophylactic or therapeutic treatment of infection with one or more respiratory pathogens, such as viruses.

In one aspect, the disclosure provides a pharmaceutical composition comprising the nucleic acid, the polypeptide encoded by the nucleic acid or the vector of the invention. In one aspect, the pharmaceutical composition comprises between 1 ng and 1 mg of nucleic acid, polypeptide or vector of the invention, preferably between 10 ng and 500 µg, more preferably between 100 ng and 400 µg, even more preferably between 1 µg and 200 µg, most preferably between 10 and 100 µg. Such dose is preferably administered 1 to 3 times at intervals of 2 to 24 weeks. The pharmaceutical compositions of the present invention may

be used to protect a subject susceptible to hMPV infection or treat a subject with an hMPV infection, by means of administering said vaccine via a systemic or mucosal route. These administrations may include injection via the intramuscular, intraperitoneal, intradermal or subcutaneous routes; or via mucosal administration to the oral/alimentary, respiratory or genitourinary tracts. Although the vaccine of the invention may be administered as a single dose, components thereof may also be co-administered together at the same time or at different times.

In one aspect, the disclosure provides a pharmaceutical composition wherein said pharmaceutical composition further comprises antigens or vectors encoding said antigens from further respiratory pathogens, in particular, RSV and/or parainfluenza virus (PIV) antigens. In a preferred aspect, the further antigens from RSV are RSV F-proteins or variants thereof or, more preferably, nucleic acids encoding RSV F-proteins or variants thereof, most preferably vectors comprising such nucleic acids. In a preferred embodiment, the RSV F-proteins or variants thereof are selected from the group consisting of post-fusion forms, monomeric native forms and prefusion forms. In a preferred embodiment, the RSV F-proteins are selected from the group consisting of a post-fusion form of hRSV as defined by SEQ ID NO: 32 (referred to herein as $sPoF_{hRSV}$; encoded by SEQ ID NO: 33); a prefusion hRSV F-protein as defined by SEQ ID NO: 34 (referred to herein as $sPrF_{hRSV}$SC-DM; encoded by SEQ ID NO: 35) or a prefusion hRSV F-protein as defined by SEQ ID NO: 36 (referred to herein as $sPrF_{hRSV}$DS-Cav1; encoded by SEQ ID NO: 37).

The pharmaceutical composition as provided herein is also suitable for use as a medicament, particularly as a vaccine for preventing or treating an infection caused by human metapneumovirus (hMPV), particularly an hMPV from A and/or B genospecies. The pharmaceutical composition as provided herein is particularly suitable for use in a method of treating or preventing an hMPV infection, particularly an hMPV infection caused by genotype A and/or B hMPV, such as genotype A1, A2, B1 and/or B2 hMPV. In a preferred aspect, the pharmaceutical composition of the invention is additionally for use as a vaccine for preventing or treating an infection cause by human Respiratory Syncytial Virus (RSV). In a preferred aspect, the pharmaceutical composition according to the current invention is for use in a method of treating or preventing a human Respiratory Syncytial Virus (RSV) infection.

In a preferred aspect, the pharmaceutical composition according to the current disclosure may contain one or more suitable auxiliary substances, such as buffer substances, pharmaceutical excipients, stabilizers or further active ingredients, especially ingredients known in connection with a pharmaceutical composition and/or vaccine production. In one aspect, the pharmaceutical composition of the disclosure further

comprises an adjuvant and/or other pharmaceutically acceptable carriers or excipients, such as buffer substances, stabilizers or further active ingredients, especially ingredients known in connection with pharmaceutical compositions and/or vaccine production. In a preferred embodiment, the pharmaceutically acceptable excipient comprises a nucleic acid delivery reagent. In a preferred embodiment, the nucleic acid delivery reagent comprises tetrafunctional non-ionic amphiphilic block copolymers comprising at least one hydrophilic block and at least one hydrophobic block.

A preferable carrier or excipient for the nucleic acid molecules according to the present invention in their diverse embodiments, is an immunostimulatory compound such as an adjuvant for further stimulating the immune response to the polypeptide encoded by the nucleic acid molecule(s) herein disclosed. There is further provided a pharmaceutical composition which is a vaccine, this vaccine may further comprise a pharmaceutically acceptable excipient. In a preferred embodiment, the excipient is a 704 or 704-M tetrafunctional non-ionic amphiphilic block copolymer.

In a preferred aspect, one or more synthetic delivery systems will be used in the formulation. A preferred compound is one of the class known as Nanotaxi®, which allow for very high *in vivo* antigen delivery and stimulation of the innate immune system, eliciting a powerful immune response. Especially preferred are tetrafunctional non-ionic amphiphilic block copolymers comprising at least one hydrophilic block and at least one hydrophobic block.

In a tetrafunctional non-ionic amphiphilic block co-polymer useful for the invention the hydrophilic block may be selected in the group consisting of polyoxyalkylenes, polyvinyl alcohols, polyvinyl-pyrrolidones, poly(2-methyl-2-oxazoline), or saccharides, and the hydrophobic block that may be selected in the group consisting of polyoxyalkylenes, aliphatic chains, alkylidene polyesters, polyethylene glycol with a benzyl polyether head, and cholesterol. The hydrophilic blocks of a block copolymer are comprised of, and preferably consist in, polyethylene oxide units. The hydrophobic blocks of a block copolymer are comprised of, and preferably consist, in polypropylene oxide units.

Especially preferred block copolymer comprises hydrophilic blocks comprising, and preferably consisting in, polyethylene oxide units, and hydrophobic blocks comprising, and preferably consisting in, polypropylene oxide units.

A preferred compound is a tetrafunctional non-ionic amphiphilic block copolymer comprising at least one terminal hydrophilic block. A “terminal hydrophilic block” is a block located at one end of a copolymer,

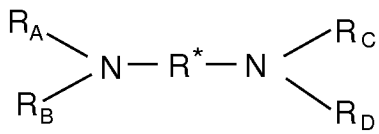
and in particular at a distal end of a branch of a tetrafunctional polymer. Preferably, a tetrafunctional non-ionic amphiphilic block copolymer comprises at least two, preferably three, and more preferably four terminal hydrophilic blocks.

A preferred compound is a block copolymer comprising at least one, preferably two, even preferably three, and more preferably four terminal oxyethylene unit(s), each at one end of each branch of the polymer. Preferably, a tetrafunctional non-ionic amphiphilic block copolymer comprises hydrophilic and hydrophobic blocks in a ratio hydrophilic block/hydrophobic block ranging from 0.7 to 1.5, preferably from 0.8 to 1.3, and more preferably from 0.8 to 1.2.

A tetrafunctional non-ionic amphiphilic tetrafunctional block copolymer useful for the invention may be a $(A\text{-}B)_n\text{-}C$ branched block copolymers, with A representing an hydrophilic block, B representing an hydrophobic block, C representing a linking moiety, and n being 4 and figuring the number of (A-B) group linked to C.

Preferably, the hydrophilic block A is a polyoxyethylene block, the hydrophobic block B is a polyoxypropylene block.

The linking moiety C may be an alkylene diamine moiety, and preferably is an ethylene diamine moiety.
A tetrafunctional non-ionic amphiphilic block copolymer useful for the invention may be of formula (I):



wherein R_A, R_B, R_C, R_D represent independently of one another

$$\begin{array}{cccccc} R^1 & R^2 & R^3 & R^4 & R^5 & R^6 \\ | & | & | & | & | & | \end{array}$$

$$[CHCHO]\text{-}[CHCHO]_i\text{-}[CHCHO]_j\text{-}H$$

in which

- i has values from about 5 to about 125, in particular from about 10 to about 100, and more particularly from about 10 to about 60, and

- j has values from 5 to about 85, in particular from about 10 to about 50, in particular from about 10 to about 20, and more particularly equal to or greater than 13,

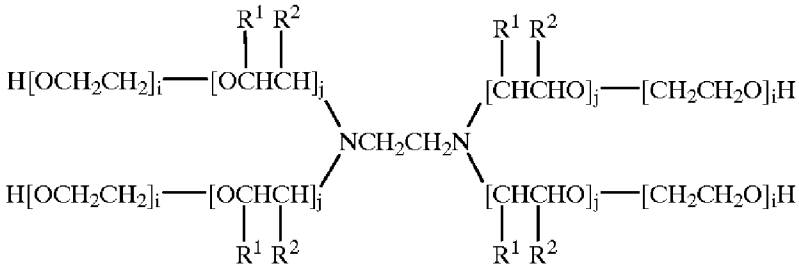
- R* is an alkylene of 2 to 6 carbons, a cycloalkylene of 5 to 8 carbons or a phenylene, and preferably is an ethylene,

- for R^1 and R^2, either (a) both are hydrogen or (b) one is hydrogen and the other is methyl,

- for R^3 and R^4 either (a) both are hydrogen or (b) one is hydrogen and the other is methyl, and

- if both of R^3 and R^4 are hydrogen, then one R^5 and R^6 is hydrogen and the other is methyl, or if one of R^3 and R^4 is methyl, then both of R^5 and R^6 are hydrogen.

More preferably, a non-ionic amphiphilic tetrafunctional block copolymer useful for the invention may be of formula (II):



in which

- i has values from about 5 to about 125, in particular from about 10 to about 100, and more particularly from about 10 to about 60, and
- j has values from about 5 to about 85, in particular from about 10 to about 50, and more particular from about 10 to about 20, and
- for each R^1, R^2 pair, R^1 shall be hydrogen and R^2 shall be a methyl group, and
- the molecular weight ranges from about 4000 to about 35000, in particular from about 4500 to about 30000, more particularly from about 5000 to about 25000, and
- the ethylene-oxide unit content is about 30% to about 80%, in particular about 35% to 50%, more preferably about 40%.

In a preferred embodiment, said tetrafunctional block co-polymer has an i-value of 13, a j-value of 14, a molecular weight of 5500 and an ethylene-oxide unit content of 40%; i.e., is a 704 block copolymer.

Preferably, i may range from about 5 to about 125, in particular from about 10 to about 100, and more particularly from about 10 to about 60, and j may range from about 5 to about 50, in particular from about 10 to about 25, in particular from about 10 to about 20, and more particularly equal to or greater than 13.

A block copolymer useful for the invention may have a molecular weight ranging from 4000 to 35000 and in particular ranging from 4500 to 30000 and more particularly ranging from 5000 to 25000.

A block copolymer useful for the invention may comprise, and preferably consist in, an ethylene-oxide units content from about 40%, in particular from about 45%, in particular ranging from about 45 to about 80%, in particular ranging from about 45 to 70%, and more particularly from about 45 to about 60%, and more preferably of about 50%.

Further details of suitable non-ionic amphiphilic block copolymers for the invention can be found in Surfactant Systems, Eds. Attwood and Florence, Chapman and Hall, London 1983, p 356-361; in The Condensed Encyclopaedia of Surfactants, Ed. Ash and Ash, Edward Arnold, London, 1989, in Non-ionic Surfactants, pp. 300-371, Ed. Nace, Dekker, New York, 1996, in Santon, Am. Perfumer Cosmet. 72(4):54-58 (1958); (Dekker, N.Y., 1967), or in US 6,353,055.

In one embodiment, a non-ionic amphiphilic block copolymer suitable for the invention is selected from the group consisting of tetrafunctional non-ionic amphiphilic block copolymer 304 (defined as i = 4; j = 4), tetrafunctional non-ionic amphiphilic block copolymer 704 (defined as i = 13; j = 14), tetrafunctional non-ionic amphiphilic block copolymer 904 (defined as i = 15; j = 17), and tetrafunctional non-ionic amphiphilic block copolymer 1107 (defined as i = 59; j = 19) or mixtures thereof. In a more preferred embodiment, the non-ionic amphiphilic block copolymer suitable for the invention is selected from the group consisting of 704 and 904 or a mixture thereof. In a preferred embodiment, the non-ionic amphiphilic block copolymer suitable for the invention is a 704 copolymer.

Another preferred compound is at least one block of a block copolymer useful for the invention, and preferably a hydrophilic block, is conjugated with a glycosyl moiety. A glycosylated tetrafunctional non-ionic amphiphilic block copolymer useful for the invention comprises at least one terminal block, and preferably one terminal hydrophilic block, conjugated with at least one glycosyl moiety.

More preferably, at least 25%, in particular at least 50%, in particular at least 75% and more particularly at least 100% of terminal blocks of a block copolymer of the invention are conjugated with a glycosyl moiety.

A non-glycosylated or glycosylated tetrafunctional non-ionic amphiphilic block copolymer may be used in an amount ranging from 0.01 to 10% by weight of the total weight of a composition containing it, in particular in a range from about 0.02 to 5%, more particularly from about 0.05 to 2%, more preferably from about 0.07 to 1%, more preferably from about 0.075 to 0.3%, and most preferably at about 0.15%, by weight of the total weight of a composition containing it.

Especially preferred are tetrafunctional non-ionic amphiphilic block copolymers. In one aspect, the tetrafunctional block co-polymers comprise at least one glycosyl moiety, wherein said at least one glycosyl moiety is a single glycosyl unit or a linear or branched polymer of glycosyl units. In a preferred embodiment, the at least one glycosyl moiety comprises mannose or galactose, preferably mannose. Especially preferred are a 704 tetrafunctional non-ionic amphiphilic block copolymer (also referred to herein as Nanotaxi®1 or 704), or a mannose-substituted 704 tetrafunctional non-ionic amphiphilic block copolymer (also referred to herein as Nanotaxi®2 or 704-M), both as described in WO2013128423, which is incorporated herein by reference in its entirety.

In a preferred embodiment, the pharmaceutical composition further comprises an immunostimulatory substance such as an adjuvant. The adjuvant can be selected based on the method of administration and may include polycationic substances, especially polycationic peptides, immunostimulatory nucleic acids molecules, preferably immunostimulatory oligo-deoxynucleotides (ODNs), especially the 26-mer oligo(dIdC)$_{13}$ (also known as "5'-(dIdC)$_{13}$-3"), peptides containing at least two KLK motifs separated by a linker of 3 to 7 hydrophobic amino acids, especially peptide KLKLLLLLKLK, a combination of KLK peptide and oligo(dIdC)$_{13}$ (also known as IC31®), alum, full-length M protein from hMPV or fragments thereof (Aerts, *et al.* (2015) Adjuvant effect of the human metapneumovirus (HMPV) matrix protein in HMPV subunit vaccines. J. of Gen. Virol. 96:767-774), or any combination of two or more of the above mentioned adjuvants. In a preferred aspect, the pharmaceutical composition comprises one or more of the 11-mer amino acid sequence KLKLLLLLKLK (SEQ ID NO: 22), the 26-mer 5'-(dIdC)$_{13}$-3' (SEQ ID NO: 23), alum or an hMPV M protein or fragment thereof, especially an M protein derived from an A1 or B1 strain of hMPV, especially an M protein as defined by SEQ ID NO: 24 or SEQ ID NO: 25.

In one aspect, the nucleic acid of the invention or a protein as encoded by the nucleic acid of the invention or the pharmaceutical composition of the disclosure is used to treat or prevent a viral infection. In a preferred aspect, the viral infection is a metapneumovirus infection. In a preferred aspect, the viral infection is a human metapneumovirus infection. In a further aspect, the viral infection is an hMPV infection caused by an A or B strain of hMPV, especially an hMPV infection caused by one or more of the group of strains selected from A1, A2, B1 and B2 strains of hMPV. In a preferred aspect, the nucleic acid of the invention or a protein as encoded by the nucleic acid of the invention or the pharmaceutical composition according to the invention provides cross-protection against more than one hMPV strain. In a preferred aspect, the pharmaceutical composition of the disclosure is useful in the prevention or treatment of RSV.

In one aspect, the invention provides a process for the production of a pharmaceutical composition of the disclosure comprising the steps of: a) providing a nucleic acid sequence encoding a polypeptide A and polypeptide B as defined above in a suitable vector; b) combining said nucleic acid with an optional adjuvant, nucleic acid delivery reagent and/or pharmaceutically acceptable excipient in order to obtain said pharmaceutical composition. In a preferred embodiment, the process is performed using a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 43 and 49.

The current disclosure further provides an hMPV F-protein complex in trimeric post-fusion conformation, wherein said complex consists of three hMPV F-protein heterodimers, and wherein said heterodimer comprises; a) a polypeptide A comprising an immunogenic F1 ectodomain of the hMPV F-protein; and b) a polypeptide B comprising an immunogenic F2 domain of the hMPV F-protein; wherein the F1 and F2 domains are covalently linked by at least one disulfide bond for use as a medicament. In a preferred embodiment, the said hMPV F-protein heterodimer comprised in the trimeric post-fusion complex is defined as SEQ ID NO: 44 or 47, preferably SEQ ID NO: 44. In a preferred aspect, the hMPV F-protein complex in trimeric post-fusion conformation as disclosed herein is used as a subunit for vaccination against hMPV infection.

The current disclosure further provides a process for producing a pharmaceutical composition comprising an hMPV F-protein complex in trimeric post-fusion conformation as defined herein, comprising the steps of: a) providing a nucleic acid sequence encoding a polypeptide A and a polypeptide B of the invention in a suitable vector; b) expressing said vector in a suitable host cell to yield polypeptide A and polypeptide B; c) optionally, purifying said hMPV F-protein complex; and d) combining said hMPV F-protein

complex with an optional adjuvant and/or other suitable excipient(s) in order to obtain said pharmaceutical composition.

SEQUENCES

SEQ ID NO: 1
Native cleavage site for proteolytic processing of hMPV F-protein (trypsin cleavage site; amino acids 99-102)
RQSR

SEQ ID NO: 2
Cleavage site II from RSV F-protein (furin cleavage site; amino acids 131-136)
KKRKRR

SEQ ID NO: 3
Cleavage sequence for TEV protease
ENLYFQG

SEQ ID NO: 4
Cleavage sequence for serine endopeptidase factor Xa
IEGR

SEQ ID NO: 5
His_6 tag
HHHHHH

SEQ ID NO: 6
Foldon domain of the T4 bacteriophage fibritin (Accession number: 1RFO_A)
GYIPEAPRDGQAYVRKDGEWVLLSTFL

SEQ ID NO: 7
F-protein of the A1 hMPV isolate “NL/1/00” (Accession No.: AAK62968)
MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRELRTVSADQ
LAREEQIENPRQSRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTR
AINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGF
LIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSGKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGIN
VAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSK
VEGEQHVIKGRPVSSSFDPVKFPEDQFNVALDQVFESIENSQALVDQSNRILSSAEKGNTGFIIVIILIAVLGSTMILVSVFIIIKKTKK
PTGAPPELSGVTNNGFIPHN

SEQ ID NO: 8
F-protein of the B1 hMPV isolate "NL/1/99” (Accession No.: AAQ90145)
MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTDGPSLIKTELDLTKSALRELKTVSADQ
LAREEQIENPRQSRFVLGAIALGVATAAAVTAGIAIAKTIRLESEVNAIKGALKQTNEAVSTLGNGVRVLATAVRELKEFVSKNLTS
AINRNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYMPTSAGQIKLMLENRAMVRRKGFGIL
IGVYGSSVIYMVQLPIFGVIDTPCWIIKAAPSCSEKNGNYACLLREDQGWYCKNAGSTVYYPNEKDCETRGDHVFCDTAAGINV
AEQSRECNINISTTNYPCKVSTGRHPISMVALSPLGALVACYKGVSCSIGSNWVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSK
VEGEQHVIKGRPVSSSFDPIKFPEDQFNVALDQVFESIENSQALVDQSNKILNSAEKGNTGFIIVVILVAVLGLTMISVSIIIIIKKTRK
PTGAPPELNGVTNGGFIPHS

SEQ ID NO: 9
F1 ectodomain of the A1 post-fusion hMPV F-protein heterodimer (aa 112-489)
VATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIADLKMAVSFSQFN
RRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYMVQLPIFGVIDTP
CWIVKAAPSCSGKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVST

GRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPVKF
PEDQFNVALDQVFESIENSQALVDQSNRILSSAEKGNT

SEQ ID NO: 10
F1 ectodomain of the A1 strain post-fusion hMPV F-protein heterodimer with a G294E mutation (aa 112-489)
VATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIADLKMAVSFSQFN
RRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYMVQLPIFGVIDTP
CWIVKAAPSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVST
GRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPVKF
PEDQFNVALDQVFESIENSQALVDQSNRILSSAEKGNT

SEQ ID NO: 11
F2 domain of the A1 strain post-fusion heterodimer (aa 20-101)
KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRELRTVSADQLAREEQIENPRQS

SEQ ID NO: 12
F1 ectodomain of the B1 post-fusion hMPV F-protein heterodimer (aa 112-489)
VATAAAVTAGIAIAKTIRLESEVNAIKGALKQTNEAVSTLGNGVRVLATAVRELKEFVSKNLTSAINRNKCDIADLKMAVSFSQFN
RRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTP
CWIIKAAPSCSEKNGNYACLLREDQGWYCKNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSRECNINISTTNYPCKVSTG
RHPISMVALSPLGALVACYKGVSCSIGSNWVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPIKFPE
DQFNVALDQVFESIENSQALVDQSNKILNSAEKGNT

SEQ ID NO: 13
F2 domain of the B1 post-fusion hMPV F-protein heterodimer (aa 20-101)
KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTDGPSLIKTELDLTKSALRELKTVSADQLAREEQIENPRQS

SEQ ID NO: 14
Polypeptide A (A1 strain) (with G294E mutation)
VATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIADLKMAVSFSQFN
RRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYMVQLPIFGVIDTP
CWIVKAAPSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVST
GRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPVKF
PEDQFNVALDQVFESIENSQALVDQSNRILSSAEKGNTSGRENLYFQGGGGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGGIEG
RHHHHHH

SEQ ID NO: 15
Polypeptide B (A1 strain)
KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRELRTVSADQLAREEQIENPRQSKKRKRR

SEQ ID NO: 16
Polypeptide A (B1 strain)
VATAAAVTAGIAIAKTIRLESEVNAIKGALKQTNEAVSTLGNGVRVLATAVRELKEFVSKNLTSAINRNKCDIADLKMAVSFSQFN
RRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTP
CWIIKAAPSCSEKNGNYACLLREDQGWYCKNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSRECNINISTTNYPCKVSTG
RHPISMVALSPLGALVACYKGVSCSIGSNWVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPIKFPE
DQFNVALDQVFESIENSQALVDQSNKILNSAEKGNTSGRENLYFQGGGGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGGIEGR
HHHHHH

SEQ ID NO: 17
Polypeptide B (B1 strain)
KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTDGPSLIKTELDLTKSALRELKTVSADQLAREEQIENPRQSKKRKRR

SEQ ID NO: 18
Coding sequence for the A1 strain hMPV post-fusion F-protein heterodimer subunit (sPoF$_{hMPV}$A1-Mfur; SEQ ID NO: 44; also shown in **Fig. 4A**) codon optimized for expression in CHO cells

GGTACCGTCGACGCTAGCGAATTCGCCGCCACCATGTCCTGGAAGGTCGTGATCATCTTCTCCCTGCTGATCACCCCCCAG
CACGGCCTGAAAGAGTCCTACCTGGAAGAGAGCTGCTCCACCATCACCGAGGGCTACCTGTCTGTGCTGCGGACCGGCTG
GTACACCAACGTGTTCACCCTGGAAGTGGGCGACGTGGAAAACCTGACCTGCGCCGATGGCCCCAGCCTGATCAAGACCG
AGCTGGACCTGACCAAGTCCGCCCTGCGGGAACTGAGAACCGTGTCTGCCGATCAGCTGGCCAGAGAGGAACAGATCGA
GAACCCCCGGCAGTCCAAGAAACGGAAGCGGAGAGTGGCCACCGCCGCTGCTGTGACAGCTGGCGTGGCCATTGCCAAG
ACCATCCGGCTGGAATCCGAAGTGACCGCCATCAAGAACGCCCTGAAAAAGACCAACGAGGCCGTGTCTACCCTGGGCAA
TGGCGTGCGAGTGCTGGCTACAGCTGTGCGCGAGCTGAAGGACTTCGTGTCCAAGAACCTGACCCGGGCCATCAACAAG
AACAAGTGTGATATCGCCGACCTGAAGATGGCCGTGTCCTTTAGCCAGTTCAACCGGCGGTTCCTGAACGTCGTGCGGCA
GTTCTCTGACAACGCCGGCATCACCCCTGCCATCTCCCTGGATCTGATGACCGACGCCGAGCTGGCTAGAGCCGTGTCCAA
CATGCCTACCTCTGCCGGCCAGATCAAGCTGATGCTGGAAAACCGGGCCATGGTGCGACGGAAGGGCTTCGGCTTTCTGA
TCGGCGTGTACGGCTCCTCCGTGATCTACATGGTGCAGCTGCCTATCTTCGGCGTGATCGACACCCCCTGCTGGATCGTGA
AGGCCGCTCCTAGCTGCTCCGAGAAGAAGGGCAACTACGCCTGCCTGCTGAGAGAGGACCAGGGCTGGTACTGTCAGAA
CGCCGGCTCCACCGTGTACTACCCCAACGAGAAGGACTGCGAGACACGGGGCGACCACGTGTTCTGTGATACCGCTGCTG
GCATCAACGTGGCCGAGCAGTCCAAAGAGTGCAACATCAACATCTCCACCACCAACTACCCCTGCAAGGTGTCCACCGGCA
GGCACCCCATCTCTATGGTGGCCCTGTCTCCTCTGGGCGCCCTGGTGGCTTGTTACAAGGGCGTGTCCTGCTCCATCGGCT
CCAACAGAGTGGGCATCATCAAGCAGCTGAACAAGGGCTGCAGCTACATCACCAACCAGGACGCCGACACCGTGACCATC
GACAATACCGTGTATCAGCTGTCCAAGGTGGAAGGCGAGCAGCACGTGATCAAGGGCAGACCCGTGTCCTCCAGCTTCGA
CCCCGTGAAGTTCCCCGAGGATCAGTTCAATGTGGCCCTGGACCAGGTGTTCGAGTCCATCGAGAACTCCCAGGCTCTGGT
GGACCAGTCCAACCGGATCCTGTCCTCTGCCGAGAAGGGAAACACCTCCGGCAGAGAGAACCTGTATTTTCAAGGCGGCG
GAGGCTCCGGCTACATCCCTGAGGCTCCTAGAGATGGCCAGGCCTACGTGCGGAAGGATGGCGAATGGGTGCTGCTGTC
CACCTTCCTGGGCGGCATCGAGGGCAGACACCACCATCATCACCACTGATGATGACCATGGTTAATTAAGTTTAAAC

