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(54) **Title:** STABILIZED PRE-FUSION HMPV FUSION PROTEINS

(57) **Abstract:** The present invention relates to stabilized pre-fusion human pneumovirus (HMPV) F proteins, to nucleic acid molecules encoding said HMPV F proteins, as well as to the use thererof.

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STABILIZED PRE-FUSION HMPV FUSION PROTEINS

The present invention relates to the field of medicine. The invention in particular relates to recombinant pre-fusion HMPV F proteins and to fragments thereof and to nucleic acid molecules encoding the HMPV F proteins and fragments thereof, and to uses thereof, e.g. in vaccines.

BACKGROUND OF THE INVENTION

Human metapneumovirus (HMPV) belongs to the family Pneumovirinae which also includes RSV. Genetic analysis of HMPV isolates have revealed two major groups A and B and four minor groups (A1, A2, B1 and B2) mainly based on the diversity of the attachment protein (G) and the fusion protein (F) (van Hoogen et al., Emerg. Infect Dis. 10(4): 658–666, 2004). Recently Noa et al. (Microorganisms. 2020 Aug 21;8(9):1280.) described the subdivision of A2 into A2a and A2b, with the latter currently circulating.

To infect a host cell, HMPV, like other enveloped viruses such as influenza virus, RSV and HIV, requires fusion of the viral membrane with a host cell membrane. For HMPV the conserved fusion protein (HMPV F protein) fuses the viral and host cell cellular membranes. The HMPV F protein initially folds into a "pre-fusion" conformation. This metastable structure has recently been solved (Battles et al., Nat Commun. Nov 16;8(1):1528, 2017.) During cell entry, the prefusion conformation undergoes refolding and conformational changes to its "post-fusion" conformation (McLellan, J. Virol. 85(15): 7788-7796, 2010; Swanson, PNAS 108(23): 9619- 9624, 2011). Thus, the HMPV F protein is a metastable protein that drives membrane fusion by coupling irreversible protein refolding to membrane juxtaposition by initially folding into a metastable form (pre-fusion conformation) that subsequently undergoes discrete/stepwise conformational changes to a lower energy conformation (post-fusion conformation).

HMPV was first identified in 2001 in clinical samples from pediatric patients who had disease resembling that of human Respiratory Syncytial Virus (RSV) but in samples from whom RSV could not be identified (van den Hoogen et al., Nat. Med. 7(6): 719-724, 2001). Subsequent studies showed that HMPV is a major cause of both upper and lower respiratory tract infections in infants, young children, the elderly and among immunocompromised persons or those with underlying chronic medical conditions. The clinical manifestation of HMPV infections is similar to that caused by RSV, ranging from mild respiratory illness to bronchiolitis and pneumonia. HPMV infections appear to be ubiquitous since virtually all children are seropositive by the age of 5 years. Previous epidemiological studies have suggested that HMPV infections cause lower respiratory tract infection in 5-15% of otherwise healthy infants (Falsey et al., J. Infect. Dis. 187: 785-790, 2003). Among children younger than 5 years who were hospitalized with acute respiratory infection or fever, HMPV was detected in 4.9% of the children, with a population similar to that of influenza and higher than that of parainfluenza virus type 3 (PIV-3) (Williams et al., J Infect Dis. 201(12): 1890-1898, 2010). Incidence of disease is the highest for children younger than 0-6 months. Recurrent infection with HMPV also has been described.

To date, most of the infection data are derived from studies in children, however evidence accumulates that HMPV can cause serious illness in adults as well. As for influenza and RSV, infection in adults is most severe in the elderly and patients with chronic underlying medical conditions. The incidence of symptomatic infection in the adult population is typically less than 5% in most studies (Falsey, Pediatr. Infect. Dis. J. 27: S80–83, 2008). It has been quantified by Gaunt et al. that influenza A, influenza B and RSV are the leading viral cause of respiratory disease in older adults (>65 years), followed by HMPV, which attributed to 2.1 disability-adjusted life years (DALYs) per 1000 hospitalized population (Gaunt et al., J. Clin. Virol. 52(3): 215-221, 2011).

There are currently no approved therapeutic or prophylactic treatments for the management of HMPV infections. Since HPMV, next to RSV, represents a major cause of acute viral respiratory tract infection, there is a need for effective therapies or vaccines against HMPV.

SUMMARY OF THE INVENTION

The present invention provides recombinant stabilized trimeric pre-fusion human pneumovirus (HMPV) fusion (F) proteins, i.e. recombinant HMPV F proteins that are stabilized in the pre-fusion conformation. The HMPV F proteins of the invention comprise at least one epitope that is specific to the pre-fusion conformation of the F protein. The invention provides both full length HMPV F proteins and soluble HMPV F proteins. In certain embodiments, the prefusion hMPV F proteins are soluble proteins (i.e. are not membrane-bound and lack the transmembrane and cytoplasmic regions). The invention also provides nucleic acid molecules encoding the pre-fusion HMPV F proteins according to the invention and vectors comprising such nucleic acid molecules. The invention also relates to pharmaceutical compositions, preferably vaccine compositions, comprising one or more HMPV F proteins, nucleic acid molecules and/or vectors according to the invention, and to the use thereof in inducing an immune response against HMPV F protein, in particular to the use thereof as a vaccine. The invention also relates to methods for inducing an anti-human pneumovirus (HMPV) immune response in a subject, comprising administering to the subject an effective amount of a pre-fusion HMPV F protein, a nucleic acid molecule encoding said HMPV F proteins, and/or a vector comprising said nucleic acid molecule. Preferably, the induced immune response is characterized by neutralizing antibodies to HMPV F, T cells and/or protective immunity against HMPV. In particular aspects, the invention relates to a method for inducing neutralizing anti-human pneumovirus (HMPV) F protein antibodies in a

subject, comprising administering to the subject an effective amount of an immunogenic composition comprising a pre-fusion HMPV F protein, a nucleic acid molecule encoding said HMPV F protein, and/or a vector comprising said nucleic acid molecule.

BRIEF DESCRIPTION OF THE FIGURES

The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. It should be understood that the invention is not limited to the precise embodiments shown in the drawings.

FIG. 1. (a) Simplified schematic drawing of full length HMPV F with indicated residue numbers and (b) soluble HMPV F based on the F ectodomain fused to the foldon domain. The immature F0 is processed in F2 and F1 fragment by TMPRSS2, or other/related proteases, between residues 102 and the N-terminus of the fusion peptide (FP) in the F1 domain, starting at position 103. Signal peptide (SP), HR1 domain (HR1), HR2 domain (HR2), transmembrane (TM) and cytoplasmic part (CM) are indicated.

FIG. 2. (A) Alignment of section of HMPV F at the border of F2 (italic) and F1 (bold) for non-stabilized HMPV F variant (MPV201285) and stabilized variants (MPV210531, MPV210502, MPV210500, MPV210507 and MPV210509). Stabilized variants contain the T69Y, A116H, A140C/A147C, D185P, H468N, E453Q substitutions). Stabilized variants have C-terminal truncations of F2 and/or additional furin sites. (B) Reduced SDS-PAGE followed by Western blot with anti-hMPV polyclonal sera of supernatants from HEK293 cells transfected with plasmids corresponding to the stabilized soluble HMPV F ectodomain with the F2-F1 borders according to (A). Incomplete processing is observed and labeled with F0. (C) Analytical SEC analysis of cell culture supernatant shows trimer peaks with a

retention time between 4.2 and 4.4 minutes (left) and trimer stability after heat treatment for 30 minutes (right). (D) Biolayer interferometry using Octet on supernatant showing binding to pre-fusion specific antibody ADI-14448 and post-fusion specific antibody DS7 (binding to non-preF conformation) (Transfections are performed with n=2. Pool was analyzed on analytical SEC. On Octet individual transfections were analyzed and average per design is reported with error bar indicating the standard deviation). The dashed line in the right panel indicates the lower limit of quantification.

FIG. 3. (A) Alignment of a of the HMPV F sequences at the border of F2 (italic) and F1 (bold) with the insertion of RSV p27 domain (double underlined) and the additional furin site C-terminal to F2 (underlined). (B) Reduced SDS-PAGE followed by Western blot with anti-hMPV polyclonal sera of supernatants from HEK293 cells transfected with plasmids corresponding to the stabilized soluble HMPV F ectodomain with the F2-F1 borders according to (A). Complete processing is indicated by F1, incomplete processing is indicated by residual F0. (C) Analytical SEC analysis of cell culture supernatant shows trimer levels (left) and trimer stability after heat treatment for 30 minutes (right). (D) Biolayer interferometry using Octet on supernatant showing binding to the pre-fusion specific antibody ADI-14448 and the post-fusion specific antibody DS7 (binding to non-preF conformation). The dashed line in the right panel indicates the lower limit of quantification.

FIG 4. HMPV F with wildtype HR2 region and foldon (MPV210751) is compared with variants without foldon or HR2 modifications (MPV211241), and with variants with modifications of HR2 positions that were truncated at residue 489 (MPV21142-MPV211292). HMPV F variants with truncated F2 at residue 89, furin cleavage site and RSV p27 with indicated HR2 regions were expressed in Expi-HEK cells. Trimer content relative to MPV210751 after harvest and after storage for 6-8 weeks at 4° C (left). Relative trimer content after heat stress compared to the 4° C control sample (right). Transfections are

performed with n=2. Average per design is reported with error bar indicating the standard deviation.