SEQ ID NO: 19
Coding sequence for the B1 strain hMPV post-fusion F-protein heterodimer derived from isolate "NL/1/99" and codon optimized (with His-tag and other modifications as described herein, also shown in **Fig. 4B**)

GGTACCGTCGACGCTAGCGAATTCGCCGCCACCATGTCCTGGAAAGTGATGATCATTATCTCCCTGCTGATCACCCCCCAG
CACGGCCTGAAAGAGTCCTACCTGGAAGAGAGCTGCTCCACCATCACCGAGGGCTACCTGTCTGTGCTGCGGACCGGCTG
GTACACCAACGTGTTCACCCTGGAAGTGGGCGACGTGGAAAACCTGACCTGCACCGATGGCCCCAGCCTGATCAAGACCG
AGCTGGACCTGACCAAGTCCGCCCTGCGCGAGCTGAAAACCGTGTCTGCCGATCAGCTGGCCAGAGAGGAACAGATCGA
GAACCCCCGGCAGTCCAAGAAACGGAAGCGGAGAGTGGCCACCGCCGCTGCTGTGACAGCTGGAATCGCTATCGCCAAG
ACCATCCGGCTGGAATCCGAAGTGAACGCCATCAAGGGCGCTCTGAAGCAGACCAACGAGGCCGTGTCTACCCTGGGCAA
TGGCGTGCGAGTGCTGGCTACAGCTGTGCGGGAACTGAAAGAATTCGTGTCCAAGAACCTGACCAGCGCCATCAACCGG
AACAAGTGTGATATCGCCGACCTGAAGATGGCCGTGTCCTTCAGCCAGTTCAACCGGCGGTTCCTGAACGTCGTGCGGCA
GTTCTCTGACAACGCCGGCATCACCCCTGCCATCTCCCTGGATCTGATGACCGACGCCGAGCTGGCTAGAGCCGTGTCTTA
CATGCCTACCTCTGCCGGCCAGATCAAGCTGATGCTGGAAAACCGGGCCATGGTGCGACGGAAGGGCTTCGGCATCCTGA
TCGGCGTGTACGGCTCCTCCGTGATCTACATGGTGCAGCTGCCTATCTTCGGCGTGATCGACACCCCCTGCTGGATTATCAA
GGCCGCTCCCAGCTGCTCCGAGAAGAACGGCAACTACGCCTGCCTGCTGAGAGAGGACCAGGGCTGGTACTGCAAGAAC
GCCGGCTCCACCGTGTACTACCCCAACGAGAAGGACTGCGAGACACGGGGCGACCACGTGTTCTGTGATACCGCTGCTGG
CATCAACGTGGCCGAGCAGTCCAGAGAGTGCAACATCAACATCTCCACCACCAACTACCCCTGCAAGGTGTCCACCGGCA
GGCACCCCATCTCTATGGTGGCCCTGTCTCCTCTGGGAGCCCTGGTGGCTTGTTACAAGGGCGTGTCCTGCTCCATCGGCT
CCAACTGGGTGGGAATCATCAAGCAGCTGCCCAAGGGCTGCAGCTACATCACCAACCAGGACGCCGACACCGTGACCATC
GACAATACCGTGTATCAGCTGTCCAAGGTGGAAGGCGAGCAGCACGTGATCAAGGGCAGACCCGTGTCCAGCTCCTTCGA
CCCCATCAAGTTCCCCGAGGATCAGTTCAATGTGGCCCTGGACCAGGTGTTCGAGTCCATCGAGAACTCCCAGGCTCTGGT
GGACCAGTCCAACAAGATCCTGAACTCCGCCGAGAAGGGCAACACCTCCGGCAGAGAGAACCTGTATTTTCAAGGCGGCG
GAGGCTCCGGCTACATCCCTGAGGCTCCTAGAGATGGCCAGGCCTACGTGCGGAAGGATGGCGAATGGGTGCTGCTGTC
CACCTTCCTGGGCGGCATCGAGGGCAGACACCACCATCATCACCACTGATGATGACCATGGTTAATTAAGTTTAAAC

SEQ ID NO: 20
DS7 VH – heavy chain variable region of DS7 monoclonal antibody specific to hMPV F-protein

EVQLLESGGGLVQPGGSRRLSCAASGFTVSSSYMSWVRQTPGKGLEWISVFYSGGTTYYADAVKGRFSISMDTSKNTLHLQMN
SLRVEDTAIYYCARVLSRASGMPDAFDIWGPGTMVTVSS

SEQ ID NO: 21
DS7 VL – light chain variable region of DS7 monoclonal antibody specific to hMPV F-protein
ELALIQPASVSVSPGQTASITCSGDKLGDKYASWYQQKPGQSPVLVIYQDSERPSGIPERFSGSNSGNTATLTISGTQAMDEADY
YCQAWDSSTAVFGGGTTLTVLGQ

SEQ ID NO: 22
KLK peptide
KLKLLLLLKLK

SEQ ID NO: 23
5'-(dIdC)$_{13}$-3'
dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC

SEQ ID NO: 24
Matrix protein from the A1 hMPV isolate "NL/1/00" (Accession No.: AAK62969)
MESYLVDTYQGIPYTAAVQVDLIEKDLLPASLTIWFPLFQANTPPAVLLDQLKTLTITTLYAASQNGPILKVNASAQGAAMSVLPK
KFEVNATVALDEYSKLEFDKLTVCEVKTVYLTTMKPYGMVSKFVSSAKSVGKKTHDLIALCDFMDLEKNTPVTIPAFIKSVSIKESE
SATVEAAISSEADQALTQAKIAPYAGLIMIMTMNNPKGIFKKLGAGTQVIVELGAYVQAESISKICKTWSHQGTRYVLKSR

SEQ ID NO: 25
Matrix protein from the B1 hMPV isolate "NL/1/99" (Accession No.: AAS92881)
MESYLVDTYQGIPYTAAVQVDLVEKDLLPASLTIWFPLFQANTPPAVLLDQLKTLTITTLYAASQNGPILKVNASAQGAAMSVLP
KKFEVNATVALDEYSKLDFDKLTVCDVKTVYLTTMKPYGMVSKFVSSAKSVGKKTHDLIALCDFMDLEKNIPVTIPAFIKSVSIKES
ESATVEAAISSEADQALTQAKIAPYAGLIMIMTMNNPKGIFKKLGAGTQVIVELGAYVQAESISRICKSWSHQGTRYVLKSR

SEQ ID NO: 26
Human furin (Accession No.: NP_001276753)
MELRPWLLWVVAATGTLVLLAADAQGQKVFTNTWAVRIPGGPAVANSVARKHGFLNLGQIFGDYYHFWHRGVTKRSLSPHR
PRHSRLQREPQVQWLEQQVAKRRTKRDVYQEPTDPKFPQQWYLSGVTQRDLNVKAAWAQGYTGHGIVVSILDDGIEKNHPD
LAGNYDPGASFDVNDQDPDPQPRYTQMNDNRHGTRCAGEVAAVANNGVCGVGVAYNARIGGVRMLDGEVTDAVEARSLG
LNPNHIHIYSASWGPEDDGKTVDGPARLAEEAFFRGVSQGRGGLGSIFVWASGNGGREHDSCNCDGYTNSIYTLSISSATQFG
NVPWYSEACSSTLATTYSSGNQNEKQIVTTDLRQKCTESHTGTSASAPLAAGIIALTLEANKNLTWRDMQHLVVQTSKPAHLNA
NDWATNGVGRKVSHSYGYGLLDAGAMVALAQNWTTVAPQRKCIIDILTEPKDIGKRLEVRKTVTACLGEPNHITRLEHAQARL
TLSYNRRGDLAIHLVSPMGTRSTLLAARPHDYSADGFNDWAFMTTHSWDEDPSGEWVLEIENTSEANNYGTLTKFTLVLYGTA
PEGLPVPPESSGCKTLTSSQACVVCEEGFSLHQKSCVQHCPPGFAPQVLDTHYSTENDVETIRASVCAPCHASCATCQGPALTDC
LSCPSHASLDPVEQTCSRQSQSSRESPPQQQPPRLPPEVEAGQRLRAGLLPSHLPEVVAGLSCAFIVLVFVTVFLVLQLRSGFSFR
GVKVYTMDRGLISYKGLPPEAWQEECPSDSEEDEGRGERTAFIKDQSAL

SEQ ID NO: 27
Coding sequence for the A1 strain hMPV post-fusion F-protein heterodimer (not containing a G294E mutation)
CGCGAATTCGGGATGTCTTGGAAAGTGGTGATCATTTTTTCATTGTTAATAACACCTCAACACGGTCTTAAAGAGAGCTACT
TAGAAGAGTCATGTAGCACTATAACTGAAGGATATCTCAGTGTTCTGAGGACAGGTTGGTACACCAATGTTTTTACACTGG
AGGTAGGCGATGTAGAGAACCTTACATGTGCCGATGGACCCAGCTTAATAAAAACAGAATTAGACCTGACCAAAAGTGCA
CTAAGAGAGCTCAGAACAGTTTCTGCTGATCAACTGGCAAGAGAGGAGCAAATTGAAAATCCCAGACAATCTAAGAAGAG
GAAGAGAAGAGTTGCAACTGCAGCTGCAGTTACAGCAGGTGTTGCAATTGCCAAAACCATCCGGCTTGAAAGTGAAGTAA
CAGCAATTAAGAATGCCCTCAAAAAGACCAATGAAGCAGTATCTACATTGGGGAATGGAGTTCGTGTGTTGGCAACTGCA
GTGAGAGAGCTGAAAGATTTTGTGAGCAAGAATCTAACACGTGCAATCAACAAAAACAAGTGCGACATTGCTGACCTGAA
AATGGCCGTTAGCTTCAGTCAATTCAACAGAAGGTTCCTAAATGTTGTGCGGCAATTTTCAGACAACGCTGGAATAACACC
AGCAATATCTTTGGACTTAATGACAGATGCTGAACTAGCCAGAGCTGTTTCCAACATGCCAACATCTGCAGGACAAATAAA
ACTGATGTTGGAGAACCGTGCAATGGTAAGAAGAAAAGGGTTCGGATTCCTGATAGGAGTTTACGGAAGCTCCGTAATTT

ACATGGTGCAACTGCCAATCTTTGGGGTTATAGACACGCCTTGCTGGATAGTAAAAGCAGCCCCTTCTTGTTCAGGAAAAA
AGGGAAACTATGCTTGCCTCTTAAGAGAAGACCAAGGATGGTATTGTCAAAATGCAGGGTCAACTGTTTACTACCCAAATG
AAAAAGACTGTGAAACAAGAGGAGACCATGTCTTTTGCGACACAGCAGCAGGAATCAATGTTGCTGAGCAGTCAAAGGA
GTGCAACATAAACATATCTACTACTAATTACCCATGCAAAGTTAGCACAGGAAGACATCCTATCAGTATGGTTGCACTATCT
CCTCTTGGGGCTTTGGTTGCTTGCTACAAGGGAGTGAGCTGTTCCATTGGCAGCAACAGAGTAGGGATCATCAAGCAACT
GAACAAAGGCTGCTCTTATATAACCAACCAAGACGCAGACACAGTGACAATAGACAACACTGTATACCAGCTAAGCAAAG
TTGAAGGCGAACAGCATGTTATAAAAGGAAGGCCAGTGTCAAGCAGCTTTGACCCAGTCAAGTTTCCTGAAGATCAATTC
AATGTTGCACTTGACCAAGTTTTCGAGAGCATTGAGAACAGTCAGGCCTTGGTGGATCAATCAAACAGAATCCTAAGCAGT
GCAGAGAAAGGAAACACTTCTGGTCGTGAAAACTTATATTTCCAAGGTGGTGGTGGCAGCGGCTATATTCCGGAAGCGCC
GCGCGATGGCCAGGCGTATGTGCGCAAAGATGGCGAATGGGTGCTGCTGAGCACCTTTCTGGGAGGCATAGAAGGAAG
ACACCACCATCATCATCATTGATTCATCATTGTAATAATTCTAATTGCTGTCCTTGGCTCTACCATGATCCTAGTGAGTGTTTT
TATCATAATAAAGAAAACAAAGAAACCCACAGGAGCACCTCCAGAGCTGAGTGGTGTCACAAACAATGGCTTCATACCAC
ATAATTAGCCATGGCGT

SEQ ID NO: 28

pVAX1™ plasmid for DNA vaccine development (Invitrogen)

GACTCTTCGCGATGTACGGGCCAGATATACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTC
ATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCC
CCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTA
TTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAA
ATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCT
ATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCAC
CCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTG
ACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCACTGCTTA
CTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGCGTTTAAACTTAAGCTTGGTACCGAGCTC
GGATCCACTAGTCCAGTGTGGTGGAATTCTGCAGATATCCAGCACAGTGGCGGCCGCTCGAGTCTAGAGGGCCCGTTTAA
ACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGA
AGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGG
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GGCTTCTACTGGGCGGTTTTATGGACAGCAAGCGAACCGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGC
CCTGCAAAGTAAACTGGATGGCTTTCTCGCCGCCAAGGATCTGATGGCGCAGGGGATCAAGCTCTGATCAAGAGACAGGA
TGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTA
TGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGT
CAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTT
CCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATC
TCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGG
CTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAG
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GGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGA
GACAATAACCCTGATAAATGCTTCAATAATAGCACGTGCTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCT
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TCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCC
GGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTA
GCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCT
GCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAA
CGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGA
AAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAG
GGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTG

ATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGGCTTTTGCTGGCCTTT
TGCTCACATGTTCTT

SEQ ID NO: 29
Expression plasmid pVVS1371 (Valneva)

GAGCTCACGGGGACAGCCCCCCCCCAAAGCCCCCAGGGATGTAATTACGTCCCTCCCCCGCTAGGGGGCAGCAGCGAGCC
GCCCGGGGCTCCGCTCCGGTCCGGCGCTCCCCCCGCATCCCCGAGCCGGCAGCGTGCGGGGACAGCCCGGGCACGGGGA
AGGTGGCACGGGATCGCTTTCCTCTGAACGCTTCTCGCTGCTCTTTGAGCCTGCAGACACCTGGGGGGATACGGGGAAAA
AGCTTTAGGCTGAAAGAGAGATTTAGAATGACAGAATCATAGAACGGCCTGGGTTGCAAAGGAGCACAGTGCTCATCCA
GATCCAACCCCCTGCTATGTGCAGGGTCATCAACCAGCAGCCCAGGCTGCCCAGAGCCACATCCAGCCTGGCCTTGAATGC
CTGCAGGGATGGGGCATCCACAGCCTCCTTGGGCAACCTGTTCAGTGCGTCACCACCCTCTGGGGGAAAAACTGCCTCCTC
ATATCCAACCCAAACCTCCCCTGTCTCAGTGTAAAGCCATTCCCCCTTGTCCTATCAAGGGGGAGTTTGCTGTGACATTGTT
GGTCTGGGGTGACACATGTTTGCCAATTCAGTGCATCACGGAGAGGCAGATCTTGGGGATAAGGAAGTGCAGGACAGCA
TGGACGTGGGACATGCAGGTGTTGAGGGCTCTGGGACACTCTCCAAGTCACAGCGTTCAGAACAGCCTTAAGGATAAGA
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CCTGTGTCTGAGCCTGCATGTTTGATGGTGTCTGGATGCAAGCAGAAGGGGTGGAAGAGCTTGCCTGGAGAGATACAGCT
GGGTCAGTAGGACTGGGACAGGCAGCTGGAGAATTGCCATGTAGATGTTCATACAATCGTCAAATCATGAAGGCTGGAA
AAGCCCTCCAAGATCCCCAAGACCAACCCCAACCCACCCACCGTGCCCACTGGCCATGTCCCTCAGTGCCACATCCCCACAG
TTCTTCATCACCTCCAGGGACGGTGACCCCCCCACCTCCGTGGGCAGCTGTGCCACTGCAGCACCGCTCTTTGGAGAAGGT
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CGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCA
ACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGG
TGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATG
ACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGT
CATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAG
TCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCC
CCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCCTCTCTTAAGGTAGCGGCCGCT
GATCACAGGTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGGCCAATAGAAACTGGGCTTGTCGAGACAGAGAAGATT
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CCATGGTATCCACCTCTCAGCTGCTGGGCCTGCTGCTGTTCTGGACCTCTGCCTCCAGATGCGACATCGTGATGACCCAGA
GCCCCGCCACCCTGTCTGTGACCCCTGGCGATAGAGTGTCCCTGTCCTGCAGAGCCTCCCAGACCATCTCCGACTACCTGCA
CTGGTACCAACAGAAGTCCCACGAGAGCCCTCGGCTGCTGATCAAGTTCGCCTCCCAGTCTATCAGCGGCATCCCCTCCAG
ATTCTCCGGCAGCGGCTCTGGCTCTGACTTCACCCTGTCCATCAACTCCGTGGAACCCGAGGACGTGGGCGTGTACTACTG
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GTGACCCACCAGGGCCTGTCCAGCCCTGTGACCAAGTCCTTCAACCGGGGCGAGTGCTGATGATTAATTAAGATAAACCC
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GGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGA
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GCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTG
CTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCT
TCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATC
ACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCT
CCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCACCCTAGGGGGAGGCTAACTGAAACACGGAAGGAGACAATACCG
GAAGGAACCCGCGCTATGACGGCAATAAAAAGACAGAATAAAACGCACGGTGTTGGGCTCGAGGCATTTATCAGGGTTC
GTCTCGTCCCGGTCTCCTCCCATGCATGGGATCCGGCGCGCCAGATCT

SEQ ID NO: 30
Polypeptide A (A1 strain) without G294E mutation

VATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIADLKMAVSFSQFN
RRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYMVQLPIFGVIDTP
CWIVKAAPSCSGKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVST
GRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPVKF
PEDQFNVALDQVFESIENSQALVDQSNRILSSAEKGNTSGRENLYFQGGGGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGGIEG
RHHHHHH

SEQ ID NO: 31
CDS 381-2765 of the *Homo sapiens* Furin, Transcript variant 3, mRNA (NM_001289824.1)
ATGGAGCTGAGGCCCTGGTTGCTATGGGTGGTAGCAGCAACAGGAACCTTGGTCCTGCTAGCAGCTGATGCTCAGGGCCA
GAAGGTCTTCACCAACACGTGGGCTGTGCGCATCCCTGGAGGCCCAGCGGTGGCCAACAGTGTGGCACGGAAGCATGGG
TTCCTCAACCTGGGCCAGATCTTCGGGGACTATTACCACTTCTGGCATCGAGGAGTGACGAAGCGGTCCCTGTCGCCTCAC
CGCCCGCGGCACAGCCGGCTGCAGAGGGAGCCTCAAGTACAGTGGCTGGAACAGCAGGTGGCAAAGCGACGGACTAAA
CGGGACGTGTACCAGGAGCCCACAGACCCCAAGTTTCCTCAGCAGTGGTACCTGTCTGGTGTCACTCAGCGGGACCTGAA
TGTGAAGGCGGCCTGGGCGCAGGGCTACACAGGGCACGGCATTGTGGTCTCCATTCTGGACGATGGCATCGAGAAGAAC
CACCCGGACTTGGCAGGCAATTATGATCCTGGGGCCAGTTTTGATGTCAATGACCAGGACCCTGACCCCCAGCCTCGGTAC
ACACAGATGAATGACAACAGGCACGGCACACGGTGTGCGGGGGAAGTGGCTGCGGTGGCCAACAACGGTGTCTGTGGT
GTAGGTGTGGCCTACAACGCCCGCATTGGAGGGGTGCGCATGCTGGATGGCGAGGTGACAGATGCAGTGGAGGCACGC
TCGCTGGGCCTGAACCCCAACCACATCCACATCTACAGTGCCAGCTGGGGCCCCGAGGATGACGGCAAGACAGTGGATGG
GCCAGCCCGCCTCGCCGAGGAGGCCTTCTTCCGTGGGGTTAGCCAGGGCCGAGGGGGGCTGGGCTCCATCTTTGTCTGG
GCCTCGGGGAACGGGGGCCGGGAACATGACAGCTGCAACTGCGACGGCTACACCAACAGTATCTACACGCTGTCCATCA
GCAGCGCCACGCAGTTTGGCAACGTGCCGTGGTACAGCGAGGCCTGCTCGTCCACACTGGCCACGACCTACAGCAGTGGC
AACCAGAATGAGAAGCAGATCGTGACGACTGACTTGCGGCAGAAGTGCACGGAGTCTCACACGGGCACCTCAGCCTCTGC
CCCCTTAGCAGCCGGCATCATTGCTCTCACCCTGGAGGCCAATAAGAACCTCACATGGCGGGACATGCAACACCTGGTGGT
ACAGACCTCGAAGCCAGCCCACCTCAATGCCAACGACTGGGCCACCAATGGTGTGGGCCGGAAAGTGAGCCACTCATATG
GCTACGGGCTTTTGGACGCAGGCGCCATGGTGGCCCTGGCCCAGAATTGGACCACAGTGGCCCCCCAGCGGAAGTGCATC
ATCGACATCCTCACCGAGCCCAAAGACATCGGGAAACGGCTCGAGGTGCGGAAGACCGTGACCGCGTGCCTGGGCGAGC
CCAACCACATCACTCGGCTGGAGCACGCTCAGGCGCGGCTCACCCTGTCCTATAATCGCCGTGGCGACCTGGCCATCCACC
TGGTCAGCCCCATGGGCACCCGCTCCACCCTGCTGGCAGCCAGGCCACATGACTACTCCGCAGATGGGTTTAATGACTGG
GCCTTCATGACAACTCATTCCTGGGATGAGGATCCCTCTGGCGAGTGGGTCCTAGAGATTGAAAACACCAGCGAAGCCAA
CAACTATGGGACGCTGACCAAGTTCACCCTCGTACTCTATGGCACCGCCCCTGAGGGGCTGCCCGTACCTCCAGAAAGCAG
TGGCTGCAAGACCCTCACGTCCAGTCAGGCCTGTGTGGTGTGCGAGGAAGGCTTCTCCCTGCACCAGAAGAGCTGTGTCC
AGCACTGCCCTCCAGGGTTCGCCCCCCAAGTCCTCGATACGCACTATAGCACCGAGAATGACGTGGAGACCATCCGGGCC
AGCGTCTGCGCCCCCTGCCACGCCTCATGTGCCACATGCCAGGGGCCGGCCCTGACAGACTGCCTCAGCTGCCCCAGCCAC
GCCTCCTTGGACCCTGTGGAGCAGACTTGCTCCCGGCAAAGCCAGAGCAGCCGAGAGTCCCCGCCACAGCAGCAGCCACC
TCGGCTGCCCCCGGAGGTGGAGGCGGGGCAACGGCTGCGGGCAGGGCTGCTGCCCTCACACCTGCCTGAGGTGGTGGCC
GGCCTCAGCTGCGCCTTCATCGTGCTGGTCTTCGTCACTGTCTTCCTGGTCCTGCAGCTGCGCTCTGGCTTTAGTTTTCGGG
GGGTGAAGGTGTACACCATGGACCGTGGCCTCATCTCCTACAAGGGGCTGCCCCCTGAAGCCTGGCAGGAGGAGTGCCC
GTCTGACTCAGAAGAGGACGAGGGCCGGGGCGAGAGGACCGCCTTTATCAAAGACCAGAGCGCCCTCTGA

SEQ ID NO: 32
PoF_{hRSV}: hRSV post-fusion F-Protein for DNA vaccine
With three naturally occurring substitutions in the ectodomain (P102A, I379V, and M447V) and deletion of amino acids 137-146 of the fusion peptide
MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTE
LQLLMQSTP**A**TNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRSAIASGVAVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLT
SKVLDLKNYIDKQLLPIVNKQSCSISNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQ
IVRQQSYSIMSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFC
DTMNSLTLPSE**V**NLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDYVSNKG**V**DTVSVGNTL
YYVNKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVNAGKSTTN

SEQ ID NO: 33

PoF$_{hRSV}$: hRSV post-fusion F-Protein coding sequence human codon optimized for DNA vaccine

ATGGAACTGCTGATCCTGAAGGCCAACGCCATCACCACAATCCTGACCGCCGTGACCTTTTGCTTCGCCAGCGGCCAG
AACATCACCGAGGAATTCTACCAGAGCACCTGTAGCGCCGTGTCCAAGGGATATCTGTCTGCCCTGAGAACCGGCTGGTACAC
CAGCGTGATCACCATCGAGCTGAGCAACATCAAAGAAAACAAGTGCAACGGCACCGACGCCAAAGTGAAGCTGATCAAGCAA
GAGCTGGACAAGTACAAGAATGCCGTGACCGAACTGCAGCTGCTGATGCAGTCTACCCCTGCCACCAACAACCGGGCCAGAA
GAGAACTGCCCAGATTCATGAACTACACCCTGAACAACGCCAAAAAGACCAACGTGACCCTGAGCAAGAAGCGGAAGAGAAG
ATCTGCCATTGCCAGCGGCGTGGCCGTGTCTAAAGTTCTGCATCTGGAAGGCGAAGTGAACAAGATCAAGAGCGCCCTGCTG
AGCACCAACAAGGCCGTGGTTTCTCTGAGCAATGGCGTGTCCGTGCTGACCAGCAAGGTGCTGGACCTGAAGAACTACATCG
ACAAACAGCTGCTGCCCATCGTGAACAAGCAGAGCTGCAGCATCAGCAACATCGAGACAGTGATCGAGTTCCAGCAGAAGAA
CAACCGGCTGCTGGAAATCACCCGCGAGTTTTCTGTGAATGCCGGCGTGACCACACCTGTGTCCACCTACATGCTGACCAACA
GCGAGCTGCTGTCCCTGATCAACGACATGCCCATCACCAACGACCAGAAAAAGCTGATGAGCAACAACGTGCAGATCGTGCG
GCAGCAGAGCTACTCCATCATGAGCATTATCAAAGAAGAGGTGCTGGCCTACGTGGTGCAGCTGCCTCTGTATGGCGTGATCG
ATACCCCTTGCTGGAAGCTGCACACAAGCCCTCTGTGCACCACCAACACCAAAGAGGGCTCCAACATCTGCCTGACCAGAACC
GATAGAGGCTGGTACTGCGATAATGCCGGCAGCGTCAGCTTCTTCCCACAAGCCGAGACATGCAAGGTGCAGAGCAACAGAG
TGTTCTGCGACACCATGAACAGCCTGACACTGCCCTCCGAAGTGAATCTGTGCAACGTGGACATCTTCAACCCTAAGTACGACT
GCAAGATCATGACCTCCAAGACCGACGTGTCAAGCTCCGTGATCACATCTCTGGGCGCCATCGTGTCCTGCTACGGCAAGACA
AAGTGCACCGCCAGCAACAAGAACCGGGGCATCATCAAGACCTTCAGCAACGGCTGCGACTACGTGTCCAACAAAGGCGTGG
ACACCGTGTCTGTGGGCAACACCCTGTACTACGTGAACAAACAAGAGGGCAAGAGCCTGTACGTGAAGGGCGAGCCCATCAT
CAACTTCTACGACCCTCTGGTGTTCCCCAGCGACGAGTTTGATGCCAGCATCTCCCAAGTGAACGAGAAGATCAACCAGAGCCT
GGCCTTCATCAGAAAGTCCGATGAGCTGCTGCACAATGTGAACGCCGGCAAGAGCACCACAAAT