FIG 5. (A) HMPV F with wildtype HR2 region and foldon (MPV210751) is compared with variants without foldon or HR2 modifications (MPV211241), and with variants with modifications of HR2 positions (MPV211247, MPV211287, MPV211917 and MPV211918) that were truncated at residue 489. HMPV F variants with truncated F2 at residue 89, furin cleavage site and RSV p27 with indicated HR2 regions were expressed in Expi-HEK cells. Supernatants were tested for amount of F trimer (middle panel) and heat stability (right panel) (B) Supernatants were further evaluated for binding to the pre-fusion specific antibody ADI-14448, apex interface binder MPV458 and antibody DS7 (binding to non-preF conformation) using biolayer interferometry on Octet. The dashed line in the right panel indicates the lower limit of quantification.

FIG. 6. (A) Comparison of HMPV F variants with truncated F2, furin cleavage site and RSV p27 domain, stabilized HR2 (I473W, S477I and A484I) and additional stabilizing substitutions (E453Q or E453P, V112R, D209E), and the natural variation V231I. Left histogram shows amount of trimer in supernatant of HEK293 cells after transfections with indicated F variants as measured by analytical SEC. Right panel shows heat stability of trimer relative to 4 °C after incubation for 30 minutes at 58 or 63 °C. (B) Supernatants were further evaluated by binding to pre-fusion specific antibody ADI-14448, apex interface antibody MPV458 and antibody DS7 (binding to non-preF conformation) using biolayer interferometry on Octet. The dashed line in the right panel indicates the lower limit of quantification.

FIG. 7. (A) Comparison of HMPV F variants with truncated F2, furin cleavage site and RSV p27 domain, stabilized HR2 (L473W, D475R, Q476K, S477F, N478D, R479E, A484I) and

additional stabilizing substitutions (E453Q or E453P, V112R, D209E) and a stabilizing substitution (V231I) based on a natural HMPV F variant. Left histogram shows amount of trimer in supernatant of HEK293 cells after transfections with indicated F variants as measured by analytical SEC. Right panel shows heat stability of trimer relative to 4° C after incubation for 30 minutes at 58 or 63° C. (B) Supernatants were further evaluated for binding to the pre-fusion specific antibody ADI-14448, the apex interface antibody MPV458 and antibody DS7 (binding to non-preF conformation) using biolayer interferometry on Octet. The dashed line in the right panel indicates the lower limit of quantification.

FIG. 8. (A) Comparison of HMPV F variants with truncated F2, furin cleavage site and RSV p27 domain, stabilized HR2 (L473W, D475R, Q476K, S477F, N478D, R479E, A484I) with additional stabilizing substitutions (E453Q, V112R, T69Y, S149Y, N313W, and S445Y) and a stabilizing substitution (N404P) based on a natural HMPV F variant. Histogram shows amount of trimer in supernatant of HEK293 cells after transfections with indicated F variants as measured by analytical SEC. (B) Supernatants were further evaluated for binding to the pre-fusion specific antibody ADI-14448, the apex interface antibody MPV458 and antibody DS7 (binding to non-preF conformation) using biolayer interferometry on Octet. The dashed line in the right panel indicates the lower limit of quantification. (C) T_{m50} by DSF as indication for heat stability of the trimer in supernatant of HEK293 cells.

FIG 9. (A) Comparison of HMPV F variants with truncated F2, furin cleavage site and RSV p27 domain, stabilizing substitutions (E453P, V112R, D209E) and the substitution based on the natural variation V231I, and HR2 stabilizing mutations (positions 473, 474, 477, 484 and 485). Histogram shows amount of trimer in supernatant of HEK293 cells after transfections with indicated F variants as measured by analytical SEC. (B) Supernatants were further evaluated for binding to the pre-fusion specific antibody ADI-14448, the apex interface antibody MPV458 and antibody DS7 (binding to non-preF conformation) using biolayer

interferometry on Octet. The dashed line in the right panel indicates the lower limit of quantification. (C) Tm50 by DSF as indication for heat stability of the trimer in supernatant of HEK293 cells. Vertical lines indicate detection of Tm50. For each sample the derivative is plotted, and the assigned melting points are indicated in ° C. For MPV220123 no Tm50 could be assigned.

Fig 10. (A) Comparison of HMPV F variants with truncated F2, furin cleavage site and RSV p27 domain, stabilizing substitutions (E453P, V112R, D209E), natural variation V231I, and HR2 stabilizing mutations S477I and A484I and further stabilizing mutations T69W, S149Y, N313W, S445Y and N404P. Histogram shows amount of trimer in supernatant of HEK293 cells after transfections with indicated F variants as measured by analytical SEC. (B) Supernatants were further evaluated for binding to the pre-fusion specific antibody ADI-14448, the apex interface antibody MPV458 and antibody DS7 (binding to non-preF conformation) using biolayer interferometry on Octet. The dashed line in the right panel indicates the lower limit of quantification. (C) Tm50 by DSF as indication for heat stability of the trimer in supernatant of HEK293 cells. Vertical lines indicate detection of Tm50. For each sample the derivative is plotted, and the assigned melting points are indicated in ° C.

FIG. 11. (A). Reduced SDS-PAGE Coomassie stained of purified HMPV F proteins. (B) Analytical SEC-MALS of trimeric purified HMPV F proteins after purification and storage at 4 ° C (species smaller than the trimer are indicates with D). (C) Heat stress at 37 ° C for 2 and/or 8 weeks (Aggregates are indicated with A). (D) Analytical SEC after one, five, or ten snap freeze cycles. (E) Melting temperature of the HMPV-F proteins measured by DSF. For each sample the derivative is plotted, and the assigned melting points are indicated in ° C. (F) Binding to the pre-fusion specific antibodies ADI-14448, the apex interface antibodies MPV458 and MPV465, to preF and postF binding antibody ADI-18992 and non-preF

specific antibody DS7 is measured using biolayer interferometry on Octet. (G) 2D class averages of negative stain EM images of MPV212047.

FIG. 12. Cryo EM structure of prefusion HMPV F MPV212047 without a foldon trimerization domain. One protomer is depicted as cartoon representation and two protomers as surface representation.

FIG. 13. Naïve cotton rats immunized at day 0 and day 21 with indicated doses AS01B-adjuvanted prefusion (PreF: MPV212047) or postfusion (PostF: MPV190470) HMPV F protein, followed by challenge with 10^5 PFU HMPV A2 on day 42 and sacrifice on day 46. Negative control was immunized twice with PBS, positive control was challenged once with 10^4 PFU HMPV A2 on day 0. (A) HMPV prefusion F binding antibody titers at day 42 in ELISA. (B) HMPV A2 neutralizing antibody titers at day 42 in HMPV A2-GFP VNA. (C) Nose viral load at day 46 as measured by plaque reduction neutralization test (PRNT). Every dot depicts the value of an individual animal, and the horizontal line indicates the median response of the group. Dotted line indicates lower limit of detection (LLoD).

FIG. 14. (A) Analytical size exclusion chromatography (SEC) profile of purified HMPV F trimer. (B) Purified HMPV F trimer binding to prefusion (ADI-14448) and apex interface (MPV458 and MPV465) HMPV F antibodies using quantitative Octet. Initial binding rate is plotted. (C) Balb/c mice immunized at day 0 and day 28 with indicated doses AS01B-adjuvanted prefusion open (MPV220215) or closed (MPV212047) HMPV F protein. Negative control was immunized twice with PBS. (C) HMPV prefusion F binding antibody titers at day 42 in ELISA. (D) HMPV A2 neutralizing antibody titers at day 42 in HMPV A2-GFP VNA. Every dot depicts the value of an individual animal, and the horizontal line indicates the median response of the group. Dotted horizontal line indicates lower limit of detection (LLoD).

Significant differences across doses indicated above groups, as determined by a Tobit model with a Bonferroni correction for multiple comparisons.

FIG. 15. Balb/c mice pre-exposed with at least $1x10^3$ up till $3x10^5$ PFU HMPV A2 at day 0, followed by immunization at week 12 with 15 μg unadjuvanted prefusion HMPV F protein (MPV212047). HMPV prefusion F binding antibody titers at week 14 and 16 in ELISA. Every dot depicts the value of an individual animal, and the horizontal line indicates the median response of the group. Dotted line indicates lower limit of detection (LLoD).

FIG. 16. (A) Trimer peak height (black bars) and melting temperature (Tm50) (grey bars) of cell culture supernatant expressing HMPV A2 F proteins as determined by analytical SEC and Differential Scanning Fluorimetry (DSF), respectively. Bars depict average of n=2 replicate measurements +SD. (B) Table describing subtype, strain identifier and Genbank ID of HMPV F variants. (C) Analytical SEC of cell culture supernatant expressing HMPV A2, B1, and B2 F proteins from (B). Trimer indicated with 'T', monomer with 'M'. (D) Tm50 of cell culture supernatant from (C) as determined by DSF. Bars depict average +SD of n=3 replicate measurements. (E) Prefusion (ADI-61026), apex interface (MPV458), and non-prefusion (DS7) HMPV F antibody binding in cell culture supernatant from (C) using quantitative Octet. Initial binding rate is plotted.

FIG. 17. (A) Expression level of HMPV F MPV220759 and MPV221190 trimer in cell culture supernatant as determined by analytical SEC. (B) Analytical SEC profile of purified HMPV F trimers. (C) Characterization of HMPV F trimers on basis of the MALS signal. (D) Melting temperature (Tm50) of purified HMPV F trimers as determined by DSF. N=3 replicate measurements, and individual and average values are reported as grey and black solid lines, respectively. (E) Purified HMPV F trimer binding to prefusion (ADI-14448 and ADI-61026), apex interface (MPV458), and non-prefusion (DS7) HMPV F antibodies using quantitative

Octet. Initial binding rate is plotted as average +SD of n=3-6 measurements. (F) Normalized trimer peak of HMPV F trimers subjected to 1-10 times snap freeze (SF) cycles in liquid nitrogen and supercooling stress towards -20°C as determined by analytical SEC. Untreated trimers were set at 100%. Bars depict average ±SD of n=5 supercooling samples or depict n=1 of SF samples. Open circles depict individual measurements. (G) Balb/c mice intramuscularly immunized at day 0 and 28 with 1.5, 5, or 15 µg AS01B-adjuvanted prefusion HMPV F protein or with PBS. HMPV prefusion F binding antibody titer ELISA at day 43. Every dot depicts the value of an individual animal, and the horizontal line indicates the median response of the group.