SEQ ID NO: 34

sPrF$_{hRSV}$SC-DM: hRSV single-chain pre-fusion F-protein for DNA vaccine

MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKIKCNGTDAKIKLIKQELDKYKNAVTEL
QLLMQSTPATNNQARGSGSGRSLGFLLGVGSAIASGVAVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQL
LPIVNKQSCSIPNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKE
EVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNL
CNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVNKQEGKSLYVK
GEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLSAIGGYIPEAPRDGQAYVRKDGEWVLLSTFL

SEQ ID NO: 35

sPrF$_{hRSV}$SC-DM: hRSV single-chain pre-fusion F-protein coding sequence human codon optimized for DNA vaccine

ATGGAACTGCTGATCCTGAAGGCCAACGCCATCACCACAATCCTGACCGCCGTGACCTTTTGCTTCGCCAGCGGCCAGAACATC
ACCGAGGAATTCTACCAGAGCACCTGTAGCGCCGTGTCCAAGGGATATCTGTCTGCCCTGAGAACCGGCTGGTACACCAGCGT
GATCACCATCGAGCTGAGCAACATCAAGAAAATCAAGTGCAACGGCACCGACGCCAAGATCAAGCTGATCAAGCAAGAGCTG
GACAAGTACAAGAATGCCGTGACCGAACTGCAGCTGCTGATGCAGTCTACCCCTGCCACCAACAATCAGGCCAGAGGCTCTGG
ATCTGGCAGAAGCCTGGGATTTCTGCTCGGCGTGGGATCTGCTATTGCTTCTGGCGTGGCCGTGTCTAAGGTGCTGCATCTGG
AAGGCGAAGTGAACAAGATTAAGAGCGCCCTGCTGAGCACCAACAAGGCCGTGGTTTCTCTGAGCAATGGCGTGTCCGTGCT
GACCAGCAAGGTGCTGGACCTGAAGAACTACATCGACAAACAGCTGCTGCCCATCGTGAACAAGCAGAGCTGCAGCATCCCC
AACATCGAGACAGTGATCGAGTTCCAGCAGAAGAACAACCGGCTGCTGGAAATCACCCGCGAGTTTTCTGTGAATGCCGGCG
TGACCACACCTGTGTCCACCTACATGCTGACCAACAGCGAGCTGCTGTCCCTGATCAACGACATGCCCATCACCAACGACCAGA
AAAAGCTGATGAGCAACAACGTGCAGATCGTGCGGCAGCAGAGCTACTCCATCATGAGCATCATCAAAGAAGAGGTGCTGGC
CTACGTGGTGCAGCTGCCTCTGTATGGCGTGATCGATACCCCTTGCTGGAAGCTGCACACAAGCCCTCTGTGCACCACCAACAC
CAAAGAGGGCAGCAACATCTGCCTGACCAGAACCGATAGAGGCTGGTACTGCGATAATGCCGGCAGCGTCAGCTTCTTCCCAC
AAGCCGAAACATGCAAGGTGCAGAGCAACAGAGTGTTCTGCGACACCATGAACAGCCTGACACTGCCCTCCGAAGTGAATCT
GTGCAACGTGGACATCTTCAACCCTAAGTACGACTGCAAGATCATGACCTCCAAGACCGACGTGTCAAGCTCCGTGATCACATC
TCTGGGCGCCATCGTGTCCTGCTACGGCAAGACAAAGTGCACCGCCAGCAACAAGAACCGGGGAATCATCAAGACCTTCAGC
AACGGCTGCGACTACGTGTCCAACAAAGGCGTGGACACCGTGTCTGTGGGCAACACCCTGTACTACGTGAACAAACAAGAGG
GCAAGAGCCTGTACGTGAAGGGCGAGCCCATCATCAACTTCTACGACCCTCTGGTGTTCCCCAGCGACGAGTTTGATGCCAGC
ATCAGCCAAGTGAACGAGAAGATCAACCAGAGCCTGGCCTTCATCAGAAAGTCCGATGAGCTGCTGAGCGCCATCGGCGGCT
ATATCCCTGAAGCTCCTAGAGATGGACAGGCCTATGTGCGGAAGGATGGCGAATGGGTGCTGCTGTCTACATTTCTG

SEQ ID NO: 36

sPrF$_{hRSV}$DS-Cav1: hRSV pre-fusion F-protein for DNA vaccine
MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTE
LQLLMQSTPATNNRARRFLGFLLGVGSAIASGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNK
QSCSISNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMCIIKEEVLAY
VVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNVDI
FNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIIN
FYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLSAIGGYIPEAPRDGQAYVRKDGEWVLLSTFL

SEQ ID NO: 37
sPrF$_{hRSV}$DS-Cav1: hRSV pre-fusion F-protein coding sequence human codon optimized for DNA vaccine
ATGGAACTGCTGATCCTGAAGGCCAACGCCATCACCACAATCCTGACCGCCGTGACCTTTTGCTTCGCCAGCGGCCAGAACATC
ACCGAGGAATTCTACCAGAGCACCTGTAGCGCCGTGTCCAAGGGATATCTGTCTGCCCTGAGAACCGGCTGGTACACCAGCGT
GATCACCATCGAGCTGAGCAACATCAAAGAAAACAAGTGCAACGGCACCGACGCCAAAGTGAAGCTGATCAAGCAAGAGCTG
GACAAGTACAAGAATGCCGTGACCGAACTGCAGCTGCTGATGCAGTCTACCCCTGCCACCAACAACCGGGCCAGAAGATTTCT
GGGCTTTCTGCTCGGCGTGGGCTCTGCTATTGCTAGCGGAGTGGCTGTGTGCAAGGTGCTGCATCTGGAAGGCGAAGTGAAC
AAGATCAAGAGCGCCCTGCTGAGCACCAACAAGGCCGTGGTTTCTCTGAGCAATGGCGTGTCCGTGCTGACCTTTAAGGTGCT
GGACCTGAAGAACTACATCGACAAACAGCTGCTGCCCATCCTGAACAAGCAGTCCTGCAGCATCAGCAACATCGAGACAGTGA
TCGAGTTCCAGCAGAAGAACAACCGGCTGCTGGAAATCACCCGCGAGTTTTCTGTGAATGCCGGCGTGACCACACCTGTGTCC
ACCTACATGCTGACCAACAGCGAGCTGCTGTCCCTGATCAACGACATGCCCATCACCAACGACCAGAAAAAGCTGATGAGCAA
CAACGTGCAGATCGTGCGGCAGCAGAGCTACAGCATCATGTGCATTATCAAAGAAGAGGTGCTGGCCTACGTGGTGCAGCTG
CCTCTGTATGGCGTGATCGATACCCCTTGCTGGAAGCTGCACACAAGCCCTCTGTGCACCACCAACACCAAAGAGGGCTCCAA
CATCTGCCTGACCAGAACCGATAGAGGCTGGTACTGCGATAATGCCGGCAGCGTCAGCTTCTTCCCACAAGCCGAAACATGCA
AAGTGCAGAGCAACCGGGTGTTCTGCGACACCATGAACAGCCTGACACTGCCCTCCGAAGTGAATCTGTGCAACGTGGACATC
TTCAACCCTAAGTACGACTGCAAGATCATGACCAGCAAGACCGACGTGTCAAGCTCCGTGATCACATCTCTGGGCGCCATCGT
GTCCTGCTACGGCAAGACAAAGTGCACCGCCAGCAACAAGAACCGGGGCATCATCAAGACCTTCAGCAACGGCTGCGACTAC
GTGTCCAACAAAGGCGTGGACACCGTGTCTGTGGGCAACACCCTGTACTACGTGAACAAACAAGAGGGCAAGAGCCTGTACG
TGAAGGGCGAGCCCATCATCAACTTCTACGACCCTCTGGTGTTCCCCAGCGACGAGTTTGATGCCAGCATCTCCCAAGTGAACG
AGAAGATCAACCAGAGCCTGGCCTTCATCAGAAAGTCCGATGAGCTGCTGAGCGCCATCGGCGGCTATATCCCTGAAGCTCCT
AGAGATGGACAGGCCTATGTGCGGAAGGATGGCGAATGGGTGCTGCTGTCTACATTTCTG

SEQ ID NO: 38
sF$_{hMPV}$: Soluble native F-protein for DNA vaccine
MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRELRTVSADQLAR
EEQIENPRQSKKRKRRVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDI
ADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYM
VQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTT
NYPCKVSTGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSF
DPVKFPEDQFNVALDQVFESIENSQALVDQSNRILSSAEKGNTG

SEQ ID NO: 39
sF$_{hMPV}$: Soluble native F-protein coding sequence human codon optimized for DNA vaccine
ATGAGCTGGAAGGTGGTCATCATCTTCAGCCTGCTGATCACCCCTCAGCACGGCCTGAAAGAGAGCTACCTGGAAGAGTCCTG
CAGCACCATCACAGAGGGCTACCTGTCTGTGCTGAGAACCGGCTGGTACACCAACGTGTTCACACTGGAAGTGGGCGACGTG
GAAAACCTGACCTGTGCCGATGGACCCAGCCTGATCAAGACCGAGCTGGATCTGACAAAGAGCGCCCTGAGAGAACTGAGGA
CCGTGTCTGCAGATCAGCTGGCCAGAGAGGAACAGATCGAGAACCCCAGACAGAGCAAGAAACGGAAGCGGAGAGTGGCCA
CAGCTGCTGCTGTTACAGCTGGCGTGGCCATTGCCAAGACCATCAGACTGGAAAGCGAAGTGACCGCCATCAAGAACGCCCT
GAAAAAGACCAACGAGGCCGTGTCTACACTCGGCAATGGCGTTAGAGTGCTGGCTACAGCCGTGCGCGAGCTGAAGGACTTC
GTGTCCAAAAATCTGACCCGGGCCATCAACAAGAACAAGTGCGACATTGCCGACCTGAAGATGGCCGTGTCCTTTAGCCAGTT
CAACCGGCGGTTTCTGAACGTCGTGCGGCAGTTTTCTGACAACGCCGGCATCACACCAGCCATCAGCCTGGACCTGATGACAG
ATGCTGAGCTGGCAAGAGCCGTGTCCAACATGCCTACATCTGCCGGCCAGATCAAGCTGATGCTGGAAAACAGAGCCATGGT
CCGACGGAAAGGCTTCGGCTTTCTGATCGGCGTGTACGGCAGCAGCGTGATCTATATGGTGCAGCTGCCTATCTTCGGCGTGA
TCGACACCCCTTGCTGGATTGTGAAGGCCGCTCCTAGCTGTAGCGAGAAGAAGGGCAATTACGCCTGCCTGCTGAGAGAGGA
CCAAGGCTGGTATTGTCAGAATGCCGGCAGCACCGTGTACTACCCCAACGAGAAGGATTGCGAGACACGGGGCGATCACGTG

TTCTGTGATACAGCCGCCGGAATCAATGTGGCCGAGCAGAGCAAAGAGTGCAACATCAACATCAGCACCACGAACTACCCCTG
CAAGGTGTCCACAGGCAGACACCCTATCTCTATGGTGGCCCTGTCTCCTCTGGGAGCCCTGGTTGCTTGTTACAAGGGCGTGTC
CTGTAGCATCGGCAGCAACAGAGTGGGCATCATCAAGCAGCTGAACAAGGGCTGCAGCTACATCACCAACCAGGACGCCGAT
ACCGTGACCATCGACAACACCGTGTATCAGCTGAGCAAGGTGGAAGGCGAACAGCACGTGATCAAGGGCAGACCTGTGTCCA
GCAGCTTCGACCCTGTGAAGTTCCCCGAGGATCAGTTCAACGTGGCCCTGGATCAGGTGTTCGAGAGCATCGAGAATAGCCAG
GCTCTGGTGGACCAGTCCAACAGAATCCTGTCTAGCGCCGAGAAGGGAAATACCGGA

SEQ ID NO: 40
FlFhMPV: Full length F-Protein of the A1 hMPV isolate "NL/1/00" (Accession No.: AAK62968)
MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRELRTVSADQLAR
EEQIENPRQSRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKN
KCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGFLIGVYGSS
VIYMVQLPIFGVIDTPCWIVKAAPSCSGKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNI
NISTTNYPCKVSTGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRP
VSSSFDPVKFPEDQFNVALDQVFESIENSQALVDQSNRILSSAEKGNTGFIIVIILIAVLGSTMILVSVFIIIKKTKKPTGAPPELSGVTNN
GFIPHN

SEQ ID NO: 41
FlF_{hMPV}: Full length F-Protein coding sequence of the A1 hMPV isolate "NL/1/00" human codon optimized for DNA vaccine
ATGAGCTGGAAGGTGGTGATCATCTTCAGCCTGCTGATCACCCCCCAGCACGGCCTGAAAGAGAGCTACCTGGAAGAGAGCT
GCAGCACCATCACCGAGGGCTACCTGAGCGTGCTGCGGACCGGCTGGTACACCAACGTGTTCACCCTGGAAGTGGGCGACGT
GGAAAACCTGACCTGCGCCGACGGCCCCAGCCTGATCAAGACCGAGCTGGACCTGACCAAGAGCGCCCTGCGCGAGCTGAGA
ACCGTGTCCGCCGATCAGCTGGCCAGAGAGGAACAGATCGAGAACCCCCGGCAGAGCAGATTCGTGCTGGGCGCCATTGCCC
TGGGCGTGGCCACAGCTGCTGCTGTGACAGCCGGCGTGGCCATTGCCAAGACCATCCGGCTGGAAAGCGAAGTGACCGCCAT
CAAGAACGCCCTGAAAAAGACCAACGAGGCCGTGTCCACCCTGGGCAACGGCGTGCGGGTGCTGGCTACAGCCGTGCGGGA
ACTGAAGGACTTCGTGTCCAAGAACCTGACCCGGGCCATCAACAAGAACAAGTGCGATATCGCCGACCTGAAGATGGCCGTG
TCCTTCAGCCAGTTCAACCGGCGGTTCCTGAACGTGGTGCGCCAGTTCAGCGACAACGCCGGCATCACCCCCGCCATCAGCCT
GGACCTGATGACCGATGCCGAGCTGGCCAGGGCCGTGTCTAACATGCCTACCTCTGCCGGCCAGATCAAGCTGATGCTGGAA
AACCGGGCCATGGTCCGCCGGAAGGGCTTCGGCTTCCTGATCGGCGTGTACGGCAGCAGCGTGATCTACATGGTGCAGCTGC
CCATCTTCGGCGTGATCGACACCCCCTGCTGGATCGTGAAGGCCGCTCCCAGCTGCAGCGGCAAGAAGGGCAACTACGCCTGC
CTGCTGAGAGAGGACCAGGGCTGGTACTGCCAGAACGCCGGCTCCACCGTGTACTACCCCAACGAGAAGGACTGCGAGACAC
GGGGCGACCACGTGTTCTGCGACACCGCCGCTGGCATCAACGTGGCCGAGCAGAGCAAAGAGTGCAACATCAACATCAGCAC
CACCAACTACCCCTGCAAGGTGTCCACCGGCAGACACCCCATCAGCATGGTGGCCCTGAGCCCTCTGGGAGCCCTGGTGGCCT
GTTACAAGGGCGTGTCCTGCAGCATCGGCAGCAACAGAGTGGGCATCATCAAGCAGCTGAACAAGGGCTGCAGCTACATCAC
CAACCAGGACGCCGACACCGTGACCATCGACAACACCGTGTACCAGCTGAGCAAGGTGGAAGGCGAGCAGCACGTGATCAA
GGGCAGACCCGTGTCCAGCAGCTTCGACCCCGTGAAGTTCCCCGAGGACCAGTTCAATGTGGCCCTGGACCAGGTGTTCGAG
AGCATCGAGAACAGCCAGGCCCTGGTGGACCAGAGCAACCGGATCCTGAGCAGCGCCGAGAAGGGAAACACCGGCTTCATC
ATCGTGATCATCCTGATCGCCGTGCTGGGCAGCACCATGATCCTGGTGTCCGTGTTCATCATCATCAAGAAAACAAAGAAGCCC
ACAGGCGCCCCTCCCGAGCTGAGCGGCGTGACCAACAATGGCTTCATCCCCCACAAC

SEQ ID NO: 42
sPoFhMPV: Post-Fusion F-protein (A1) for DNA vaccine
MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRELRTVSADQLAR
EEQIENPRQSKKRKRRVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDI
ADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYM
VQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTT
NYPCKVSTGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSF
DPVKFPEDQFNVALDQVFESIENSQALVDQSNRILSSAEKGNTSGRENLYFQGGGGSGYIPEAPRDGQAYVRKDGEWVLLSTFL

SEQ ID NO: 43
sPoFhMPV: Post-Fusion F-protein (A1) human codon optimized DNA Sequence for DNA vaccine

ATGAGCTGGAAGGTGGTGATCATCTTCAGCCTGCTGATCACCCCCCAGCACGGCCTGAAAGAGAGCTACCTGGAAGAGAGCT
GCAGCACCATCACCGAGGGCTACCTGAGCGTGCTGCGGACCGGCTGGTACACCAACGTGTTCACCCTGGAAGTGGGCGACGT
GGAAAACCTGACCTGCGCCGACGGCCCCAGCCTGATCAAGACCGAGCTGGACCTGACCAAGAGCGCCCTGCGCGAGCTGAGA
ACCGTGTCCGCCGATCAGCTGGCCAGAGAGGAACAGATCGAGAACCCCCGGCAGAGCAAGAAACGGAAGCGGAGAGTGGCC
ACCGCCGCTGCCGTGACAGCTGGCGTGGCCATTGCCAAGACCATCCGGCTGGAAAGCGAAGTGACCGCCATCAAGAACGCCC
TGAAAAAGACCAACGAGGCCGTGTCCACCCTGGGCAACGGCGTGCGGGTGCTGGCTACAGCCGTGCGGGAACTGAAGGACT
TCGTGTCCAAGAACCTGACCCGGGCCATCAACAAGAACAAGTGCGATATCGCCGACCTGAAGATGGCCGTGTCCTTCAGCCAG
TTCAACCGGCGGTTCCTGAACGTGGTGCGCCAGTTCAGCGACAACGCCGGCATCACCCCCGCCATCAGCCTGGACCTGATGAC
CGATGCCGAGCTGGCCAGGGCCGTGTCTAACATGCCTACCTCTGCCGGCCAGATCAAGCTGATGCTGGAAAACCGGGCCATG
GTCCGCCGGAAGGGCTTCGGCTTCCTGATCGGCGTGTACGGCAGCAGCGTGATCTACATGGTGCAGCTGCCCATCTTCGGCGT
GATCGACACCCCCTGCTGGATCGTGAAGGCCGCTCCCAGCTGCAGCGAGAAGAAGGGCAACTACGCCTGCCTGCTGAGAGAG
GACCAGGGCTGGTACTGCCAGAACGCCGGCTCCACCGTGTACTACCCCAACGAGAAGGACTGCGAGACACGGGGCGACCAC
GTGTTCTGCGACACCGCTGCCGGCATCAACGTGGCCGAGCAGAGCAAAGAGTGCAACATCAACATCAGCACCACCAACTACCC
CTGCAAGGTGTCCACCGGCAGACACCCCATCAGCATGGTGGCCCTGAGCCCTCTGGGAGCCCTGGTGGCCTGTTACAAGGGC
GTGTCCTGCAGCATCGGCAGCAACAGAGTGGGCATCATCAAGCAGCTGAACAAGGGCTGCAGCTACATCACCAACCAGGACG
CCGACACCGTGACCATCGACAACACCGTGTACCAGCTGAGCAAGGTGGAAGGCGAGCAGCACGTGATCAAGGGCAGACCCGT
GTCCAGCAGCTTCGACCCCGTGAAGTTCCCCGAGGACCAGTTCAATGTGGCCCTGGACCAGGTGTTCGAGAGCATCGAGAACA
GCCAGGCCCTGGTGGACCAGAGCAACCGGATCCTGAGCAGCGCCGAGAAGGGCAATACCAGCGGCAGAGAGAACCTGTATT
TTCAAGGCGGAGGCGGCAGCGGCTACATCCCCGAAGCCCCTAGAGATGGCCAGGCCTACGTGCGGAAGGACGGCGAGTGGG
TGCTGCTGAGCACCTTCCTG

SEQ ID NO: 44
sPoF$_{hMPV}$A1-Mfur: post-fusion configuration F-protein polypeptide with His-tag for purification (encoded by SEQ ID NO: 18)
KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRELRTVSADQLAREEQIENPRQSKKRKRR
VATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIADLKMAVSFSQFN
RRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYMVQLPIFGVIDTP
CWIVKAAPSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVST
GRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPVKF
PEDQFNVALDQVFESIENSQALVDQSNRILSSAEKGNTSGRENLYFQGGGGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGGIEG
RHHHHHH

SEQ ID NO: 45
sF$_{hMPV}$A1-V: soluble configuration F-protein polypeptide in monomer form for purification
MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRELRTVSADQ
LAREEQIENPRQSRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTR
AINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGF
LIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGIN
VAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSK
VEGEQHVIKGRPVSSSFDPVKFPEDQFNVALDQVFESIENSQALVDQSNRILSSAEKGNTGHHHHHH

SEQ ID NO: 46
sF$_{hMPV}$A1-K soluble F-protein subunit polypeptide in prefusion trimer form for purification
MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRELRTVSADQ
LAREEQIENPRQSRFVLGAIALGVCTAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRVLAFAVRELKDFVSKNLTR
ALNKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGF
LIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTACGIN
VAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSK
VEGEQHVIKGRPVSSSFDPVKFPEDQFNVALDQVFESIENSQALVDQSNRILSSAESAIGGYIPEAPRDGQAYVRKDGEWVLLST
FLGGLVPRGSHHHHHHSAWSHPQFEK

SEQ ID NO: 47

sPoF$_{hMPV}$: Post-Fusion hMPV F-protein (B1) adapted from the sequence of the "NL/1/99" isolate (Accession number AY304361.1)
MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTDGPSLIKTELDLTKSALRELKTVSADQ
LAREEQIENPRQSKKRKRRVATAAAVTAGIAIAKTIRLESEVNAIKGALKQTNEAVSTLGNGVRVLATAVRELKEFVSKNLTSAINR
NKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYMPTSAGQIKLMLENRAMVRRKGFGILIGVY
GSSVIYMVQLPIFGVIDTPCWIIKAAPSCSEKNGNYACLLREDQGWYCKNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQS
RECNINISTTNYPCKVSTGRHPISMVALSPLGALVACYKGVSCSIGSNWVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGE
QHVIKGRPVSSSFDPIKFPEDQFNVALDQVFESIENSQALVDQSNKILNSAEKGNTSGRENLYFQGGGGSGYIPEAPRDGQAYVR
KDGEWVLLSTFL

SEQ ID NO: 48
sPoF$_{hMPV}$: Post-Fusion hMPV F-protein (B1) adapted from the sequence of the "Arg/2/02" isolate (Accession number DQ362937.1)
MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRELRTVSADQ
LAREEQIENPRQSKKRKRRVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINK
NKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGFLIGV
YGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAE
QSKECNINISTTNYPCKVSTGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEG
EQHVIKGRPVSSSFDPVKFPEDQFNVALDQVFESIENSQALVDQSNRILSSAEKGNTSGRENLYFQGGGGSGYIPEAPRDGQAY
VRKDGEWVLLSTFL

SEQ ID NO: 49
sPoF$_{hMPV}$: Post-Fusion hMPV F-protein (B1) adapted from the sequence of the "Arg/2/02" isolate (Accession number DQ362937.1) human codon optimized DNA Sequence for DNA vaccine
ATGAGCTGGAAGGTGGTGATCATCTTCAGCCTGCTGATCACCCCCCAGCACGGCCTGAAAGAGAGCTACCTGGAAGAGAG
CTGCAGCACCATCACCGAGGGCTACCTGAGCGTGCTGCGGACCGGCTGGTACACCAACGTGTTCACCCTGGAAGTGGGCG
ACGTGGAAAACCTGACCTGCGCCGACGGCCCCAGCCTGATCAAGACCGAGCTGGACCTGACCAAGAGCGCCCTGCGCGA
GCTGAGAACCGTGTCCGCCGATCAGCTGGCCAGAGAGGAACAGATCGAGAACCCCCGGCAGAGCAAGAAACGGAAGCG
GAGAGTGGCCACCGCCGCTGCCGTGACAGCTGGCGTGGCCATTGCCAAGACCATCCGGCTGGAAAGCGAAGTGACCGCC
ATCAAGAACGCCCTGAAAAAGACCAACGAGGCCGTGTCCACCCTGGGCAACGGCGTGCGGGTGCTGGCTACAGCCGTGC
GGGAACTGAAGGACTTCGTGTCCAAGAACCTGACCCGGGCCATCAACAAGAACAAGTGCGATATCGCCGACCTGAAGAT
GGCCGTGTCCTTCAGCCAGTTCAACCGGCGGTTCCTGAACGTGGTGCGCCAGTTCAGCGACAACGCCGGCATCACCCCCG
CCATCAGCCTGGACCTGATGACCGATGCCGAGCTGGCCAGGGCCGTGTCTAACATGCCTACCTCTGCCGGCCAGATCAAG
CTGATGCTGGAAAACCGGGCCATGGTCCGCCGGAAGGGCTTCGGCTTCCTGATCGGCGTGTACGGCAGCAGCGTGATCTA
CATGGTGCAGCTGCCCATCTTCGGCGTGATCGACACCCCCTGCTGGATCGTGAAGGCCGCTCCCAGCTGCAGCGAGAAGA
AGGGCAACTACGCCTGCCTGCTGAGAGAGGACCAGGGCTGGTACTGCCAGAACGCCGGCTCCACCGTGTACTACCCCAAC
GAGAAGGACTGCGAGACACGGGGCGACCACGTGTTCTGCGACACCGCTGCCGGCATCAACGTGGCCGAGCAGAGCAAA
GAGTGCAACATCAACATCAGCACCACCAACTACCCCTGCAAGGTGTCCACCGGCAGACACCCCATCAGCATGGTGGCCCTG
AGCCCTCTGGGAGCCCTGGTGGCCTGTTACAAGGGCGTGTCCTGCAGCATCGGCAGCAACAGAGTGGGCATCATCAAGCA
GCTGAACAAGGGCTGCAGCTACATCACCAACCAGGACGCCGACACCGTGACCATCGACAACACCGTGTACCAGCTGAGCA
AGGTGGAAGGCGAGCAGCACGTGATCAAGGGCAGACCCGTGTCCAGCAGCTTCGACCCCGTGAAGTTCCCCGAGGACCA
GTTCAATGTGGCCCTGGACCAGGTGTTCGAGAGCATCGAGAACAGCCAGGCCCTGGTGGACCAGAGCAACCGGATCCTG
AGCAGCGCCGAGAAGGGCAATACCAGCGGCAGAGAGAACCTGTATTTTCAAGGCGGAGGCGGCAGCGGCTACATCCCCG
AAGCCCCTAGAGATGGCCAGGCCTACGTGCGGAAGGACGGCGAGTGGGTGCTGCTGAGCACCTTCCTG

EXAMPLES

Materials and Methods

DNA sequences encoding F-proteins for DNA vaccines

Three Nanotaxi®-DNA hMPV vaccine candidate constructs encoding hMPV F-proteins were designed to trigger expression of proteins by the host cell as outlined in Table 1A.