FIG. 18. (A) Expression level of HMPV F MPV221190 and MPV220552 trimer in cell culture supernatant as determined by analytical SEC. Trimer peak is indicated with a black arrow for MPV221190, and with a grey arrow for MPV220552. (B) Characterization of MPV220552 trimer on basis of the MALS signal. (C) Melting temperature (Tm50) of MPV220552 trimer as determined by DSF. N=3 replicate measurements, and individual and average values are reported as grey and black solid lines, respectively. (D) HMPV F trimer binding to prefusion (ADI-14448 and ADI-61026), apex interface (MPV458), and non-prefusion (DS7) HMPV F antibodies using quantitative Octet. Initial binding rate is plotted. (E) Analytical SEC profile of purified MPV221190 and MPV220552 trimer after purification (t=0), after storage for 2 weeks at 4°C or 2 weeks at 37°C.

FIG. 19. (A) Analytical SEC of cell culture supernatant expressing backbone MPV220847 and variants carrying substitutions at position 477. (B) Melting temperature (Tm50) of cell culture supernatant from (A) as determined by DSF. N=3 replicate measurements, and individual and average values are reported as grey and black solid lines, respectively. (C) Prefusion (ADI-14448) and non-prefusion (DS7) HMPV F antibody binding in cell culture supernatant from (A) using quantitative Octet. Initial binding rate is plotted.

FIG. 20. Analytical SEC of cell culture supernatant expressing backbone MPV211241 and variants carrying substitutions at position 477. Black lines depict supernatant at harvest (4°C) and grey lines after 30-minute incubation at 58°C. HMPV F trimer retention time is indicated with T', monomer with 'M'.

FIG. 21. Median fluorescence intensity of prefusion (ADI-61026) and non-prefusion (DS7) antibody binding to cell surface-expressed full-length HMPV F proteins, as determined by flow cytometry.

FIG. 22. (A) Alignment of HMPV F p27 variants. Glycosylation sites are underlined, N-to-Q mutations are in bold. Deletions indicated by dashed line. (B) Analytical SEC of cell culture supernatant expressing HMPV F proteins from (A), co-transfected with (grey) or without (black) 20% furin. (C) Reduced SDS-PAGE followed by Western blot with anti-hMPV polyclonal sera of selected supernatants from (B).

DETAILED DESCRIPTION OF THE INVENTION

The fusion protein (F) of the human pneumovirus (HMPV or hMPV) is a trimeric class I fusion protein involved in fusion of the viral membrane with a host cell membrane, which is required for infection. The HPMV F mRNA is translated into a 539 amino acid precursor protein designated F0, which contains a signal peptide sequence at the N-terminus (i.e. amino acid residues 1-18 of SEQ ID NO: 1) (Ulbrandt et al, Journal of General Virology (2008), 89, 3113–3118) which is removed by a signal peptidase in the endoplasmic reticulum. To become activated, the precursor form F0 is proteolytically cleaved (processed) by cellular proteases, generating two domains (F1 and F2) in a metastable, disulfide-linked heterodimer (F1+F2). The newly formed N terminus of F1 is believed to be the fusion peptide. Three F2-F1 dimers associate to form a mature F protein, which adopts a metastable prefusogenic

("prefusion") conformation that is triggered to undergo a conformational change upon contact with a target cell membrane. This conformational change exposes the fusion peptide, which associates with the host cell membrane and promotes fusion of the membrane of the virus, or an infected cell, with the target cell membrane. Immediately adjacent to the fusion peptide and the transmembrane domains (see Fig. Fig.1) are two heptad repeat (HR) regions, HR1 and HR2, respectively. Once cleaved, HMPV F can be triggered to undergo an essentially irreversible and energetically favorable conformational change from the pre-fusion form to the post-fusion state with released potential energy driving membrane fusion.

The F1 domain (corresponding to amino acid residues 103-539 of SEQ ID NO: 1) contains a 23 hydrophobic fusion peptide at its N-terminus (corresponding to amino acids 103-126 of SEQ ID NO: 1), the refolding region 2 (RR2) (corresponding to amino acids 426-491 of SEQ ID NO: 1), with HR2 comprising amino acid 453 to 484, and the C-terminus contains the transmembrane region (TM) (corresponding to amino acid residues 492-513 of SEQ ID NO: 1) and the cytoplasmic region (corresponding to amino acid residues 514 – 539) (Ulbrandt et al, Journal of General Virology (2008), 89, 3113–3118).