Three Nanotaxi®-DNA hRSV vaccine candidate constructs were designed for combination hMPV/RSV vaccine candidates as detailed in Table 1A. The constructs trigger the expression of secreted (soluble) forms of the pre- or post-fusion conformations of the hRSV F-protein as a trimer complex by the host cells. The amino acid sequences of the different hRSV F-proteins are derived from the sequence of the F-protein of the hRSV A2 strain (Pubmed accession No.: P03420) belonging to the A2 sublineage.

Synthesis and subcloning of codon optimized F-protein DNA sequences

Because examination of the native sequence of the hMPV F-protein revealed numerous rare mammalian codons, AT-rich sequences, mRNA instability motifs including poly(A) regions and cryptic splice sites, as well as several potential RNA secondary structural elements, codon optimization of the DNA sequences for use in DNA vaccination was performed to increase expression in humans. The codon-optimized sequences for the six F-protein constructs in Table 1A below were *de novo* synthesized and spliced into the immunization plasmid (pVax1, Invitrogen; SEQ ID NO: 28) using HindIII and XhoI restriction sites. The resulting vector DNA was amplified in *E. coli* (DH5α™), purified with a HiSpeed Plasmid Giga EF Kit (Qiagen S.A., Courtaboeuf, France) according to the manufacturer's protocol and quantified by measuring the absorbance (260 nm) of diluted plasmid solution (1/250 & 1/500) in triplicate. The plasmids were additionally checked by enzymatic digestion, followed by agarose gel electrophoresis.

Table 1A. Sequences for hMPV F-protein DNA vaccine preparation and hRSV F-protein DNA vaccine preparation. All DNA sequences were codon optimized for human expression and sub-cloned into pVAX1.

Name	Description	Nucleotide SEQ ID NO:	Amino acid SEQ ID NO:
Soluble post-fusion hMPV F-protein ($sPoF_{hMPV}$)	Encodes soluble post-fusion form of hMPV F-protein, A1 genotype, which assembles into homotrimers; as described in detail in **Figure 3**.	43	42
Full-length hMPV F-protein (FlF_{hMPV})	Encodes the full-length wild-type amino acid sequence of the hMPV F-protein (Accession No.: AAK62968) from the A1 hMPV isolate "NL/1/00" (Accession No.: AF371337.2) with no modifications.	41	40
Soluble hMPV F-	Encodes a soluble monomeric form of hMPV F-protein comprising	39	38

protein (sF_{hMPV})	the ectodomain in native form with only a G294E mutation. The C-terminus is truncated, removing the transmembrane domain and cytoplasmic tail. Additionally added was a KKRKRR cleavage site for enhanced processing. The sequence is modified from "fusion protein [Human metapneumovirus]" with accession number AAK62968.		
Soluble single-chain prefusion hRSV F-Protein ($sPrF_{hRSV}SC$-DM)	Soluble form of hRSV F-protein in a pre-fusion configuration (Krarup et al., 2015), including a modified ectodomain with three naturally occurring substitutions (found in other RSV isolates) to enhance expression (P102A, I379V, and M447V) as well as four mutations to improve expression and to stabilize the prefusion state (E66K, N67I, V76I and S215P). Further, the P27 interchain region from amino acids 108-134 were replaced by a linker (GSGSG) and two substitutions to impair furin cleavage (R106Q and F137S). This is a single chain (SC) mutant, which is resistant to cleavage into F1 and F2 fragments.	35	34
Soluble pre-fusion hRSV F-Protein (Disulfide-Cavity-filling) ($PrF_{hRSV}DS$-Cav1)	Soluble form of hRSV F-protein (McLellan et al., 2013), including an ectodomain to which several modifications have been made including three substitutions to enhance expression (P102A, I379V, and M447V), the addition of a cysteine pair (S155C; S290C) and cavity-filling hydrophobic substitutions (S190F; V207L) to stabilize the prefusion state and, further, the P27 interchain region from amino acids 108-134 were removed by keeping only the first furin site intact.	37	36
Post-fusion hRSV F-Protein (PoF_{hRSV})	Post-fusion form of hRSV F-protein, including a truncated ectodomain (TM and CT removed) in which three naturally occurring substitutions were introduced to enhance expression (P102A, I379V, and M447V), additionally, a part of the fusion peptide has been removed (aa 137 to 146). Notably, this RSV post-fusion ectodomain properly trimerizes without the addition of foldon (McLellan et al., 2011; Swanson et al., 2011)	33	32

Plasmid for F-protein DNA vaccines

The plasmid pVAX1 (SEQ ID NO: 28) from Invitrogen was developed for use in DNA vaccines by modification of the pcDNA™3.1 vector according to considerations put forth by the FDA Center for Biologics Evaluation and Research (CBER) in the document "Points to Consider on Plasmid DNA Vaccines for Preventive Infectious Diseases Indications" (2007; Docket no. 96N-0400). The pVAX1™ vector was used for all DNA constructs used in the DNA vaccination studies described herein and contains:

-a human cytomegalovirus immediate-early (CMV) promoter for high-level expression in a wide range of mammalian cells

-a bovine growth hormone (BGH) polyadenylation signal for efficient transcription termination and polyadenylation of mRNA

-a kanamycin resistance gene for selection in *E. coli.*

Constructs for hMPV F-protein subunit vaccines

In nature, the mature F-protein is a homotrimer of F2/F1 heterodimers covalently linked by two disulfide bridges. Each F2/F1 heterodimer is initially expressed as a single polypeptide precursor,

designated F0 (**Figure 1**). The F0 precursor proteins form trimers in the endoplasmic reticulum and are proteolytically processed by extracellular proteases at a single site located immediately upstream of the hydrophobic fusion peptide, which lies at the N-terminus of the F1 domain. The mature trimeric F-protein adopts a metastable pre-fusion conformation in the mature virus particle that is triggered to undergo a conformational change when the viral and target-cell membranes are brought into proximity. Final refolding of the paramyxovirus F-protein into a stable post-fusion conformation leads to the merging of the viral and host cell membranes and the formation of the fusion pore (**Figure 2**).

Modifications to the subunit F-proteins

The post-fusion hMPV F-protein construct ($sPoF_{hMPV}$) in trimeric form is stabilized by fusion of the ectodomain (F-protein excluding the C-terminal transmembrane and cytoplasmic tail domains) to a foldon trimerization domain derived from the T4 phagehead fibritin (SEQ ID NO: 6). In addition, the polybasic cleavage site II of hRSV (KKRKRR; SEQ ID NO: 2) was added after the native proteolytic cleavage site present in the hMPV F-protein (RQSR; SEQ ID NO: 1) to facilitate proteolytic processing in the absence of added trypsin. Furthermore, in order to avoid aggregation of the mature post-fusion hMPV F-protein homotrimer, the first eight amino acids of the fusion peptide were deleted (Δ103-111). A His6-tag (SEQ ID NO: 5) was added downstream of the trimerization domain for purification purposes. Finally, also added were a TEV protease cleavage site (SEQ ID NO: 3) for the removal of the foldon if desired and an Xa cleavage site (SEQ ID NO: 4) to remove the His6 tag after purification if desired, for example, for use in humans, and the sequence was codon optimized for expression in CHO cells.

The soluble monomeric subunit hMPV F-protein construct (sF_{hMPV}A1-V) is both soluble and monomeric by virtue of truncation of the transmembrane and cytoplasmic tail domains. The construct contains also a G294E substitution for enhanced production and a His6-tag (SEQ ID NO: 5) for purification purposes. (Herfst et al. (2007) Journal of General Virology, 88:2702-2709.)

In both constructs above, a G294E mutation was introduced to potentially improve the cell surface exposure and fusion characteristics of the F-protein (Mas *et al.* (2011) Residues of the Human Metapneumovirus Fusion (F) Protein Critical for Its Strain-Related Fusion Phenotype: Implications for the Virus Replication Cycle. J. Virol. 85(23): 12650-12661). The prefusion trimeric hMPV F-protein construct (sF_{hMPV}A1-K) assumes a prefusion trimeric configuration.

Table 1B. Sequences for hMPV F-protein subunit preparation; DNA sequences were codon optimized for expression in CHO cells and sub-cloned into pVVS1371. For the post-fusion hMPV F-protein, the pVVS1371 plasmid also contained the furin coding sequence of SEQ ID NO: 31 to cleave the introduced furin site. (The other two constructs contain the native trypsin cleavage site.)

Name	Description	Nucleic acid SEQ ID NO:	Amino acid SEQ ID NO:
$sPoF_{hMPV}A1$-Mfur	Encodes soluble post-fusion form of hMPV F-protein, A1 genotype, which assembles into homotrimers. Includes a furin site for processing, a His6-tag for purification and an Xa cleavage site for optional removal of the His6-tag; described in detail in **Figure 3**	18	44
$sF_{hMPV}A1$-V	Encodes a soluble monomeric form of hMPV F-protein comprising the ectodomain in native form with a G294E mutation (C-terminus is truncated, removing the transmembrane domain and cytoplasmic tail)	-	45
$sF_{hMPV}A1$-K	Soluble pre-fusion form of hMPV F-protein, in trimeric form	-	46

Plasmid for expression of hMPV F-proteins

The plasmid pVVS1371 (SEQ ID NO: 28) was designed at Valneva for transient or stable expression of one or, optionally, two proteins of interest in CHO cells.

The plasmid contains:

-an HS4 insulator sequence from chicken β-globin locus,

-two cytomegalovirus (CMV) promoters,

-two chimeric introns, downstream from the CMV promoter, composed of the 5´-donor site from the first intron of the human β-globin gene and the branch and 3´-acceptor sites from the intron of an immunoglobulin gene heavy chain variable region. The sequences of the donor and acceptor sites, along with the branchpoint site, were changed to match the consensus sequences for splicing. The intron is located upstream of the cDNA insert in order to prevent utilization of possible cryptic 5´-donor splice sites within the cDNA sequence,

-the bovine growth hormone polyadenylation signal sequence (bgh-polyA),

-the neomycin phosphotransferase gene from Tn5 under the regulation of the SV40 enhancer and early promoter region. An HSV TK polyadenylation signal based on the highly efficient polyadenylation signal of the thymidine kinase gene of Herpes Virus is located downstream of the neomycin phosphotransferase gene. Expression of the neomycin phosphotransferase gene in mammalian cells confers resistance to the antibiotic G-418,

-a kanamycin resistance gene under the regulation of a bacterial promoter, and

-a pUC origin of replication.

Constructs for DNA vaccines

All DNA sequences used for DNA vaccines (see Table 1A) were cloned into the pVAX1 plasmid backbone containing a CMV promoter to control gene expression. Plasmids were purified from recombinant *E. coli* using the EndoFree plasmid purification kit (Qiagen, France).

Construction of expression vectors for production of hMPV F-protein polypeptides

The coding sequences for the post-fusion hMPV F-protein construct $sPoF_{hMPV}$A1-MFur (polynucleotide sequence provided by SEQ ID NO: 18), the soluble monomeric hMPV F-protein construct sF_{hMPV}A1-V (polypeptide of SEQ ID NO: 45), the prefusion trimeric hMPV F-protein construct sF_{hMPV}A1-K (polypeptide of SEQ ID NO: 46) as well as the coding sequence for human furin (polynucleotide sequence provided by SEQ ID NO: 31) were cloned into pVVS1371 for transient or stable protein expression in CHO cells. The human furin protease for processing F0 into F1 and F2 was provided by cloning a furin coding sequence (SEQ ID NO: 31) into the same plasmid under the EF1 promoter (see below for details).

The hMPV F-protein coding sequences for A1-Mfur (SEQ ID NO: 18), A1-V and A1-K were inserted between the chimeric intron and the bGH A polyadenylation site of pVVS1371 vector using the restriction sites *SalI* and *PacI*. Briefly, vector and synthetic coding sequences for recombinant F-proteins (synthesis done by GeneArt) were double-digested with *SalI* and *PacI* followed by purification following separation on an agarose gel. The vector and coding sequence fragments were ligated with T4 DNA ligase and the ligation product was used to transform Max efficiency DH5α™ competent cells. Selected clones were checked for mutation by sequencing.

Because proteolytic cleavage by furin is key to the proteolytic processing of the $sPoF_{hMPV}$A1-Mfur protein (due to the introduced furin cleavage site) and only very low levels of furin are expressed by CHO cells, a codon optimized coding sequence for the human furin gene (SEQ ID NO: 31) was inserted into the same plasmid downstream from the recombinant hMPV post-fusion F-protein sequence for co-expression of the two proteins. The expression of the furin gene (accession No.: NP_001276753; encoded by SEQ ID NO: 26) was under the regulation of an EF1 promoter and a bovine growth hormone polyadenylation signal (bgh-polyA). The synthetic gene, including a bgh-polyA sequence, the EF1 promoter sequence and the hFUR gene were cloned between the HS4 insulator and the bgh-polyA sequence by using *XbaII/PmeI* restriction sites following the same steps as described previously. For tandem expression, the same synthetic gene was cloned between the

hMPV F-protein coding sequence and the bgh-polyA sequence using *PacI/PmeI* restriction sites following the same steps as described previously.

Cell lines, bacteria and viruses

Vero and HeLa cell lines were acquired from ATCC, CHO cells from ECACC and LLC-MK2 cells from HPA Culture Collections. Max Efficiency™ DH5α™ Competent Cells (ThermoFisher), a chemically-competent *E. coli* strain, were used for amplification of the plasmids used in this work. Strain A1 hMPV was a kind gift from CHU Caen.

Animals

Mice (*e.g.*, C57Bl/6 and BALB/c, Janvier) were used for immunogenicity studies of the vaccine candidates. Cotton rats for use in hMPV and/or RSV challenge studies were purchased from Sigmovir Biosystems (Rockville, MD).

Monoclonal antibodies to F-proteins

DS7 Antibody: A neutralizing mouse monoclonal antibody that specifically binds to an epitope on hMPV F-protein that is present on both pre- and post-fusion conformations of the MPV F-protein. The DS7 antibody and methods for its production have been described previously (*e.g.*, Wen, *et al.*, 2012, Structure of the Human Metapneumovirus Fusion Protein with Neutralizing Antibody Identifies a Pneumovirus Antigenic Site. Nat. Struct. Mol. Biol. 19:461-463). The amino acid sequences of the heavy and light variable regions of the DS7 antibody are provided as SEQ ID NOs: 20 and 21, respectively, and are deposited in PDB as Nos. 4DAG_H (DS7 VH) and 4DAG_L (DS7 VL), each of which is incorporated by reference herein as present in the database. The DS7 antibody was manufactured in-house.

MPE8 Antibody: A neutralizing monoclonal antibody that binds selectively to the prefusion form of F-proteins of both hRSV and hMPV (Corti et al., 2013, Cross-neutralization of four paramyxoviruses by a human monoclonal antibody Nature 501, 439-443).

Nanotaxi® and preparation of DNA formulations

The 704 tetrafunctional non-ionic block copolymers (Nanotaxi®) in non-glycosylated (704) and mannosylated (704-M) form were provided by In-Cell-Art (Nantes, France). Stock solutions of the 704 tetrafunctional non-ionic amphiphilic block copolymers were prepared at 2% in sterile deionized water and stored at 4°C. Formulations of DNA with 704 or 704-M tetrafunctional block copolymers were

prepared by mixing equal volumes of tetrafunctional block copolymer working solution at 0.3% in water with plasmid DNA solution at the desired concentration in buffered solutions.

Two Nanotaxi® delivery systems (Nanotaxi®1 and Nanotaxi®2, i.e., copolymer 704 without or with mannose; i.e., 704 and 704-M, respectively) were used in the immunization studies based upon their ability to stimulate the innate immune response and to trigger a balanced Th_1/Th_2 response. The Nanotaxi®1 (704) was used in the first immunogenicity study. Each plasmid was separately formulated with each Nanotaxi® prior to injection into mice. Briefly, stock solutions of the 704 tetrafunctional non-ionic amphiphilic block copolymers were prepared at 2% in sterile deionized water and stored at 4°C. Plasmid DNAs were amplified and purified using endotoxin free kit and controlled by enzymatic restriction analysis and concentration measured by optical density. Formulations of DNA with non-glycosylated or glycosylated tetrafunctional block copolymers were prepared by mixing equal volumes of tetrafunctional block copolymer stock solution in water with plasmid DNA solution at the desired concentrations in buffered solutions. In the Examples, the 704 and 704-M working solutions were at a concentration of 0.3% for a final concentration of 0.15%. Intramuscular injections of glycosylated or not tetrafunctional non-ionic block copolymer formulating various amounts of plasmid DNA ranging from 5 µg to 50 µg were performed in both anterior tibial muscles of C57Bl/6 mice.

Example 1 Expression of protein from DNA pVAX1 constructs in HeLa cells

Preparation of ICAfectin®441 transfection solution

Formulations of nucleic acids with ICAfectin®441 were prepared by mixing equal volume of DNA solution containing 0.5µg per p24-well plate with ICAfectin®441 as recommended by the provider (In-Cell-Art, France). Twenty four hours prior to transfection HeLa cells were seeded in 24-well culture plates at a density of 55 000 cells per well in 1 mL of complete medium and incubated at 37°C in a humidified 5% CO_2 / 95% air containing atmosphere.

Thirty minutes before transfection, medium was removed and replaced by 400 µL of fresh complete medium and 100 µL of ICAfectin®441/DNA complexes were added to the HeLa cells. After 24h of incubation at 37°C, 5% CO_2, samples of cells (10^5-10^6) were collected and re-suspended in phosphate-buffered saline (PBS). The resulting cell suspension was assayed for the expression of hMPV F-protein by flow cytometry analysis using a FACSverse flow cytometer (Becton Dickinson) after labelling by anti-F antibody DS7. All measurements were performed in triplicate. Results are shown in **Figure 5**.

Example 2 Construction, expression, characterization and formulation of F-protein polypeptides for subunit vaccines and/or use in ELISA

Expression in Chinese Hamster Ovary (CHO) cells

Subunit protein production was based on transient transfection of CHO cells using a MaxCyte® STX Scalable Transfection System device and following experimental recommendations of the supplier. Briefly, prior to electroporation, CHO cells were pelleted, suspended in MaxCyte® electroporation buffer and mixed with corresponding expression plasmid DNA. The cell-DNA mixture was transferred to a cassette processing assembly and loaded onto the MaxCyte® STX Scalable Transfection System. Cells were electroporated using the preprogrammed "CHO" protocol and immediately transferred to culture flasks and incubated for 30 to 40 minutes at 37°C with 8% CO_2. Following the recovery period, cells were resuspended at high density in EX-CELL ACF CHO medium (Sigma Aldrich). Post-electroporation, cells were incubated at 37°C with 8% CO_2 and orbital shaking for 24 hours.

The production kinetics consisted of decreasing the culture temperature to 32°C and feeding the transfected cells daily with a fed-batch medium developed for transient protein expression in CHO cells (CHO CD EfficientFeed™ (ThermoFisher Scientific), supplemented with yeastolate, glucose and glutaMax). After 7 or 14 days of culture, cell viability was checked and conditioned medium was harvested after cell clarification corresponding to two runs of centrifugation at maximum speed for 10 minutes. Clarified product was 0.2 µm sterile filtered and stored at -80°C before protein purification.

Concentration and purification

After a period of storage at -80°C, the clarified supernatant was brought to room temperature and concentrated 50-60 times the initial volume with a tangential flow system of 50 kDa (Vivaflow 200, Sartorius). Subsequently, it was equilibrated with 50 mM Na_2HPO_4 buffer at pH 8.0, 300 mM NaCl. Purification of the protein was performed using Immobilized metal ion affinity chromatography (IMAC) as described below, followed by diafiltration on a Slide-A-Lyzer™ Dialysis Cassette (ThermoFisher Scientific; 10kDa MWCO) against a storage buffer (50mM Na_2HPO_4, 300mM NaCl, 5mM EDTA, 10% sucrose, 0.05% Tween-20, pH 8).

Immobilized metal ion affinity chromatography (IMAC)

For metal ion affinity chromatography (IMAC), agarose resin containing Ni^{2+} (His-Select Nickel Affinity Gel, Sigma) was manually packed into chromatography columns. The resin was washed with two volumes of deionized water and equilibrated with three volumes of equilibration and wash buffer (50 mM sodium phosphate, pH 8.0, with 0.3 M sodium chloride and 10 mM imidazole) as indicated by the manufacturer. The sample was loaded onto the column via a peristaltic pump having a flow rate of about 0.8 mL/minute. After all extract was loaded, the column was washed with wash buffer at a flow rate of about 10-20 column volumes/hour. The column was washed extensively until the A_{280} of the eluate was stable and near that of the wash buffer. The His-tagged protein was eluted from the column using 3-10 column volumes of elution buffer as indicated by the manufacturer (50 mM sodium phosphate, pH 8.0, with 0.3 M sodium chloride and 250 mM imidazole). The fractions (0.5 mL) with the highest absorbance were pooled and concentrated to a volume of 0.5 mL with Amicon Ultra-4 centrifugal filters (Millipore, Merck) having a pore size of 50 kDa.

Coomassie blue staining and Western Blot

The $sPoF_{hMPV}$A1-Mfur and sF_{hMPV}A1-V proteins were diluted in sample buffer (0.08 M Tris-HCl at pH 8.8, 2% SDS, 10% glycerol, 0.01% bromophenol blue), boiled for five minutes and electrophoretically separated on polyacrylamide gels (Criterion XT precast gel Bis-Tris 12% 12+2 wells) in the presence of 100 mM dithiothreitol (DTT). The gels were stained for 1 hour in a solution of 0.05% Coomassie blue, 45% methanol and 7% acetic acid in water. The excess stain was removed with 25% methanol and 7% acetic acid in water. Results are shown in **Figure 6A**.

When the gel was used to perform a Western blot, the proteins were electro-transferred to Immobilon-P paper (Millipore) in transfer buffer (25 mM Tris, 192 mM glycine, 0.1% SDS and 20% methanol) for 2 hours at 250 mA. Nonspecific binding sites were blocked for one hour at room temperature or overnight at 4°C with 2% Membrane blocking agent (GE Healthcare) in 0.01% Tween-20/PBS. The membrane was incubated with stirring for one hour at room temperature with antibodies diluted in blocking solution (monoclonal IgG1 anti Penta-His (50µg/ml; Qiagen, ref. 34660). Then the membrane was washed three times with stirring for 5 minutes in 0.1% Tween-20/PBS. The antigen-antibody complexes were detected by incubation with α-Ig specific to the peroxidase-conjugated species, and after washing the membrane again in 0.1% Tween-20/PBS, it was developed with the luminescent substrate ECL (ECL Prime Western Blotting Detection Reagent, GE Healthcare) following the instructions of the manufacturer. The bands were visualized using an Imaging System camera (Imager 600RGB, GE HealthCare). Results are shown in **Figure 6A**.

FACS analysis of expressed subunit proteins

The presence of F-protein in the transfected CHO cells was confirmed by flow cytometry (**Figure 6**) by staining with anti-F antibodies MPE8 and DS7. Analysis by flow cytometry was done as follows: Seven days after transfection, cells were washed once in PBS and fixed in 4% paraformaldehyde for 10 minutes at room temperature. After two washes in PBS, cells were permeabilized in BD Perm wash buffer for 15 minutes at room temperature. Then the cells were stained with anti-F primary antibodies in BD Perm wash buffer for one hour at 4°C. Cells were washed with BD Perm wash buffer and incubated with a Goat anti-mouse IgG and IgM FITC (Jackson ImmunoResearch, JIR 115-096-068) for one hour at 4°C. After washing, the cells were resuspended in 100-150 µL/well of PBS-SVF 2% for FACS analysis.

Example 3 Immunogenicity comparison of Post-fusion hMPV and full-length hMPV DNA vaccines versus post-fusion hMPV F-protein subunit vaccine in mice

To investigate the immunogenicity of recombinant Nanotaxi®-associated DNA, 8 groups of 5 mice (C57Bl/6; Groups A, B and C: $sPoF_{hMPV}$; Groups D, E and F: FlF_{hMPV}, Group G: subunit $sPoF_{hMPV}$A1-Mfur; Group H: empty vector at the highest DNA dose) were used to determine humoral responses. The immunization protocol was carried out as described in **Table 2**. Briefly, 6 groups of 5 mice (6-8 weeks of age) were injected with different doses (5, 10 and 50µg) of both DNA vector constructs ($sPoF_{hMPV}$ and FlF_{hMPV}). All DNA vaccines were formulated with a final concentration of 0.15% 704 (Nanotaxi®1). For the DNA vector constructs and the negative control (empty vector), three immunizations by intramuscular route (anterior tibial muscle) were performed at D0, D21 and D42. In parallel, the empty vector and alum-adjuvanted $sPoF_{hMPV}$ subunit vaccine were injected as a negative control and a benchmark, respectively, in two additional groups of 5 mice. The sub-unit vaccine was delivered three times at two week intervals.

During the study, mice were bled 5 times: at D0 (before and after immunization), D21, D42 and D56 (termination day) or d0, d14, d28, d42 for the subunit-vaccinated mice. Pooled sera from each group were collected and terminal pools (d56 for Nanotaxi groups; d42 for subunit group) were analyzed in virus neutralization (PRNT) assays as outlined in **Table 3** below. The results are shown in **Figure 7**. Additionally, individual terminal sera were assessed for binding to hMPV-FL ICAFectin®441 transfected HeLa cells by flow cytometry. FlF_{hMPV} DNA and $sPoF_{hMPV}$A1-Mfur subunit vaccination results are shown in **Figure 8**; $sPoF_{hMPV}$ DNA and $sPoF_{hMPV}$A1-Mfur subunit vaccination results are shown in **Figure 9**. Each FACS plot as shown represents the binding results of sera from individual vaccinated mice at day 0 and day 56 after immunization.

Table 2. Immunization protocol for Post-fusion and Full-length hMPV F-protein-encoding DNA constructs formulated with Nanotaxi®1 (0.15%) and compared with alum-adjuvanted post-fusion subunit (polypeptide) vaccination.

Groups of 5 mice (C57Bl/6)	DNA encoding for A1-F hMPV protein	Quantity injected	Immunizations	Serum harvests
Group A	$sPoF_{hMPV}$ encoding DNA* + Nanotaxi®1	3 x 5 µg	d0, d21, d42	d0, d21, d42, d56
Group B		3 x 10 µg		
Group C		3 x 50 µg		
Group D	FlF_{hMPV} encoding DNA + Nanotaxi®1	3 x 5 µg		
Group E		3 x 10 µg		
Group F		3 x 50 µg		
Group G	Benchmark 1-Subunit $sPoF_{hMPV}$A1-MFur *	3 x 10 µg	d0, d14, d28	d0, d14, d28, d42
Group H	Empty plasmid (pVAX1) + Nanotaxi®1	3 x 50 µg	d0, d21, d42	d0, d21, d42, d56

*see **Fig. 3**; polypeptide formulated in Alhydrogel® adjuvant 2%, Brenntag

Table 3. PRNT protocol

Timepoint	Steps	Protocol
Day -1	Seeding of LLC-MK2 cells	60 x 10^5 cells/cm^2 (1.2 x 10^5 cells per well in 1 mL for 24-well plate)
Day 0	Neutralizing step [virus (A1 strain VR1605-001 CNR16) + (serum or CTRL antibody [DS7])*]	Incubation 1 hr at 37°C/ 5% CO_2
	Infection of cells	Incubation 2 hr at 37°C, 5% CO_2
	Addition of overlay	Methylcellulose 0.75% + EMEM-2mM L-glutamine*
	Incubation	37°C, 5% CO_2
Day 5	Cell fixation	PFA 4% 10 min at RT
	Cell permeabilization/blocking	PBS Tween 0.5% T BSA 0.1% 30 min at 4°C
	Primary antibody incubation**	45 min at 37°C under shaking
	Secondary antibody incubation	45 min at 37°C under shaking
	Substrate addition	Minimum 5 min incubation
	Foci counting	With binocular microscope

*with or without trypsin depending on the virus strain used
**serum or control (DS7) antibody diluted in EMEM-2mM L-glutamine + 1% NEAA

Example 4 Analysis of immunogenic response to hMPV F-protein-encoding DNA constructs with two Nanotaxi® formulations in two different strains of mice

To investigate the immunogenicity of recombinant Nanotaxi®-formulated hMPV F-protein DNA vectors, the immunization protocol as outlined in **Table 4** was used. Briefly, 18 groups of 5 animals each (Groups 1 to 4: Benchmark 1 [subunit $sPoF_{hMPV}$A1-MFur]; Groups 5 to 8: Post-fusion hMPV F-protein DNA [$sPoF_{hMPV}$]; Groups 9 to 12: full length hMPV F-protein DNA [FlF_{hMPV}]; Groups 13 and 14: Benchmark 2 [subunit sF_{hMPV}A1-V]; Groups 15 to 18: soluble hMPV F-protein DNA [sF_{hMPV}]) were used to compare three different hMPV F-protein-encoding DNA constructs combined with two different Nanotaxi® DNA delivery systems at one dose of DNA/injection (50 µg) in two mouse strains (C57BL/6 and BALB/c).

Three immunizations by subcutaneous or intramuscular routes (anterior tibial muscle) as indicated were performed at D0, D14 and D28 for subunit vaccines and D0, D21, D42 for Nanotaxi®/DNA formulations (and one subunit group: Group 4). During the study, mice were bled 4 times: D0 before immunization, D14, D28 and D42 (termination day) or D0, D21, D42 and D56 (termination day), depending on the treatment. At D56, splenocytes and bronchoalveolar lavage (BALs) cells were also collected. All samples from mice were stored at -80°C until analysis. Antibody titers as well as PRNT values were assessed.

Table 4. Experimental design for comparison of hMPV F-protein DNA constructs combined with 0.15% 704 (Nanotaxi®1) or 704-M (Nanotaxi®2) in Balb/c and C57Bl/6 mice. Four experiments in total were done as indicated, using two different subunit (polypeptide) vaccines as Benchmarks (shaded grey).

Harvest day	Group No	Exp't No	Antigen	Dose	Adjuvant/ Delivery reagent	Injection volume	Route	Mouse strain	Immunization schedule
Day 42	14	4531	subunit $sF_{hMPV}A1$-V (Benchmark 2)	10 µg	2% alum*	100 µl	SC	BALB/c	3x2 weeks
	2	4531	subunit $sPoF_{hMPV}A1$-MFur (Benchmark 1)	10 µg	2% alum*	100 µl	SC	BALB/c	3x2 weeks
Day 56	7	4532	DNA encoding $sPoF_{hMPV}$**	50 µg	704	30 µl	IM	BALB/c	3x3 weeks
	8	4532	DNA encoding $sPoF_{hMPV}$**	50 µg	704-M	30 µl	IM	BALB/c	3x3 weeks
	11	4532	DNA encoding FlF_{hMPV}**	50 µg	704	30 µl	IM	BALB/c	3x3 weeks
	12	4532	DNA encoding FlF_{hMPV}**	50 µg	704-M	30 µl	IM	BALB/c	3x3 weeks
	17	4532	DNA encoding sF_{hMPV}**	50 µg	704	30 µl	IM	BALB/c	3x3 weeks
	18	4532	DNA encoding sF_{hMPV}**	50 µg	704-M	30 µl	IM	BALB/c	3x3 weeks
	4	4532	subunit $sPoF_{hMPV}A1$-MFur (Benchmark 1)	10 µg	2% alum*	100 µl	SC	BALB/c	3x3 weeks
Day 42	13	4533	subunit $sF_{hMPV}A1$-V (Benchmark 2)	10 µg	2% alum*	100 µl	SC	C57Bl/6	3x2 weeks
	1	4533	subunit $sPoF_{hMPV}A1$-MFur (Benchmark 1)	10 µg	2% alum*	100 µl	SC	C57Bl/6	3x2 weeks
Day 56	5	4534	DNA encoding $sPoF_{hMPV}$**	50 µg	704	30 µl	IM	C57Bl/6	3x3 weeks
	6	4534	DNA encoding $sPoF_{hMPV}$**	50 µg	704-M	30 µl	IM	C57Bl/6	3x3 weeks
	9	4534	DNA encoding FlF_{hMPV}**	50 µg	704	30 µl	IM	C57Bl/6	3x3 weeks
	10	4534	DNA encoding FlF_{hMPV}**	50 µg	704-M	30 µl	IM	C57Bl/6	3x3 weeks
	15	4534	DNA encoding sF_{hMPV}**	50 µg	704	30 µl	IM	C57Bl/6	3x3 weeks
	16	4534	DNA encoding sF_{hMPV}**	50 µg	704-M	30 µl	IM	C57Bl/6	3x3 weeks
	3	4534	subunit $sPoF_{hMPV}A1$-MFur (Benchmark 1)	10 µg	2% alum*	100 µl	SC	C57Bl/6	3x3 weeks

*5 µl Alhydrogel (Brenntag)

**comprised in pVAX1 vector

Comparison of antibody responses in Balb/c and C57Bl/6 mice

For antibody titer determination, 96-well half-area plates were coated with 1 µg/mL of capture antigen overnight at 4°C in pH 9.6 carbonate-bicarbonate buffer. After washing in PBS 0.05% Tween-20, 1 hour of blocking in PBS/ 0.05% Tween-20/ 5% bovine serum albumin (BSA) at 37°C and another wash step, serially diluted sera (or control DS7 IgG_{2A} antibody) were added to the plates and incubated for 1 hour at 37°C. Subsequently, plates were washed, incubated for 1 hour at 37°C with secondary antibodies, washed and finally incubated in TMB substrate solution (KPL) for 20 minutes at RT. The substrate reaction was stopped by addition of 85% orthophosphoric acid and signal was detected by optical density reading at 450 nm. For IgG_1 and IgG_{2A} isotype ELISAs, the capture antigen was Post-Fusion subunit hMPV F-protein ($sPoF_{hMPV}A1$-Mfur) and the secondary antibodies were anti-mouse IgG_1-HRP (horseradish peroxidase; Bio-rad) and anti-mouse IgG_{2A}-HRP (Abcam) at 1:10,000 dilution. For total IgG ELISA, the capture antigen was either post-fusion subunit hMPV F-protein ($sPoF_{hMPV}A1$-Mfur), native monomer hMPV F-protein ($sF_{hMPV}A1$-V), or prefusion trimer protein ($sF_{hMPV}A1$-K). The secondary antibody anti-mouse IgG-HRP (Covalab) was used at 1:5000 dilution.

As shown in **Figure 10A**, in comparison with the subunit vaccines, the hMPV neutralizing titers in C57Bl/6 mice on day 56 were generally higher in response to the DNA vaccine preparations, with the exception of the post-fusion subunit delivered at three week intervals. These observations are in contrast to those in Balb/c mice on day 56, as shown in **Figure 11A**, which showed somewhat higher neutralizing antibody titers in response to subunit vaccines. The IgG2a/IgG1 ratio (i.e. Th1/Th2 ratio) in Balb/c mice on d42, however, was more favorable with the DNA vaccine formulations (i.e. >1) (see **Figure 11B**), demonstrating the ability of DNA vaccination to generate strong Th1 responses even in a Th2-biased mouse model. The IgG1 titers in C57Bl/6 mice on d42, as shown in **Figure 10B**, indicated that both subunit vaccine and DNA vaccine preparations can generate strong IgG1/Th2 immune responses despite the well-established Th1 bias of C57Bl/6 mice (Watanabe, et al., 2004, Innate Immune Response in Th1- and Th2-Dominant Mouse Strains, SHOCK 22(5): 460-466). In sum, the results demonstrated that DNA vaccines performed robustly in both Th1- and Th2-biased mouse models.

Finally, the hyperimmune sera from Balb/c groups immunized two times at three week intervals (d42 sera) with either subunit post-fusion hMPV F-protein or DNA encoding post-fusion hMPV F-protein were compared for their binding ability to different bound antigens *in vitro* by ELISA. The coating antigens used were a post-fusion trimeric form ($sPoF_{hMPV}A1$-Mfur; SEQ ID NO: 44), a pre-fusion trimeric form ($sF_{hMPV}A1$-K; SEQ ID NO: 46) and a native monomeric form of hMPV F-protein

($sF_{hMPV}A1$-V; SEQ ID NO: 45). The mAb DS7, which binds both pre- and post-fusion forms of hMPV F-protein, was used as a control.

As shown in **Figure 12A**, it was observed that the antibodies raised in $sPoF_{hMPV}$-DNA-vaccinated mice bound approximately equally to all three forms of the protein *in vitro*. By contrast, however, the sera raised in $sPoF_{hMPV}A1$-Mfur subunit-vaccinated mice bound preferentially to the post-fusion trimeric form ($sPoF_{hMPV}A1$-Mfur) of the protein *in vitro*. This observation is shown quantitatively by endpoint titer in the Table in **Figure 12B**. This finding suggests that the DNA-delivered coding sequence of the post-fusion form of the hMPV F-protein is superior to the subunit vaccine in terms of diversity of epitope presentation *in vivo*, which would likely translate to a more robust protective response.

Example 5 Testing of hMPV/RSV combination DNA vaccine in mice

To investigate immunogenicity of recombinant hMPV and RSV F-protein Nanotaxi®-associated DNA vectors, C57Bl/6 mice were immunized as outlined in **Table 5**. Briefly, 7 groups of 5 mice (C57Bl/6; 6-8 weeks of age) were immunized with 25 µg of the indicated DNA constructs (single or in combination), to a total of 50 µg of DNA in combination with 0.15% Nanotaxi® 704 (Nanotaxi®1). pVAX1 vector comprising DNA encoding $sPoF_{hMPV}$ as described above, as well as three pVAX1 constructs containing the coding sequences for three different hRSV F-proteins; soluble pre-fusion hRSV F-protein "SC-DM" ($sPrF_{hRSV}$SC-DM), soluble pre-fusion hRSV F-protein "DS-Cav1" ($sPrF_{hRSV}$DS-Cav1) and post-fusion hMPV F-protein (PoF_{hRSV}) were used. For immunizations comprising a single construct, the 25 µg of recombinant DNA plasmid was supplemented with 25 µg empty pVAX1 plasmid. Three immunizations by the intramuscular route (anterior tibial muscle) were performed at D0, D21 and D42. During the study, mice were bled 4 times: at D0 (before immunization), D21, D42 and D56 (termination day). At D56, splenocytes and bronchoalveolar lavage (BALs) cells were collected. All samples from mice were stored at -80°C until analysis. Humoral responses in pooled D56 serum samples were assessed by neutralization of hMPV as outlined in **Table 3**. Sera collected at D0 from group 1 were used as a negative control.

Results are shown in **Figure 13**. The findings indicated that the addition of RSV F-protein encoding vectors did not reduce the immune response to post-fusion hMPV F-protein encoding vectors.

Table 5. Immunization protocol for combination hMPV/RSV DNA vaccines in C57Bl/6 mice. Three different hRSV F-protein DNA coding sequences in the pVAX1 vector were delivered alone or together with a pVAX1 vector comprising a DNA sequence encoding a post-fusion form of hMPV F-protein. All treatments were combined with all with 0.15% Nanotaxi®1 (704).

Group	Treatment (IM)	Dose/site	Volume/ site	Immunization Schedule	Day of terminal bleed
1	$sPoF_{hMPV}$ encoding DNA/Nanotaxi®1	25 µg*	30 µl / tibia	D0+D21+D42	D56
2	$sPoF_{RSV}$ encoding DNA/Nanotaxi®1	25 µg*	30 µl / tibia		
3	$sPrF_{hRSV}$SC-DM encoding DNA/Nanotaxi®1	25 µg*	30 µl / tibia		
4	$sPrF_{hRSV}$DS-Cav1 encoding DNA/Nanotaxi®1	25 µg*	30 µl / tibia		
5	$sPoF_{hMPV}$ + PoF_{RSV} encoding DNAs/Nanotaxi®1	25 µg + 25 µg	30 µl / tibia		
6	$sPoF_{hMPV}$ + $sPrF_{hRSV}$SC-DM encoding DNAs/Nanotaxi®1	25 µg + 25 µg	30 µl / tibia		
7	$sPoF_{hMPV}$ + $sPrF_{hRSV}$DS-Cav1 encoding DNAs/Nanotaxi®1	25 µg + 25 µg	30 µl / tibia		

*Includes additionally 25 µg empty pVAX1 vector for a final dose of 50 µg total DNA

Example 6 Virus challenge studies following immunization

A1, B1 and B2 hMPV virus isolates are grown on LLC-MK2 cells, banked and used for animal challenge experiments. Cotton rats previously vaccinated as indicated above are challenged intra-nasally on day 28 with 10^5 PFU of the hMPV viruses. A few days later, the animals are sacrificed and individual serum samples are prepared and frozen. Nasal and lung tissues are harvested separately, weighed and either snap frozen in liquid nitrogen and conserved for viral titer determination or fixed with 4% buffered formalin for histopathological examination after paraffin embedding and staining with hematoxylin and eosin. Viral load in nasal and lung tissues is determined by virus foci immunostaining as described above. Alternatively or additionally, PCR is used to determine viral load in the harvested tissues. Optionally, immunogenicity is determined as described for mouse studies (above).

Preferred aspects:

1. An hMPV F-protein complex in trimeric post-fusion conformation, wherein said complex consists of three hMPV F-protein heterodimers, and wherein said heterodimer comprises
   a. a polypeptide A comprising an immunogenic F1 ectodomain of the hMPV F-protein; and
   b. a polypeptide B comprising an immunogenic F2 domain of the hMPV F-protein;
   wherein the F1 and F2 domains are covalently linked by at least one disulfide bond for use as a medicament.

2. The complex according to aspect 1 for use as a prophylactic or therapeutic treatment against a viral infection.

3. A pharmaceutical composition comprising the complex according to aspect 1 for use as a medicament.

4. A pharmaceutical composition comprising the complex according to aspect 1 for use as a prophylactic or therapeutic treatment against a viral infection.

5. The complex for use according to aspect 1 or 2 or the composition for use according to aspect 3 or 4, wherein both domains, i.e. the F1 ectodomain and the F2 domain, are selected from an A1 strain or a B1 strain of hMPV.

6. The complex for use according to aspect 1 or 2 or the composition for use according to aspect 3 or 4, wherein the immunogenic F1 ectodomain consists of (i) amino acids 112 to 489 of the hMPV F-protein (A1 genotype), i.e. the amino acid sequence of SEQ ID NO: 10 and (ii) the immunogenic F2 domain consists of amino acid 20 to 101 of the hMPV F-protein (A1 genotype), i.e. amino acid sequence of SEQ ID NO: 11.

7. The complex for use according to aspect 1 or 2 or the composition for use according to aspect 3 or 4, wherein the immunogenic F1 ectodomain consists of (i) amino acids 112 to 489 of the hMPV F-protein (B1 genotype), i.e. the amino acid sequence of SEQ ID NO: 12 and (ii) the immunogenic F2 domain consists of amino acid 20 to 101 of the hMPV F-protein (B1 genotype), i.e. amino acid sequence of SEQ ID NO: 13.

8. The complex for use according to aspects 1 or 2 or the composition for use according to any of aspects 3 to 7, wherein the polypeptide A additionally comprises a trimerization domain C-terminal to the F1 ectodomain.

9. The complex or composition for use according to aspect 8, wherein the trimerization domain comprises or consists of the fibritin T4 foldon domain (SEQ ID NO: 6).

10. The complex or composition for use according to aspect 8 or 9, wherein the polypeptide A additionally comprises another cleavage site B between the F1 ectodomain and the trimerization domain.

11. The complex or composition for use according to aspect 10, wherein the cleavage site B is a TEV protease cleavage site.

12. The complex or composition for use according to aspect 11, wherein the TEV protease cleavage site is defined by SEQ ID NO: 3.

13. The complex or composition for use according to any of aspects 1 to 5 or 8 to 12, wherein the strain A1 F1 ectodomain is the wild-type sequence; i.e., does not contain a G294E mutation (SEQ ID NO: 9).

14. The complex or composition for use according to any of aspects 1 to 13, wherein the polypeptide A additionally comprises a tag at the C-terminal end of the trimerization domain.

15. The complex or composition for use according to aspect 14, wherein the tag is a His6 tag (SEQ ID NO: 5).

16. The complex or composition for use according to aspect 14 or 15, wherein the polypeptide A additionally comprises another cleavage site C between the trimerization domain and the tag.

17. The complex or composition for use according to aspect 16, wherein the cleavage site C is a factor Xa cleavage site.

18. The complex or composition for use according to aspect 17, wherein the factor Xa cleavage site is SEQ ID NO: 4.

19. The complex or composition for use according to any of aspects 1 to 6 or 8 to 18 wherein said heterodimer consists of a polypeptide A with SEQ ID NO: 14 or a functional variant thereof that is more than 80% identical to the polypeptide with SEQ ID NO: 14, and a polypeptide B with SEQ ID NO: 15 or a functional variant thereof that is more than 80% identical to the polypeptide with SEQ ID NO: 15.

20. The complex or composition for use according to any of aspects 1 to 6 or 8 to 18 wherein said heterodimer consists of a polypeptide A with SEQ ID NO: 16 or a functional variant thereof that is more than 80% identical to the polypeptide with SEQ ID NO: 16, and a polypeptide B with SEQ ID NO: 17 or a functional variant thereof that is more than 80% identical to the polypeptide with SEQ ID NO: 17.

21. The composition for use according to any of aspects 3 to 20, additionally comprising an adjuvant and/or other pharmaceutically acceptable excipient(s).

22. The composition for use according to aspect 21, wherein the adjuvant is an hMPV M protein and/or alum and/or IC31.

23. The composition for use according to aspect 22, wherein the hMPV M protein is defined by SEQ ID NO: 24 or SEQ ID NO: 25.

24. The complex or composition for use according to any of aspects 2 or 4 to 23, wherein the viral infection is an hMPV infection.

25. The composition for use according to aspect 24, wherein the viral infection is an hMPV infection caused by the A or B strain of hMPV.

26. The composition for use according to aspect 25, wherein the viral infection is an hMPV infection caused by one or more of the group of strains selected from the A1, A2, B1 and B2 strains of hMPV.

27. The composition for use according to any of aspects 3 to 26, wherein the composition further comprises RSV and/or PIV antigens.

28. The composition for use according to any of aspects 3 to 27, wherein the composition is a vaccine.

29. A process for producing the composition for use according to any of aspects 3 to 28 comprising the steps of:

a. providing a nucleic acid sequence encoding a polypeptide A and polypeptide B as defined in aspects 1 to 20 in a suitable vector;

b. expressing said vector in a suitable host cell to yield polypeptide A and polypeptide B;

c. optionally, purifying resulting complex; and

d. combining said complex with an optional adjuvant and/or other suitable excipient(s) in order to obtain said pharmaceutical composition.

30. The process according to aspect 29, wherein the nucleic acid sequence is selected from the group consisting of

a. SEQ ID NO: 18,

b. SEQ ID NO: 27 and

c. SEQ ID NO: 19.

31. The process according to aspect 29 or 30, wherein the vector is pVVS1371 (SEQ ID NO: 28).

32. The process according to any of aspect 29 to 31, wherein the host cells are Chinese Hamster Ovary (CHO) cells.

CLAIMS

1. A nucleic acid which encodes a single polypeptide capable of being processing into two fragments consisting of
   a) a fragment A comprising an immunogenic F1 ectodomain of an hMPV F-protein, wherein fragment A does not comprise the transmembrane domain; and
   b) a fragment B comprising an immunogenic F2 domain of an hMPV F-protein.

2. The nucleic acid according to claim 1, wherein said nucleic acid encodes four cysteine residues which can form disulfide bridges between said F1 ectodomain and F2 domain, resulting in the formation of a soluble heterodimeric protein.

3. The nucleic acid according to claim 1 or 2, wherein said encoded heterodimeric protein combines to form a trimeric post-fusion conformation.

4. The nucleic acid according to any of claims 1 to 3, wherein said encoded immunogenic F1 ectodomain and said encoded immunogenic F2 domain, are selected from a genotype A or a genotype B hMPV, such as a genotype A1, A2, B1 or B2 hMPV.

5. The nucleic acid according to any of claims 1 to 4, wherein said encoded immunogenic F1 ectodomain consists of (i) amino acids 112 to 489 of an A1 genotype hMPV F-protein with a G294E point mutation, i.e. the amino acid sequence of SEQ ID NO: 10 and (ii) said encoded immunogenic F2 domain consists of amino acids 20 to 101 of an A1 genotype hMPV F-protein, i.e. the amino acid sequence of SEQ ID NO: 11.

6. The nucleic acid according to any of claims 1 to 5, wherein said encoded immunogenic F1 ectodomain consists of (i) amino acids 112 to 489 of a B1 genotype hMPV F-protein, i.e. the amino acid sequence of SEQ ID NO: 12 and (ii) said encoded immunogenic F2 domain consists of amino acids 20 to 101 of a B1 genotype hMPV F-protein, i.e. the amino acid sequence of SEQ ID NO: 13.

7. The nucleic acid according to any of claims 1 to 6, wherein said encoded polypeptide A additionally comprises a trimerization domain C-terminal to the F1 ectodomain.

8. The nucleic acid according to claim 7, wherein said encoded trimerization domain comprises or consists of the fibritin T4 foldon domain, i.e. the amino acid sequence of SEQ ID NO: 6.

9. The nucleic acid according to any of claims 1 to 8, wherein said encoded polypeptide B additionally comprises a cleavage site A at its C-terminal end.

10. The nucleic acid according to claim 9, wherein said encoded cleavage site A comprises or consists of the RSV cleavage site II, i.e. the amino acid sequence of SEQ ID NO: 2 (KKRKRR).

11. The nucleic acid according to any of claims 7 to 10, wherein said encoded polypeptide A additionally comprises another cleavage site B between the F1 ectodomain and the trimerization domain.

12. The nucleic acid according to claim 11, wherein said encoded cleavage site B comprises or consists of a TEV protease cleavage site, preferably as defined by the amino acid sequence of SEQ ID NO: 3 (ENLYFQG).

13. The nucleic acid according to any of claims 1 to 12, wherein said encoded F1 ectodomain from the A1 genotype is the wild-type sequence not containing a G294E mutation, i.e., the amino acid sequence of SEQ ID NO: 9.

14. The nucleic acid according to any of claims 1 to 13 wherein said encoded heterodimeric protein consists of a polypeptide A with the amino acid sequence of SEQ ID NO: 14 or a functional variant thereof that is more than 80% identical to the polypeptide defined by SEQ ID NO: 14, and a polypeptide B with the amino acid sequence of SEQ ID NO: 15 or a functional variant thereof that is more than 80% identical to the polypeptide defined by SEQ ID NO: 15.