After cleavage, the F2 domain (corresponding to amino acid residues 19-102 of SEQ ID NO: 1) is covalently linked to F1 by two disulfide bridges (Ulbrandt et al, Journal of General Virology (2008), 89, 3113–3118). The F1-F2 heterodimers are assembled as homotrimers in the virion.

A vaccine against HMPV infection is not currently available but is desired. One potential approach to producing a vaccine is a subunit vaccine based on purified HMPV F protein. However, for this approach it is desirable that the purified HMPV F protein is in a conformation which resembles the conformation of the pre-fusion state of HMPV F protein, and which is stable over time, and can be produced in sufficient quantities. In addition, for a subunit-based vaccine, the HMPV F protein needs to be truncated by deletion of the

transmembrane (TM) and the cytoplasmic region to create a soluble secreted F protein (F or sF). Because the TM region is responsible for membrane anchoring and trimerization, the anchorless soluble F protein is considerably more labile than the full-length protein and will readily refold into the post-fusion end-state. In order to obtain soluble F protein in the stable pre-fusion conformation that shows high expression levels and high stability, the pre-fusion conformation thus needs to be stabilized. Because also the full-length (membrane-bound) HMPV F protein is metastable, stabilization of the pre-fusion conformation is also desirable for the full-length HMPV F protein, i.e. including the TM and cytoplasmic region, e.g. for any live attenuated, vector-based vaccine approach, or RNA vaccines.

The present invention provides trimeric recombinant pre-fusion HMPV F proteins, i.e. HMPV F proteins that are stabilized in the pre-fusion conformation. In the research that led to the present invention, several modifications, such as mutations, deletions, insertions, and/or fusions of amino acids as compared to the amino acid sequence of a wild-type HMPV F protein, in particular the amino acid sequence of SEQ ID NO: 1, were introduced in order to obtain said stable pre-fusion HMPV F proteins. The stable pre-fusion HMPV F proteins of the invention are in the pre-fusion conformation, i.e. they comprise (display) at least one epitope that is specific to the pre-fusion conformation F protein. An epitope that is specific to the pre-fusion conformation F protein is an epitope that is not present in the post-fusion conformation. Without wishing to be bound by any particular theory, it is believed that the pre-fusion conformation of HMPV F protein may contain epitopes that are the same as those present on the HMPV F protein expressed on natural HMPV virions, and therefore may provide advantages for eliciting protective neutralizing antibodies. In certain embodiments, the proteins of the invention comprise at least one epitope that is recognized by a pre-fusion specific anti-HMPV monoclonal antibody. Examples of such pre-fusion HMPV antibodies are MPE8 (Corti et. al., Nature 50(7467): 439-443, 2013) and ADI-14448 (Gilman et al, Sci

Immunol. 2016 Dec 16;1(6):eaaj1879. doi: 10.1126/SciImmunol.aaj1879. Epub 2016 Dec 9). In certain embodiments, the recombinant pre-fusion HMPV F proteins comprise at least one epitope that is recognized by at least one pre-fusion specific monoclonal antibody, as described above, and are trimeric. In certain embodiments, the stable pre-fusion HMPV F proteins according to the invention are soluble and thus comprise a truncated F1 domain (i.e. the transmembrane and cytoplasmic region have been (partially) deleted).

The present invention in particular provides pre-fusion human pneumovirus (HMPV) F precursor F0 proteins, comprising an F1 and an F2 domain, and comprising at least one modification in the amino acid sequence of the F1 and/or F2 domain.

In certain embodiments, the at least one modification stabilizes the pre-fusion conformation and/or increases trimer formation and/or the (thermal) stability of the HMPV F protein.

With reference to the primary amino acid sequence of the HMPV F0 protein, the following terms are utilized to describe structural features of the F proteins. The term F0 refers to a full-length translated HMPV F protein precursor. During maturation, the F0 polypeptide undergoes proteolytic cleavage at a cleavage site situated between F2 and F1, i.e. between the amino acids at positions 102 and 103.

As used herein, an F2 domain includes at least a portion of amino acids 19-102, and the F1 domain includes at least a portion of the amino acids 103-539. A soluble F protein includes an F2 domain and an F1 domain of the HMPV F protein and does not include the transmembrane domain of the HMPV F protein. A soluble portion of an F1 domain includes at least a portion, and up to all, of amino acids 103-491 of the F0 protein. As indicated above, these amino acid positions (and all subsequent amino acid positions designated herein) are given in reference to the exemplary HMPV F protein precursor polypeptide (F0) of SEQ ID NO:1.