15. The nucleic acid according to any of claims 1 to 13 wherein said encoded heterodimeric protein consists of a polypeptide A with the amino acid sequence of SEQ ID NO: 16 or a functional variant thereof that is more than 80% identical to the polypeptide defined by SEQ ID NO: 16, and a polypeptide B with the amino acid sequence of SEQ ID NO: 17 or a functional variant thereof that is more than 80% identical to the polypeptide defined by SEQ ID NO: 17.

16. A vector comprising the nucleic acid according to any of claims 1 to 15.

17. The vector according to claim 16, wherein said vector is preferably pVAX1 vector as provided by SEQ ID NO: 28 or a vector for *in vitro* expression of the heterodimeric protein encoded by the nucleic acid of any of claims 1 to 14.

18. The vector according to claim 16 or 17, wherein said vector optionally comprises at least one additional coding sequence.

19. The vector according to claim 18, wherein said at least one additional coding sequence encodes an additional antigen or a furin coding sequence according to SEQ ID NO: 31.

20. The nucleic acid according to any of claims 1 to 15 or the vector according to any of claims 16 to 19 for use as a medicament.

21. The nucleic acid according to any of claims 1 to 15 or the vector according to any of claims 16 to 19 for use as a prophylactic or therapeutic treatment against a viral infection, preferably an hMPV infection.

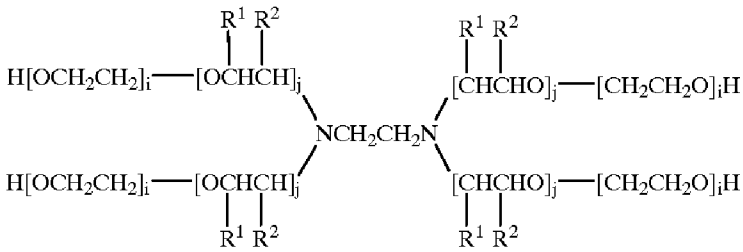
22. A pharmaceutical composition comprising the nucleic acid according to any of claims 1 to 15 or the vector according to any of claims 16 to 19.

23. A pharmaceutical composition comprising a nucleic acid encoding an A1 post-fusion protein and a B1 post-fusion protein, preferably an A1 post-fusion protein as defined by SEQ ID NO: 44 and a B1 post-fusion protein as defined by SEQ ID NO: 47 or 48.

24. The pharmaceutical composition according to claim 22 or 23, additionally comprising an adjuvant, a nucleic acid delivery reagent and/or a pharmaceutically acceptable excipient.

25. The pharmaceutical composition according to claim 24, wherein said nucleic acid delivery reagent is a tetrafunctional block co-polymer.

26. The pharmaceutical composition according to claim 25, wherein said tetrafunctional block co-polymer is a non-ionic amphiphilic tetrafunctional block copolymer of the formula:



in which

- i has values from about 5 to about 125, in particular from about 10 to about 100, and more particularly from about 10 to about 60, and
- j has values from about 5 to about 85, in particular from about 10 to about 50, and more particular from about 10 to about 20, and
- for each R^1, R^2 pair, R^1 shall be hydrogen and R^2 shall be a methyl group, and
- the molecular weight ranges from about 4000 to about 35000, in particular from about 4500 to about 30000, more particularly from about 5000 to about 25000, and
- the ethylene-oxide unit content is about 30% to about 80%, in particular about 35% to 50%, more preferably about 40%.

27. The pharmaceutical composition according to claim 26, wherein said tetrafunctional block co-polymer has an i-value of 13, a j-value of 14, a molecular weight of 5500 and an ethylene-oxide unit content of 40%.

28. The pharmaceutical composition according to claim 26 or 27, wherein said tetrafunctional block co-polymer is a 704 block co-polymer.

29. The pharmaceutical composition according to any of claims 26 to 28 wherein said tetrafunctional block co-polymer optionally comprises at least one glycosyl moiety, wherein said at least one glycosyl moiety is a single glycosyl unit or a linear or branched polymer of glycosyl units.

30. The pharmaceutical composition according to claim 29 wherein said at least one glycosyl moiety comprises mannose or galactose, preferably mannose.

31. The pharmaceutical composition according to any of claims 26 to 30, wherein said tetrafunctional block co-polymer is present at about 0.01 to 5%, preferably at about 0.05 to 2%, preferably at about 0.1 to 1%, most preferably at about 0.15 to 1%.

32. The pharmaceutical composition according to any of claims 22 to 31, wherein said composition further comprises a nucleic acid encoding an RSV antigen, preferably an RSV F-protein.

33. The pharmaceutical composition according to claim 32, wherein said encoded RSV antigens are selected from the group consisting of SEQ ID NO: 33, SEQ ID NO: 35 and SEQ ID NO: 37.

34. A pharmaceutical composition comprising SEQ ID NO: 43 and/or SEQ ID NO: 49, optionally comprising about 0.15 to 1% 704 or 704-M.

35. The pharmaceutical composition according to any of claims 22 to 34, wherein said composition is a vaccine.

36. The pharmaceutical composition according to any of claims 22 to 35, for use as a medicament.

37. The pharmaceutical composition according to any of claims 22 to 35, for use in a method of treatment or prevention against a viral infection.

38. The pharmaceutical composition according to claim 37, wherein said viral infection is an hMPV infection and/or and RSV infection.

39. The pharmaceutical composition according to claims 37 or 38, wherein said viral infection is an hMPV infection.

40. The pharmaceutical composition according to claim 39, wherein said hMPV infection is caused by A and/or B genotypes of hMPV.

41. A process for producing the pharmaceutical composition according to any of claims 22 to 40 comprising the steps of:
    a) providing a nucleic acid sequence according to any of claims 1 to 15;
    b) combining said nucleic acid with an optional adjuvant, nucleic acid delivery reagent and/or pharmaceutically acceptable excipient in order to obtain said pharmaceutical composition.

42. The process according to claim 41, wherein the nucleic acid sequence is selected from the group consisting of

a) SEQ ID NO: 43, and

b) a nucleic acid sequence encoding the protein according to SEQ ID NO: 49.

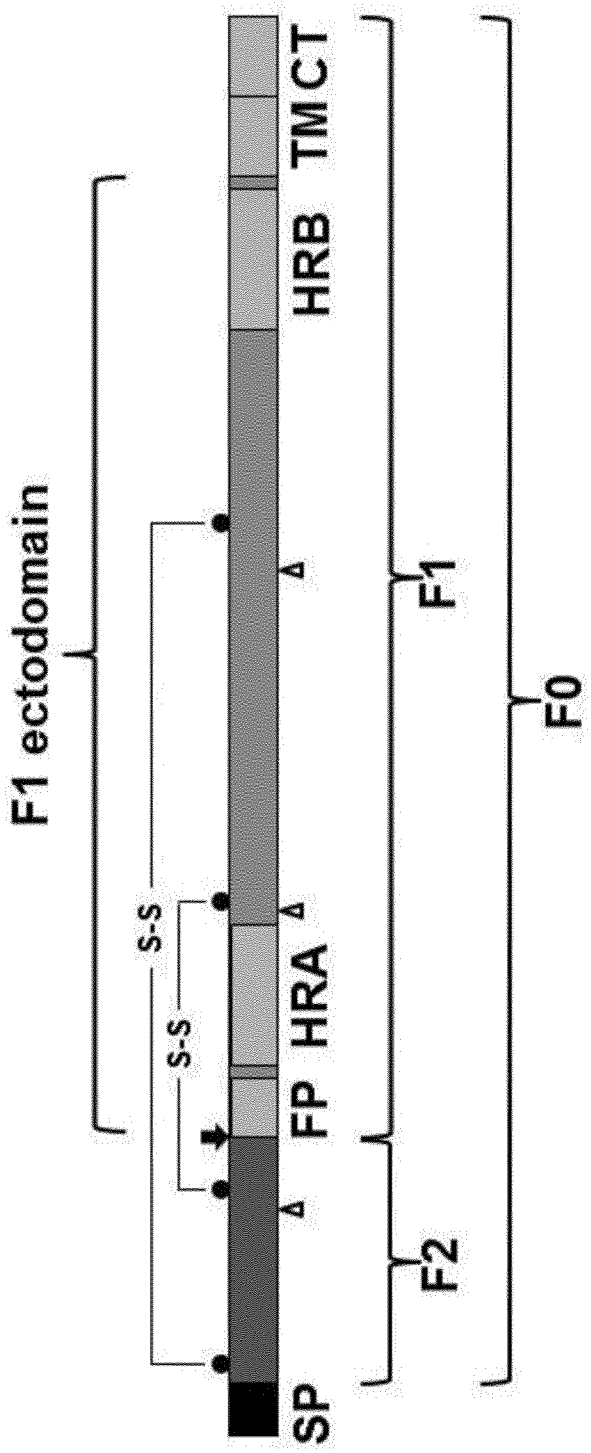


Figure 1

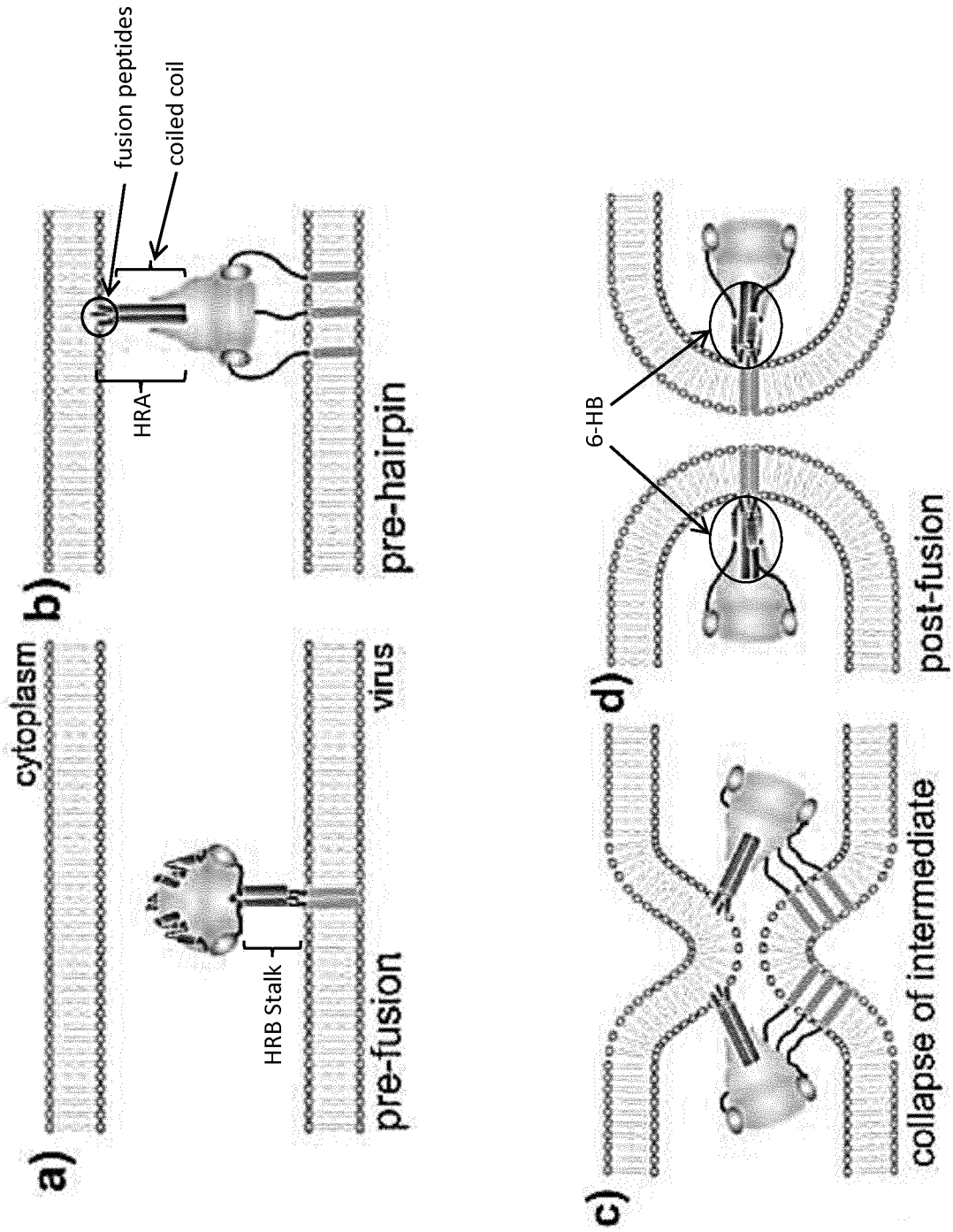

a)
cytoplasm
virus
HRB Stalk
pre-fusion
b)
fusion peptides
coiled coil
HRA
pre-hairpin
c)
collapse of intermediate
d)
6-HB
post-fusion

Figure 2

Figure 3

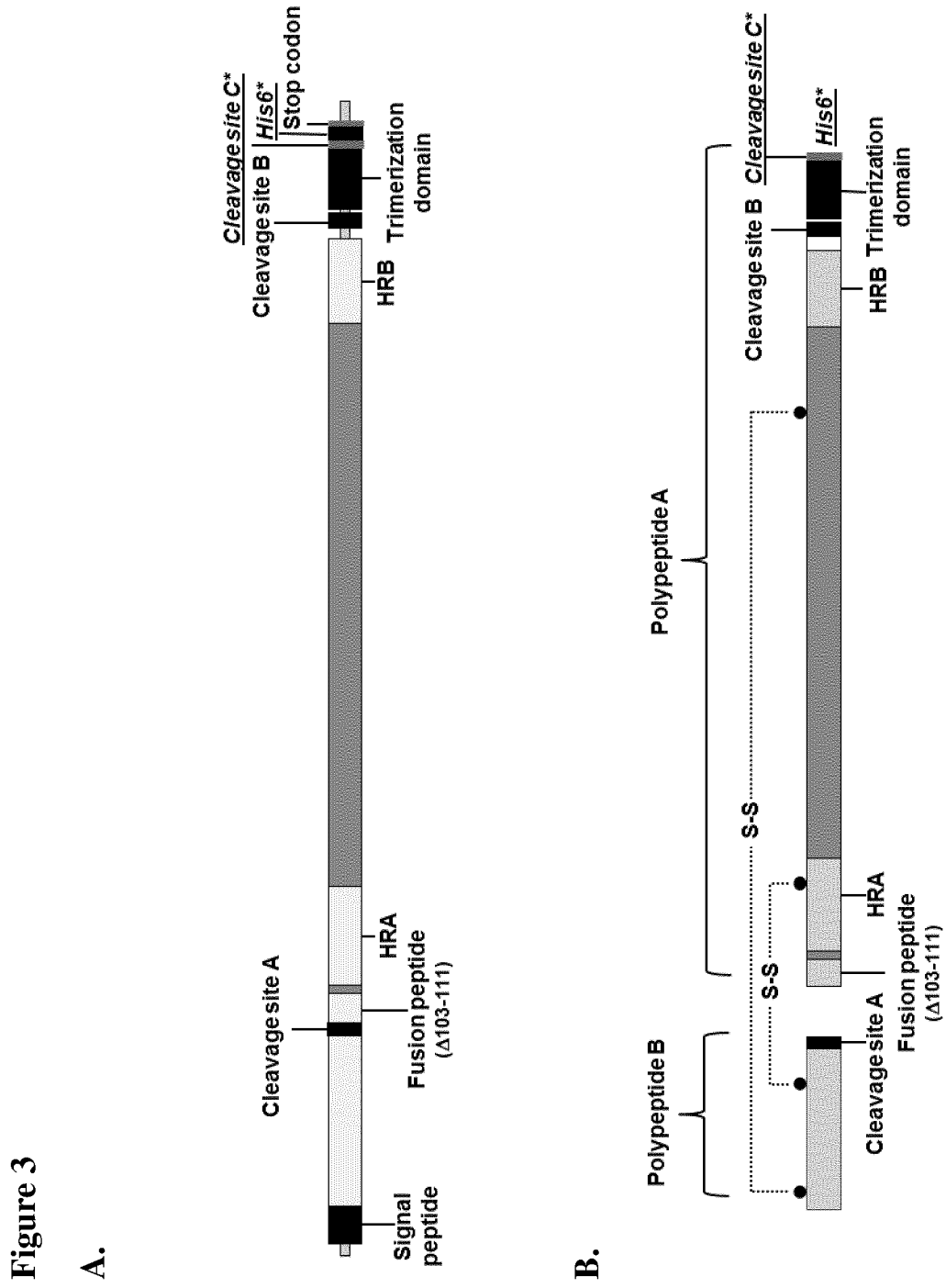

A.
Cleavage site C*
Cleavage site B
His6*
Stop codon
Trimerization domain
HRB
HRA
Cleavage site A
Fusion peptide (Δ103-111)
Signal peptide
B.
Polypeptide A
Polypeptide B
S-S
S-S
Cleavage site C*
Cleavage site B
His6*
HRB
Trimerization domain
HRA
Cleavage site A
Fusion peptide (Δ103-111)

Figure 4A

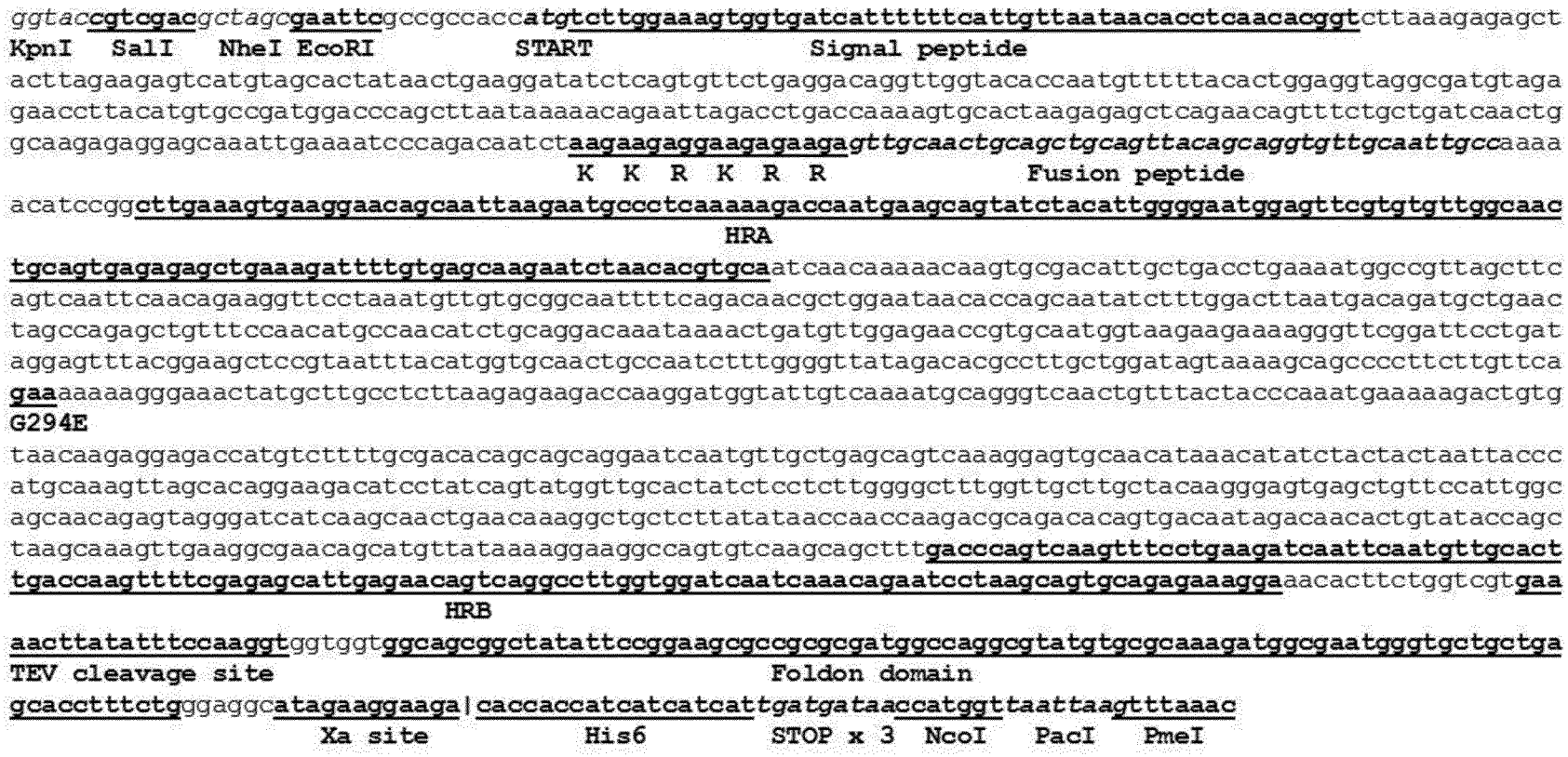

ggtaccgtcgacgctagcgaattcgccgccaccatgtcttggaaagtggtgatcatttttcattgttaataacacctcaacacggtcttaaagagagct
KpnI SalI NheI EcoRI START Signal peptide
acttagaagagtcatgtagcactataactgaaggatatctcagtgttctgaggacaggttggtacaccaatgttttacactggaggtaggcgatgtaga
gaaccttacatgtgccgatggacccagcttaataaaaacagaattagacctgaccaaaagtgcactaagagagctcagaacagtttctgctgatcaactg
gcaagagaggagcaaattgaaaatcccagacaatctaagaagaggaagagaagagttgcaactgcagctgcagttacagcaggtgttgcaattgccaaaa
K K R K R R Fusion peptide
acatccggcttgaaagtgaaggaacagcaattaagaatgccctcaaaaagaccaatgaagcagtatctacattggggaatggagttcgtgtgttggcaac
HRA
tgcagtgagagagctgaaagattttgtgagcaagaatctaacacgtgcaatcaacaaaaacaagtgcgacattgctgacctgaaaatggccgttagcttc
agtcaattcaacagaaggttcctaaatgttgtgcggcaattttcagacaacgctggaataacaccagcaatatctttggacttaatgacagatgctgaac
tagccagagctgtttccaacatgccaacatctgcaggacaaataaaactgatgttggagaaccgtgcaatggtaagaagaaaagggttcggattcctgat
aggagtttacggaagctccgtaatttacatggtgcaactgccaatctttggggttatagacacgccttgctggatagtaaaagcagccccttcttgttca
gaaaaaaggaaactatgcttgcctcttaagagaagaccaaggatggtattgtcaaaatgcagggtcaactgtttactacccaaatgaaaaagactgtg
G294E
taacaagaggagaccatgtcttttgcgacacagcagcaggaatcaatgttgctgagcagtcaaaggagtgcaacataaacatatctactactaattaccc
atgcaaagttagcacaggaagacatcctatcagtatggttgcactatctcctcttggggctttggttgcttgctacaagggagtgagctgttccattggc
agcaacagagtagggatcatcaagcaactgaacaaaggctgctcttatataaccaaccaagacgcagacacagtgacaatagacaacactgtataccagc
taagcaaagttgaaggcgaacagcatgttataaaaggaaggccagtgtcaagcagctttgacccagtcaagtttcctgaagatcaattcaatgttgcact
tgaccaagttttcgagagcattgagaacagtcaggccttggtggatcaatcaaacagaatcctaagcagtgcagagaaaggaaacacttctggtcgtgaa
HRB
aacttatatttccaaggtggtggtggcagcggctatattccggaagcgccgcgcgatggccaggcgtatgtgcgcaaagatggcgaatgggtgctgctga
TEV cleavage site Foldon domain
gcacctttctggaggcatagaaggaaga|caccaccatcatcatcattgatgataaccatggttaattaagtttaaac
Xa site His6 STOP x 3 NcoI PacI PmeI

Figure 4B

*ggtacc***gtcgacgc***tagc***gaattc**gccgccacc**atgtcttggaaagtgatgatcatcatttcgttactcataacaccccagcacgggg**ctaaaggagagtt
KpnI SalI NheI EcoRI START Signal peptide
atttggaagaatcatttagtagtataactgagggatacctcagtgttttaagaacaggctggtacactaatgtcttcacattagaagttggtga
tgttgaaaatcttacatgtactgatggacctagcttaatcaaaacagaacttgatctaacaaaaagtgctttaagggaactcaaaacagtctctg
ctgatcagttggcgagagaggagcaaattgaaaatcccagacaatca**aagaaaaggaaaagaaga*gttgctacagcagcagcagtcacagcaggc***
K K R K R R Fusion peptide
attgcaatagccaaaaccataatg**cttgagagtgaggtgaatgcaattaaaggtgctctcaaacaaactaatgaagcagtatccacattagggaa**
HRA
tggtgtgcgggtcctagccacttcagtgagagagctaaaagaatttgtgagcaaaaacctgactagtgcaatcaacaggaacaaatgtgacattg
ctgatctgaagatggctgtcagcttcagtcaattcaacagaagatttctaaatgttgtgcggcagttttcagacaatgcagggataacaccagca
atatcattggacctgatgactgatgctgagttggccagagctgtatcatacatgccaacatctgcagggcagataaaactgatgttggagaaccg
cgcaatggtaaggagaaaaggatttggaatcctgataggggtctacggaagctctgtgatttacatggttcaattgccgatctttggtgtcatag
atacaccttgttggatcatcaaggcagctccctcttgctcagaaaaaaacgggaattatgcttgcctcctaagagaggatcaagggtggtattgt
aaaaatgcaggatctactgtttactacccaaatgaaaaagactgcgaaacaagaggtgatcatgtttttgtgacacagcagcagggatcaatgt
tgctgagcaatcaagagaatgcaacatcaacatatctactaccaactacccatgcaaagtcagcacaggaagacaccctataagcatggttgcac
tatcacctctcggtgctttggtggcttgctataaaggggtaagctgctcgattggcagcaattgggttggaatcatcaaacaattacccaaaggc
tgctcatacataaccaaccaggatgcagacactgtaacaattgacaataccgtgtatcaactaagcaaagttgaaggtgaacagcatgtaataaa
agggagaccagtttcaagcagtttt**gatccaatcaagtttcctgaggatcagttcaatgttgcgcttgatcaagtcttcgaaagcattgagaaca**
HRB
gtcaggcactagtggaccagtcaaacaaaattctaaacagtgcagaaaaaggaaacacttctggtcgt**gaaaacttatatttccaaggt**ggtggt
TEV cleavage site
Ggcagcggctatattccggaagcgccgcgcgatggccaggcgtatgtgcgcaaagatggcgaatgggtgctgctgagcacctttctgggaggc**at**
Foldon domain
agaaggaaga|caccaccatcatcatcat*tgatgataaccatggttaattaagtttaaac*
Xa site His6 STOP x 3 NcoI PacI PmeI