According to the invention, the HMPV F protein includes at least one modification that stabilizes the prefusion conformation of the F protein, such that the HMPV F protein retains at least one immunodominant epitope of the prefusion conformation of the F protein and/or increases trimer formation and/or the (thermal) stability of the HMPV F protein.

In certain embodiments, the at least one modification is the introduction of at least one non-native cleavage site. Thus, in certain embodiments, the present invention provides stabilized pre-fusion human pneumovirus (HMPV) F precursor (F0) proteins, comprising an F1 and an F2 domain, and comprising a first non-native cleavage site between the F1 and the F2 domain, for example at the C-terminal end of the F2 domain.

In addition, or alternatively, the F2 domain may be C-terminally truncated. Thus, in certain embodiments, the first non-native cleavage site is positioned at the C-terminal end of a truncated F2 domain, for example, the F2 domain may be C-terminally truncated after position 89, and the first non-native cleavage site is positioned after the C-terminal amino acid residue at position 89. In the research that led to the present invention, it has been shown that the cleavage site is between residue 92-93. Thus, if e.g. a furin site that contains 4 residues is added after residue 89, the position of the terminus is ~ 92, but the last residue at the C-terminus is not native.

According to the present invention, it has been shown that by introducing a non-native cleavage site between the F1 and the F2 domain, the processing (cleavage) of the HMPV F0 protein is improved, as compared to HMPV F proteins with a native cleavage site. According to the invention it was shown that improved processing increased the stability and antigenicity of the HMPV F proteins.

According to the invention, the term non-native cleavage site refers to a cleavage site which is not present in an F protein of a naturally occurring HMPV.

A cleavage site is a proteolytic site utilized by cellular proteases that activate a wide range of precursor proteins, including class I type viral fusion proteins, such as HMPV F. Thus, various cellular proteases, such as furin, TMPRSS2, cathepsins, and other trans-membrane serine proteases, that catalyze the proteolytic activation process are known to cleave various viral cell surface proteins, which is required for the viral entry to host cells. A cleavage site typically comprises, or consists of, a specific amino acid sequence that is recognized by cellular proteases. The protease thus recognizes the site as to where it cuts via a specific sequence of amino acids along the polypeptide chain. For example, furin-like proteases preferably cleave proteins just after a basic amino acid target sequence (canonically, Arg-X-(Arg/Lys) -Arg). According to the present invention, the non-native cleavage site may be introduced N-terminally from the native cleavage site, i.e. in addition to the native cleavage site. In a preferred embodiment, the first non-native cleavage site replaces a native cleavage site.

In certain embodiments, the proteins of the invention further comprise a second non-native cleavage site in the F2 domain. According to the invention, the second non-native cleavage site is introduced in the F2 domain positioned N-terminally from the first non-native cleavage site, wherein a spacer sequence is present between the first and second non-native cleavage sites. Thus, a second non-native cleavage site is introduced within the F2 domain, positioned N-terminal from the first native or non-native cleavage site and a spacer sequence is present between the first and second non-native cleavage site, i.e the first and second cleavage site are separated by a spacer sequence and are not directly adjacent. The spacer sequence can be part of the native sequence of the F2 domain or can be a heterologous sequence separating the first and second non-native cleavage sites.

In certain embodiments, the second non-native cleavage site is positioned after amino acid residue 88 of the F2 domain, or after amino acid residue 89, or after amino acid residue

90 of the F2 domain, and the first non-native cleavage site is positioned at the C-terminal position of the F2 domain. In preferred embodiments, the second non-native cleavage site is position after amino acid 88 or 89 of the F2 domain. In preferred embodiments, the second non-native cleavage site is position after amino acid 88 or 89 of the F2 domain, such that it is cleaved after residue 92 or 93.

In certain embodiments, the first non-native cleavage site comprises an amino acid sequence RX_1X_2R, wherein X_1 and X_2 can be any amino acid.

In preferred embodiments, the first non-native cleavage is a furin cleavage site comprising the sequence $RX_1[K/R]R$.

In certain preferred embodiments, the F0 protein comprises a p27 peptide of an RSV F protein between the F1 and the (truncated) F2 domain, wherein the p27 peptide comprises the first cleavage site at its C-terminal end. In this case, the spacer sequence thus is (part of) the p27 peptide sequence. The p27 peptide can be any p27 peptide of any RSV F protein, or a variant thereof. In a particular embodiment, the p27 peptide is from an RSV A and comprises the amino acid sequence ELPRFMNYTLNNAKKTNVTLSKKRKRR (SEQ ID NO: 2, cleavage site underlined), or an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 2. In certain embodiments, the p27 peptide has an amino acid sequence having at least 90%, preferably, at least 95%, more preferably at least 97%, even more preferably at least 98%, most preferably at least 99% sequence identity to SEQ ID NO: 2. In these embodiments, the p27 peptide comprises the first non-native cleavage site at its C-terminus, and the second non-native cleavage site is positioned N-terminally from the p27 peptide. Again, the F2 domain may be truncated at its C-terminal side. The F domain may be truncated after the amino acid residue at position 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 101. In preferred embodiments, the F2 domain is truncated after the amino acid residue at

position 89, followed by a cleavage site which results in inclusion of Arg at position 91 of the F2 C-terminus.