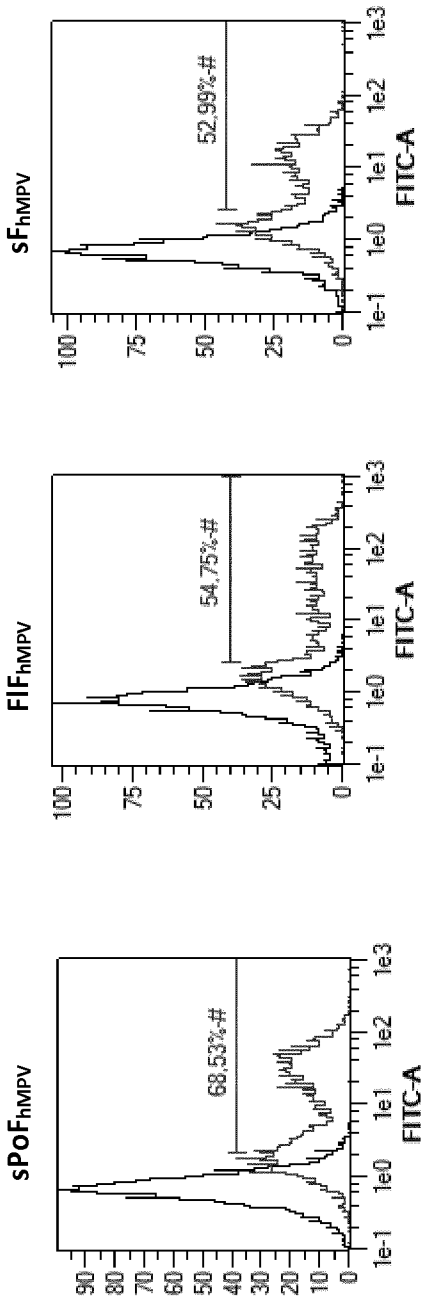
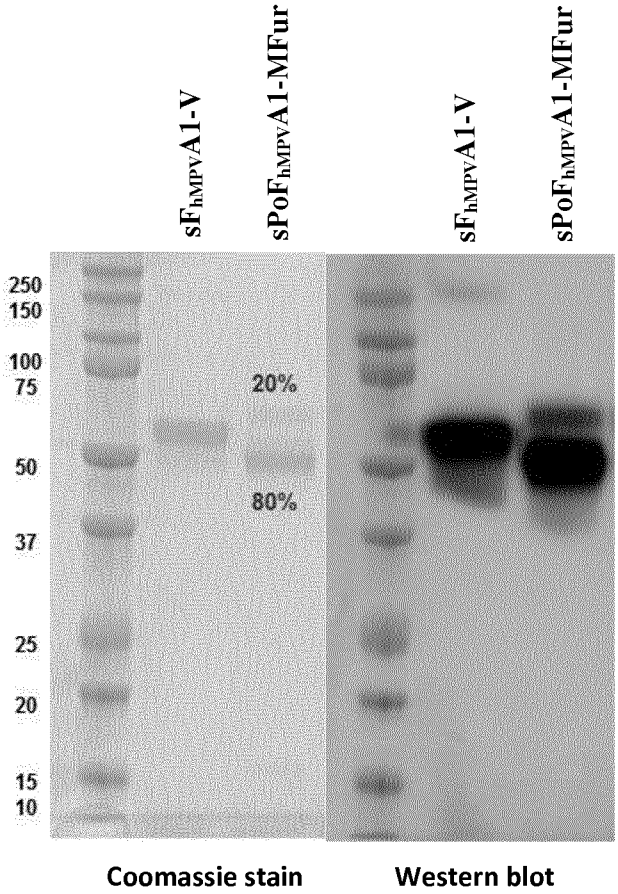


Figure 5

Figure 6

A.


sF$_{hMPV}$A1-V
sPoF$_{hMPV}$A1-MFur
sF$_{hMPV}$A1-V
sPoF$_{hMPV}$A1-MFur
250
150
100
75
50
37
25
20
15
10
20%
80%
Coomassie stain
Western blot

B.

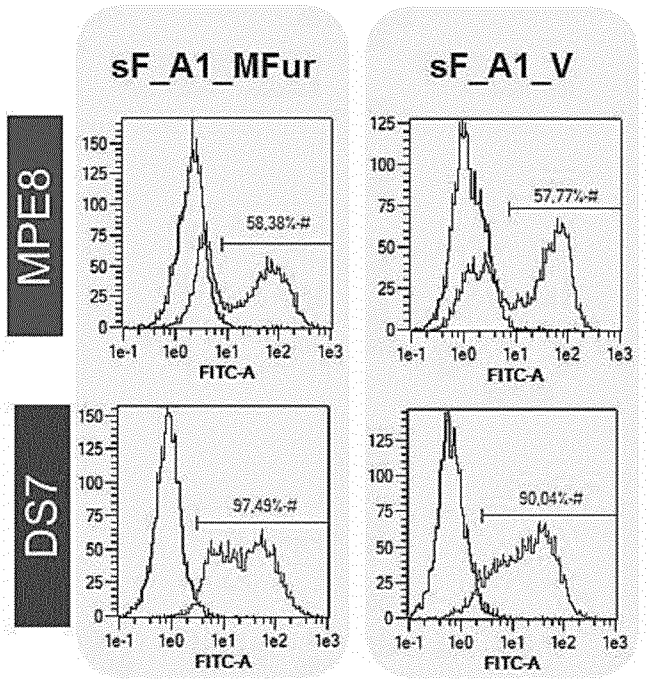

sF_A1_MFur
sF_A1_V
MPE8
DS7
58,38%-#
57,77%-#
97,49%-#
90,04%-#
FITC-A

Figure 7

Construct		Dose	IC_{50}
DNA	**sPoFhMPV ("PF")**	5 µg	2552
		10 µg	2192
		50 µg	4552
DNA	**sFlFhMPV ("FL")**	5 µg	845.6
		10 µg	3030
		50 µg	2751
Sub-unit (polypeptide)	**sPoFhMPV ("PF subunit")**	10 µg	852.5
DNA control	**pVAX1 vector ("empty plasmid")**	50 µg	---

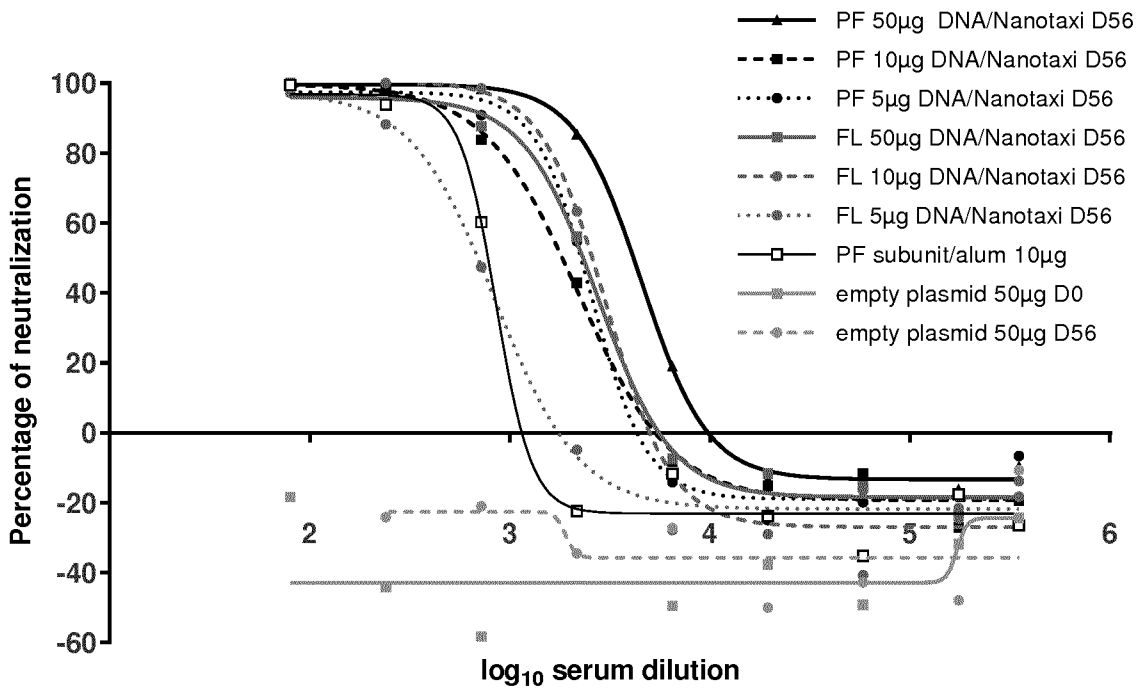


Figure 8

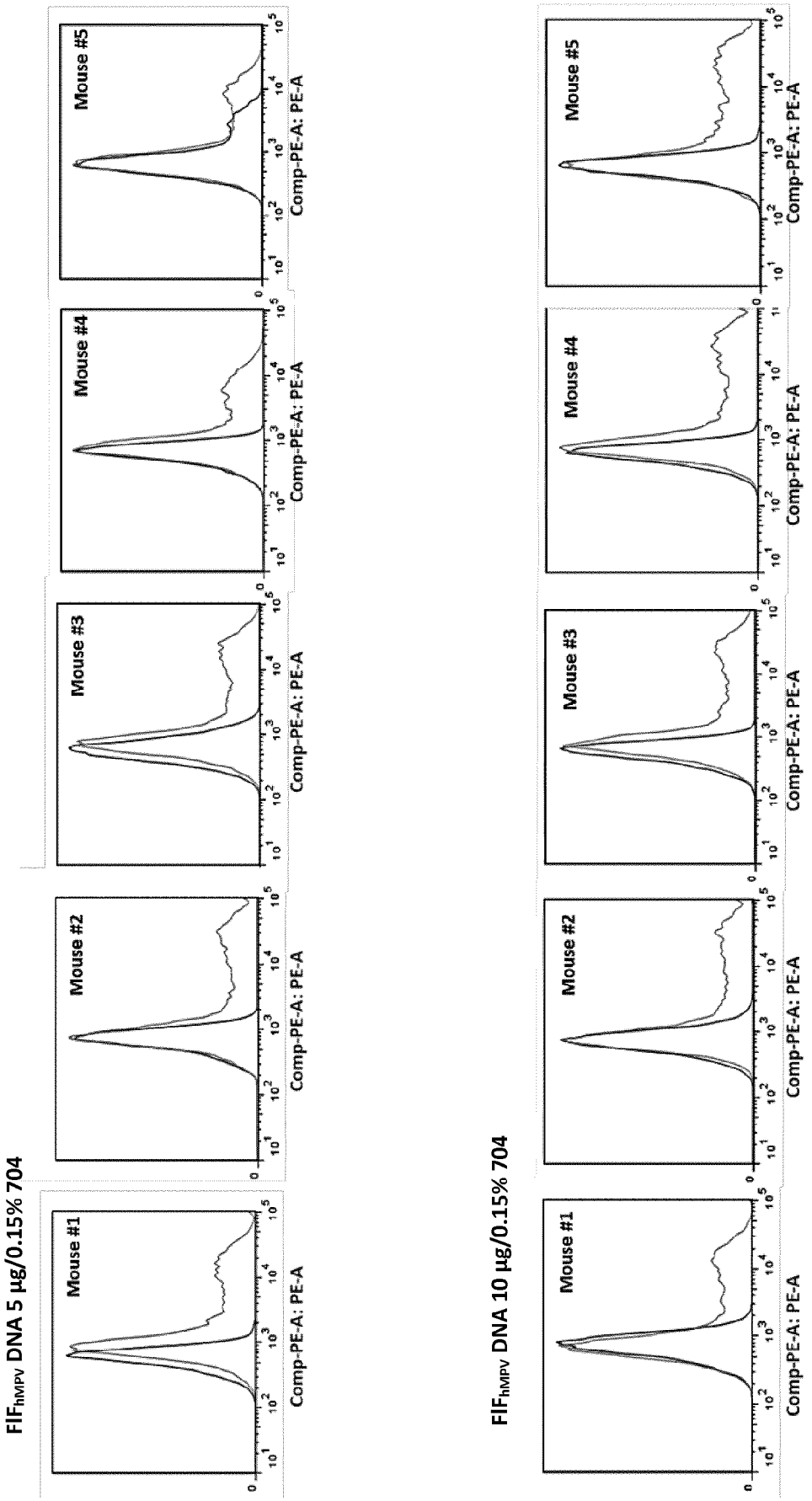

FIF$_{hMPV}$ DNA 5 µg/0.15% 704
FIF$_{hMPV}$ DNA 10 µg/0.15% 704
Mouse #1
Mouse #2
Mouse #3
Mouse #4
Mouse #5
Comp-PE-A: PE-A

Figure 8 (continued)

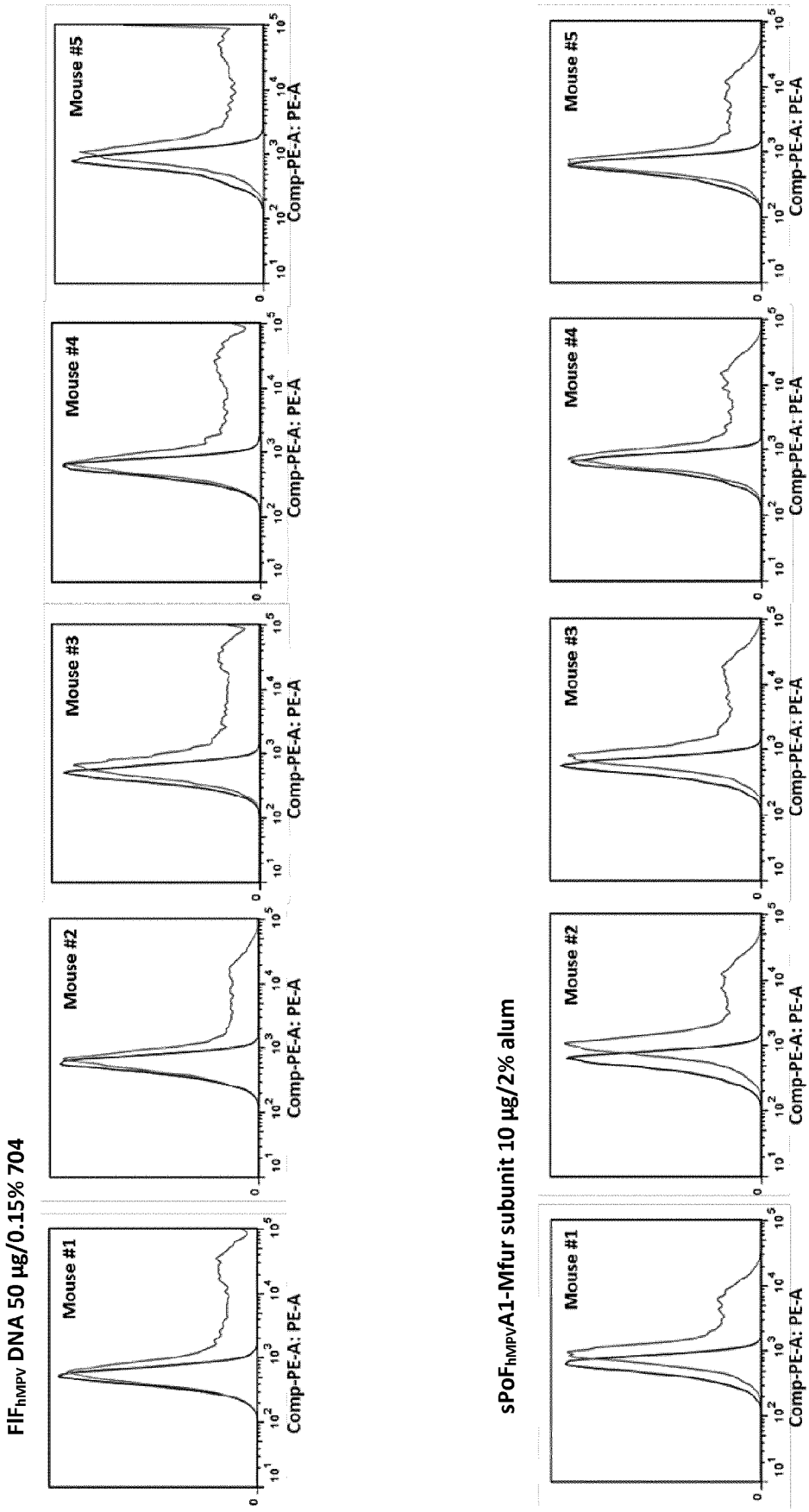


Figure 9

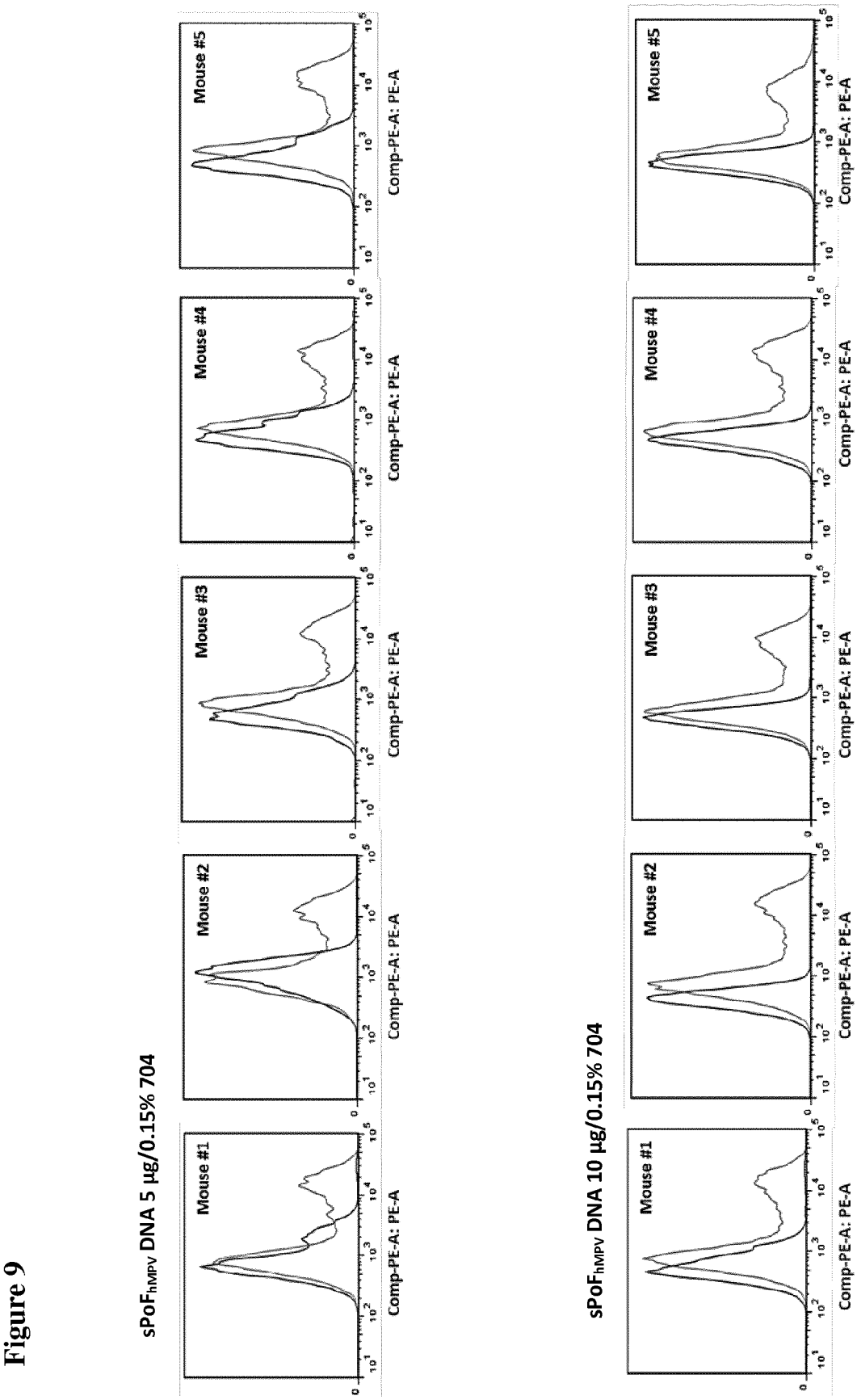

sPoF$_{hMPV}$ DNA 5 µg/0.15% 704
sPoF$_{hMPV}$ DNA 10 µg/0.15% 704
Mouse #1
Mouse #2
Mouse #3
Mouse #4
Mouse #5
Comp-PE-A: PE-A

Figure 9 (continued)

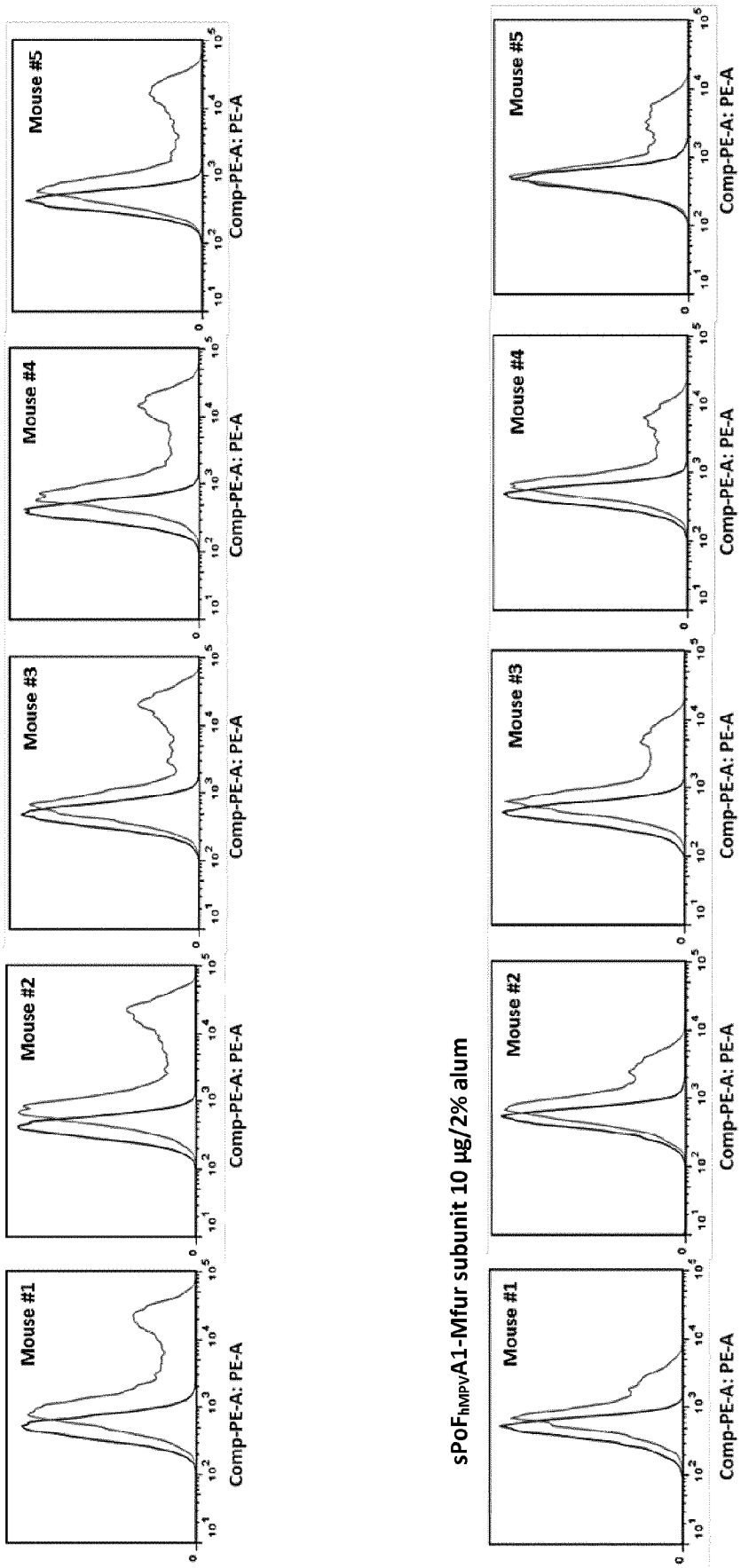


Figure 8

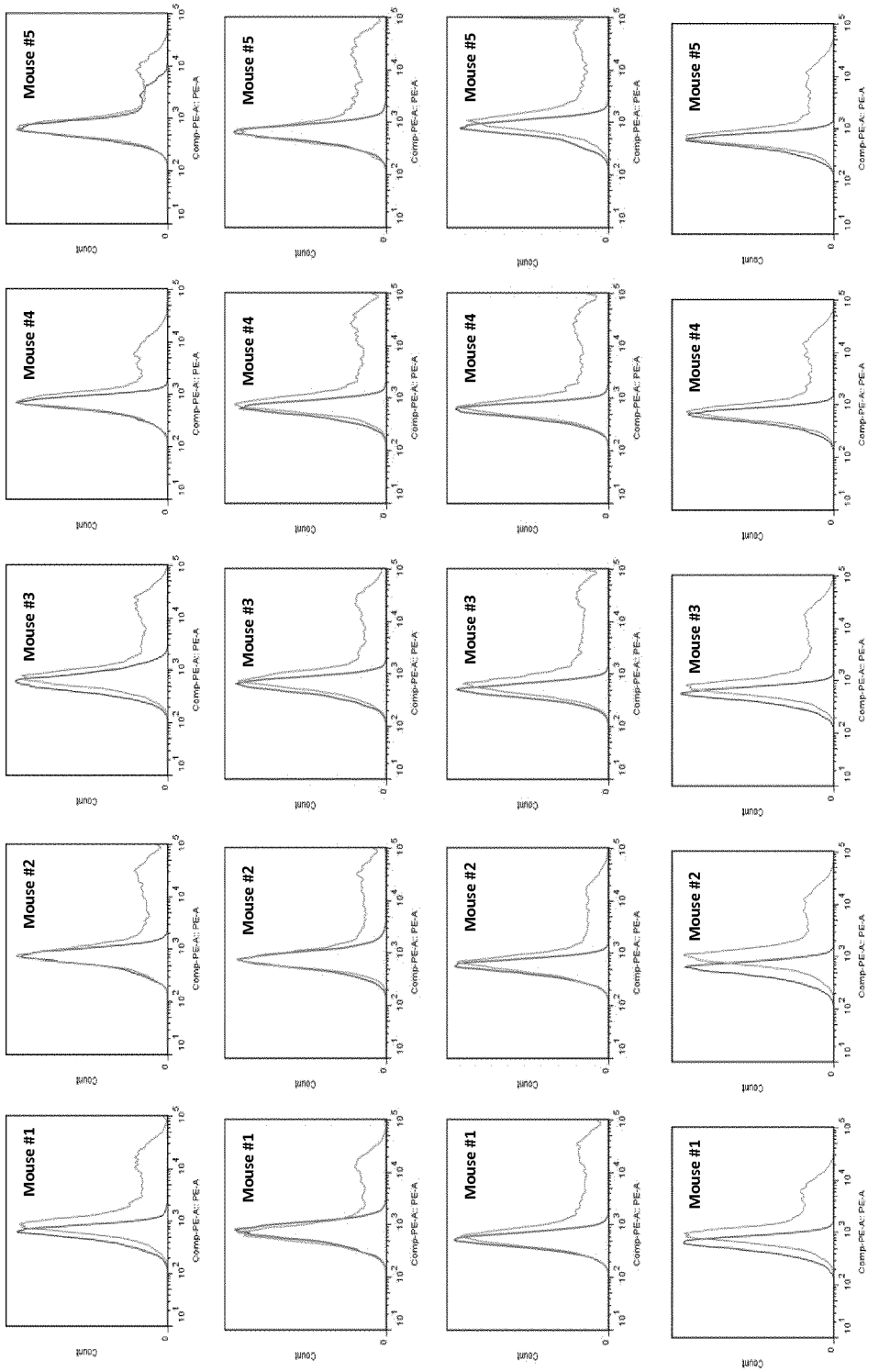

Mouse #1
Mouse #2
Mouse #3
Mouse #4
Mouse #5
Count
Comp-PE-A:: PE-A
FIF$_{hMPV}$ DNA 5 µg/0.15%
FIF$_{hMPV}$ DNA 10 µg/0.15% 704
FIF$_{hMPV}$ DNA 50 µg/0.15% 704
sPoF$_{hMPV}$A1-Mfur subunit 10 µg/2% alum

Figure 9

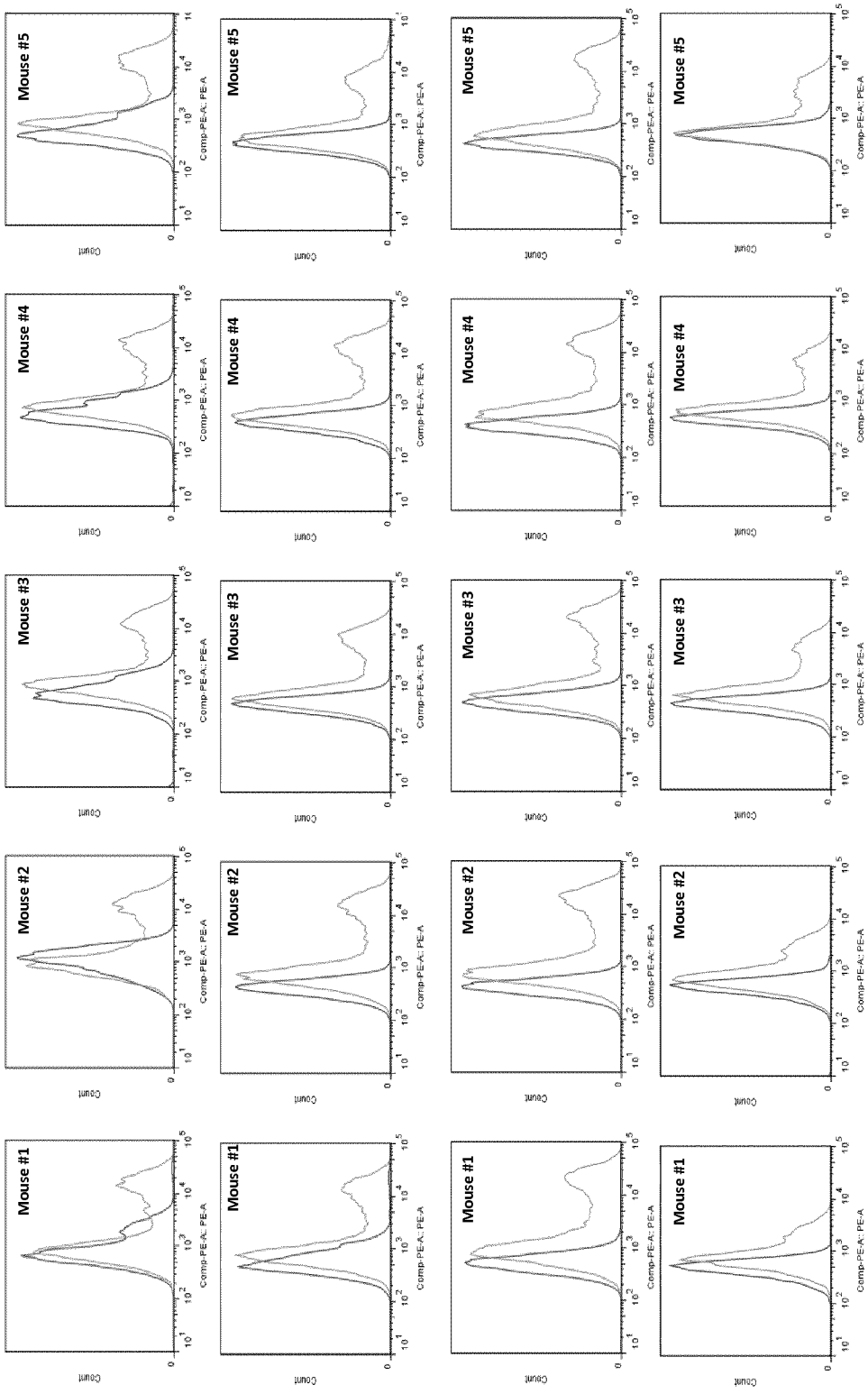
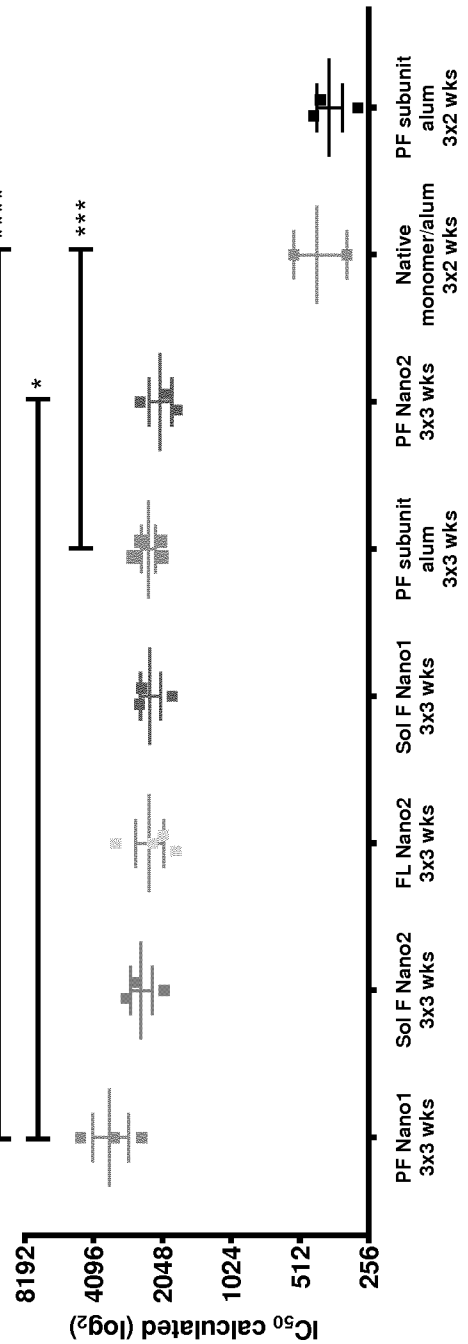

Mouse #1
Mouse #2
Mouse #3
Mouse #4
Mouse #5
Count
Comp-PE-A:: PE-A
sPoF$_{hMPV}$ DNA
5 µg/0.15% 704
sPoF$_{hMPV}$ DNA
10 µg/0.15% 704
sPoF$_{hMPV}$ DNA
50 µg/0.15% 704
sPoF$_{hMPV}$A1-Mfur
subunit
10 µg/2% alum

Figure 10

A.


IC50 calculated (log2)
8192
4096
2048
1024
512
256
****
*
***
PF Nano1 3x3 wks
Sol F Nano2 3x3 wks
FL Nano2 3x3 wks
Sol F Nano1 3x3 wks
PF subunit alum 3x3 wks
PF Nano2 3x3 wks
Native monomer/alum 3x2 wks
PF subunit alum 3x2 wks

B.

Serum α-F protein
IgG1 titer (log10), 0.5 cutoff
0
1
2
3
4
5
6
PF Nano1 2x3 wks
Sol F Nano1 2x3 wks
FL Nano2 2x3 wks
PF Nano2 2x3 wks
Sol F Nano2 2x3 wks
PF subunit alum 2x3 wks
Native monomer/alum 2x2 wks
PF subunit alum 2x2 wks

Figure 11

A.

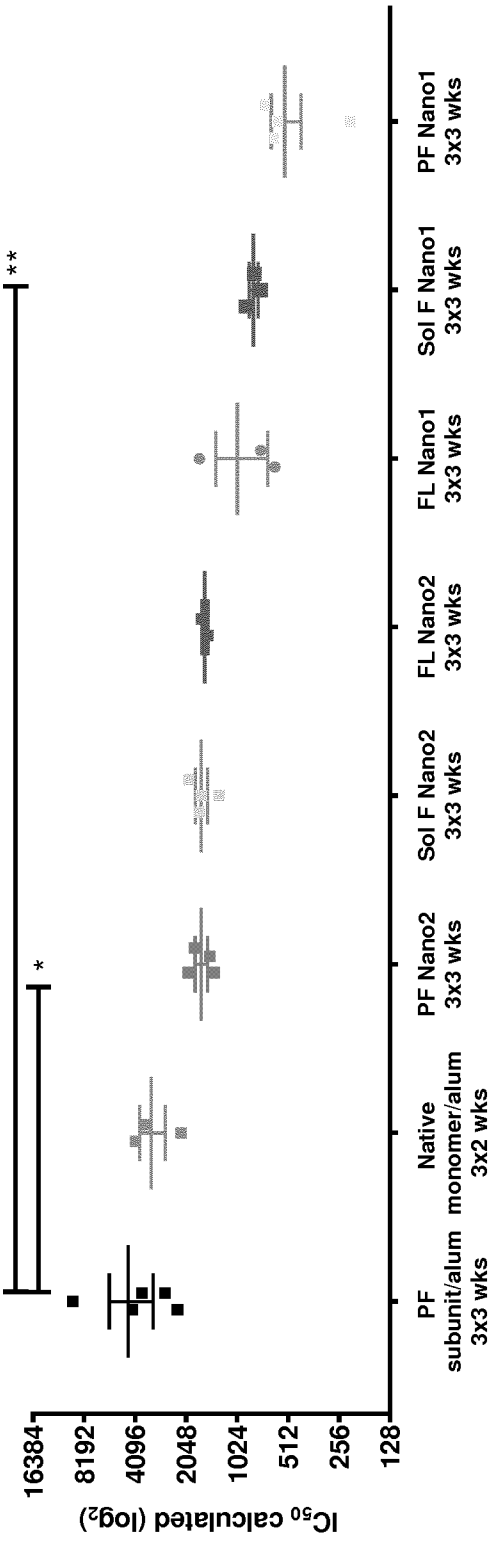

IC50 calculated (log2)
16384
8192
4096
2048
1024
512
256
128
*
**
PF subunit/alum 3x3 wks
Native monomer/alum 3x2 wks
PF Nano2 3x3 wks
Sol F Nano2 3x3 wks
FL Nano2 3x3 wks
FL Nano1 3x3 wks
Sol F Nano1 3x3 wks
PF Nano1 3x3 wks

Figure 11 (continued)

B.

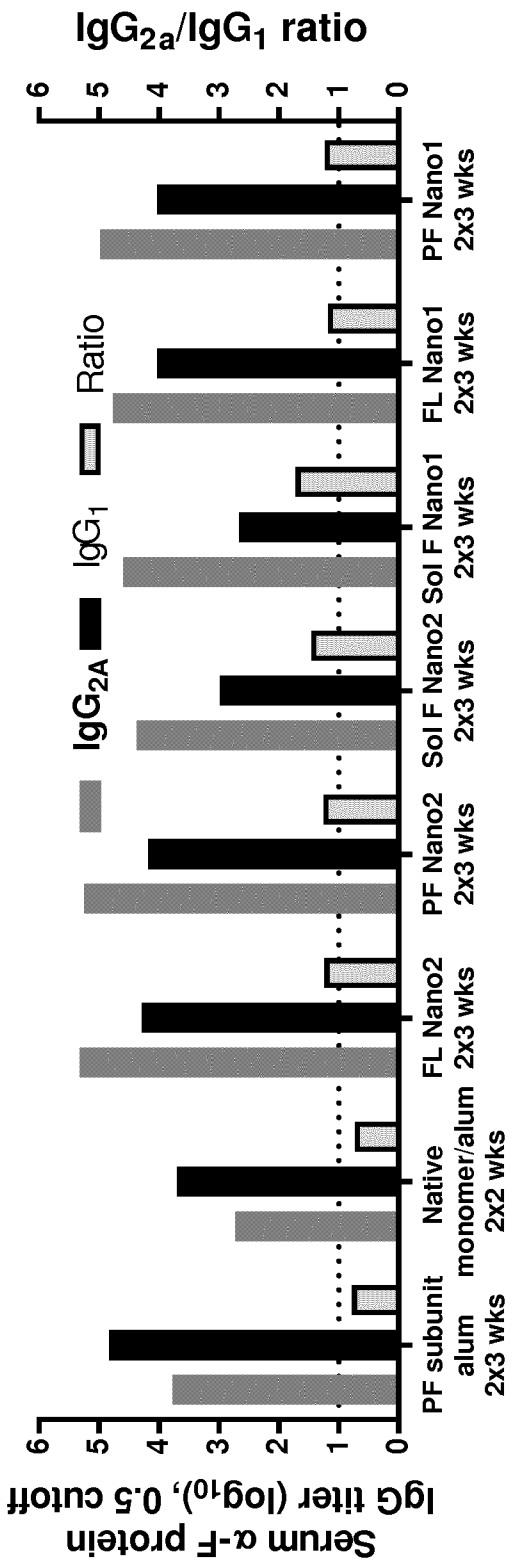


Figure 12

A.

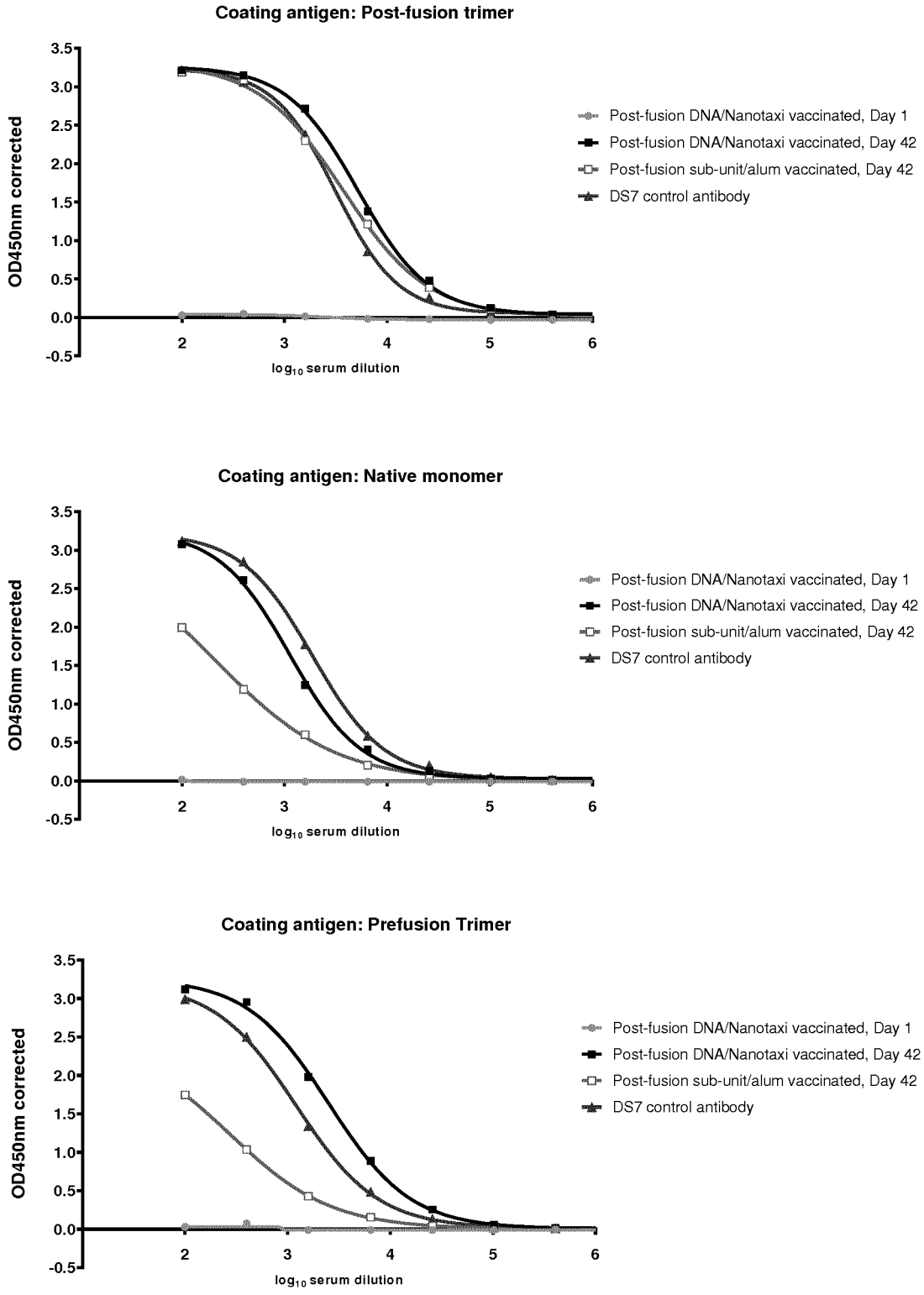

Coating antigen: Post-fusion trimer
OD450nm corrected
log10 serum dilution
Post-fusion DNA/Nanotaxi vaccinated, Day 1
Post-fusion DNA/Nanotaxi vaccinated, Day 42
Post-fusion sub-unit/alum vaccinated, Day 42
DS7 control antibody
Coating antigen: Native monomer
OD450nm corrected
log10 serum dilution
Post-fusion DNA/Nanotaxi vaccinated, Day 1
Post-fusion DNA/Nanotaxi vaccinated, Day 42
Post-fusion sub-unit/alum vaccinated, Day 42
DS7 control antibody
Coating antigen: Prefusion Trimer
OD450nm corrected
log10 serum dilution
Post-fusion DNA/Nanotaxi vaccinated, Day 1
Post-fusion DNA/Nanotaxi vaccinated, Day 42
Post-fusion sub-unit/alum vaccinated, Day 42
DS7 control antibody

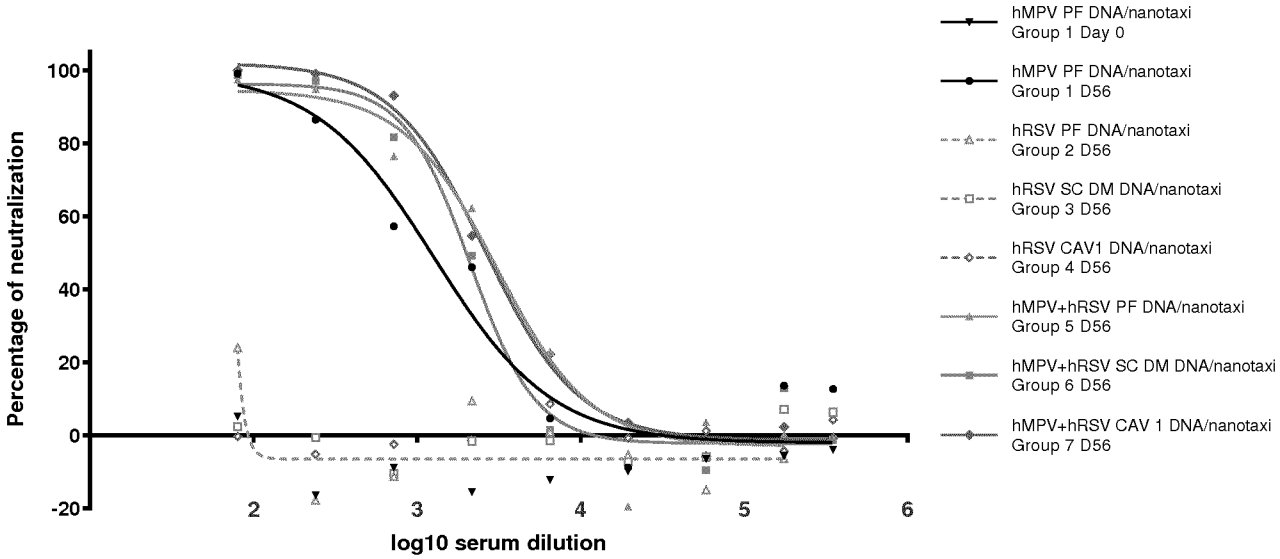
Figure 12 (continued)

B.

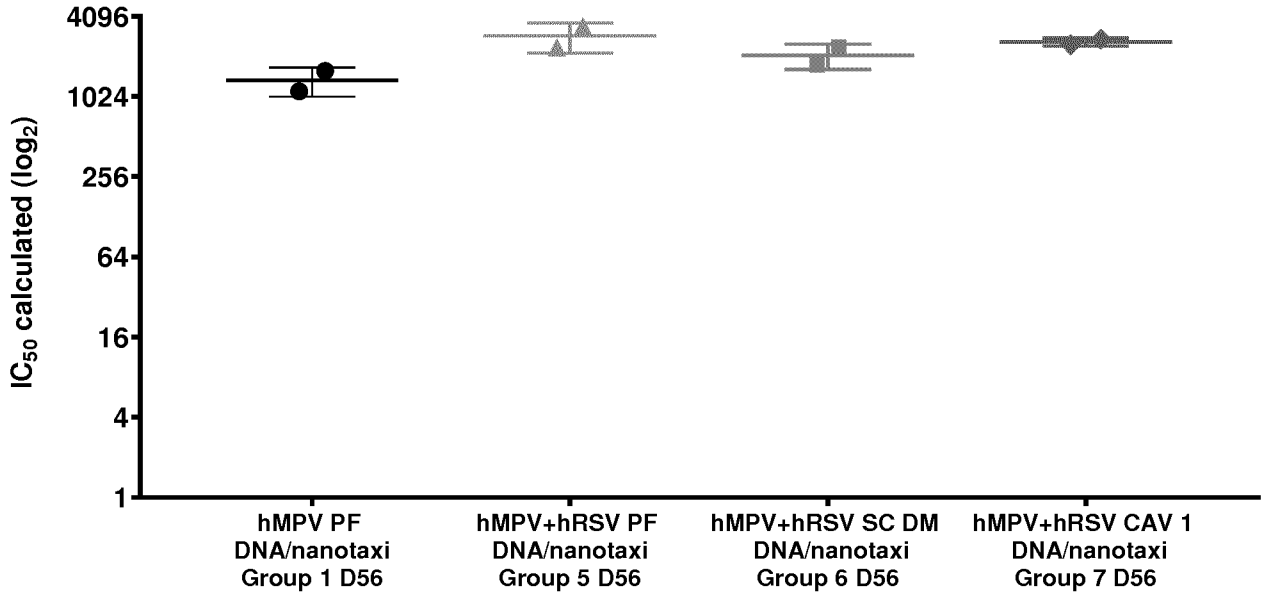
	50 µg Post-fusion DNA/0.15% 704 D42 Balb/c sera	10 µg Post-fusion sub-unit/2% alum D42 Balb/c sera	DS7 control antibody
Coating antigen	**Endpoint titer**		
Post-fusion trimer	5202	3705	3142
Native monomer	1125	214	1873
Prefusion trimer	2513	277	1207

Figure 13

A.


Percentage of neutralization
100
80
60
40
20
0
-20
2
3
4
5
6
log10 serum dilution
hMPV PF DNA/nanotaxi Group 1 Day 0
hMPV PF DNA/nanotaxi Group 1 D56
hRSV PF DNA/nanotaxi Group 2 D56
hRSV SC DM DNA/nanotaxi Group 3 D56
hRSV CAV1 DNA/nanotaxi Group 4 D56
hMPV+hRSV PF DNA/nanotaxi Group 5 D56
hMPV+hRSV SC DM DNA/nanotaxi Group 6 D56
hMPV+hRSV CAV 1 DNA/nanotaxi Group 7 D56

B.


IC50 calculated (log2)
4096
1024
256
64
16
4
1
hMPV PF DNA/nanotaxi Group 1 D56
hMPV+hRSV PF DNA/nanotaxi Group 5 D56
hMPV+hRSV SC DM DNA/nanotaxi Group 6 D56
hMPV+hRSV CAV 1 DNA/nanotaxi Group 7 D56

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/080424

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/155 A61K39/39 C07K14/005
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, CHEM ABS Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SKIADOPOULOS M H ET AL: "Individual contributions of the human metapneumovirus F, G, and SH surface glycoproteins to the induction of neutralizing antibodies and protective immunity", VIROLOGY, ELSEVIER, AMSTERDAM, NL, vol. 345, no. 2, 20 February 2006 (2006-02-20), pages 492-501, XP024896787, ISSN: 0042-6822, DOI: 10.1016/J.VIROL.2005.10.016 [retrieved on 2006-02-20] the whole document	1-42

X	WO 2010/149743 A2 (ID BIOMEDICAL CORP QUEBEC [CA]; GLAXOSMITHKLINE BIOLOG SA [BE]; BLAIS) 29 December 2010 (2010-12-29)	1-4
Y	the whole document	5-42

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 December 2018	04/01/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Meyer, Wolfram

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/080424

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/103238 A1 (US HEALTH [US]; INST RESEARCH IN BIOMEDICINE [CH]) 30 June 2016 (2016-06-30)	1-4
Y	the whole document -----	5-42

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2018/080424

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