In other embodiments, the p27 peptide is from an RSV B and comprises the amino acid sequence EAPQYMNYTINTTKNLNVSISKKRKRR (SEQ ID NO: 150), or an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 150. In certain embodiments, the p27 peptide has an amino acid sequence having at least 90%, preferably, at least 95%, more preferably at least 97%, even more preferably at least 98%, most preferably at least 99% sequence identity to SEQ ID NO: 150.

In certain embodiments, the F0 protein comprises an optimized variant of a p27 peptide of an RSV F protein located between the F1 and the (truncated) F2 domain, wherein the p27 peptide comprises the first cleavage site at its C-terminal end.

In certain embodiments, the p27 peptide has been modified by deleting one or more of the glycosylation sites in the p27 peptide sequence. The p27 peptide typically has 2 or 3 N-linked glycosylation sites. A glycosylation site typically is a 'NXS/T' motif that allows N-linked glycosylation in mammalian cells. The motif may or may not be flanked by short linkers (Gly/Ser). Preferably, all glycosylation sites in the p27 sequence have been deleted. Deletion of the glycosylation sites can be done by mutation of certain amino acids in the NXS/T motif, such that the glycosylation site is no longer present, or by deleting one or more amino acids, such that a glycosylation site is no longer present.

According to the invention it has been shown that using a deglycosylated variant of a p27 peptide, cleavage is improved without having to add furin.

In other embodiments, the p27 peptide has been modified by a deletion of 1-11 amino acids from the p27 sequence, preferably 9 or 11 amino acids.

In certain preferred embodiments, the p27 peptide comprises an amino acid sequence selected from SEQ ID NO: 185 and 186.

In certain embodiments, the second non-native cleavage site comprises a sequence RX_1X_2R, wherein X_1 and X_2 can be any amino acid (https://doi.org/10.1371/journal.pone.0054290).

In preferred embodiments, the second non-native cleavage is a furin cleavage site comprising the sequence $RX_1[K/R]R$, preferably RRRR.

In addition, or alternatively, the proteins may comprise an F1 domain which is C-terminally truncated. Thus, in order to obtain a soluble F protein, the TM and cytoplasmic regions may be removed in order to provide an HMPV F ectodomain. Thus, in certain embodiments, the proteins comprise a truncated F1 domain. In particular embodiments, the truncated F1 domain does not comprise the transmembrane and cytoplasmic regions. The F1 domain may be truncated after the amino acid at position 481, 482, 483, 484, 485, 486, 487, 488 or 489. In certain embodiments, the F1 domain is truncated after the amino acid residue at position 481 or 489. Preferably, the F1 domain is truncated after the amino acid residue at position 489. In preferred embodiment, the truncated F1 domain thus comprises, or consists of, the amino acids 103-481 or 103-489 of the HMPV F protein, preferably the amino acids 103-489 of the HMPV F protein.

As used throughout the present application, the position of the amino acid residues (or amino acid positions) are given in reference to the sequence of the HMPV F protein of SEQ ID NO: 1. As used in the present invention, the wording "the amino acid residue at position e.g. 88 of the HMPV F protein thus means the amino acid corresponding to the amino acid at position 88 in the HMPV F protein of SEQ ID NO: 1. It is noted that, in the numbering system used throughout this application 1 refers to the N-terminal amino acid of an immature

F0 protein (SEQ ID NO: 1), i.e. including the signal peptide. When a HMPV strain other than the strain TN/00/3-14 of SEQ ID NO: 1 is used, the amino acid positions of the F protein are to be numbered with reference to the numbering of the F protein of the strain of SEQ ID NO: 1, by aligning the sequences of the other HMPV strain with the F protein of SEQ ID NO: 1 with the insertion of gaps as needed. Sequence alignments can be done using methods well known in the art, e.g. by CLUSTALW, Bioedit or CLC Workbench.

An amino acid according to the invention can be any of the twenty naturally occurring (or 'standard' amino acids) or variants thereof, such as e.g. D-amino acids (the D-enantiomers of amino acids with a chiral center), or any variants that are not naturally found in proteins, such as e.g. norleucine. The standard amino acids can be divided into several groups based on their properties. Important factors are charge, hydrophilicity or hydrophobicity, size and functional groups. These properties are important for protein structure and protein–protein interactions. Some amino acids have special properties such as cysteine, that can form covalent disulfide bonds (or disulfide bridges) to other cysteine residues, proline that induces turns of the protein backbone, and glycine that is more flexible than other amino acids. Table 2 shows the abbreviations and properties of the standard amino acids. It will be appreciated by a skilled person that the mutations can be made to the protein by routine molecular biology procedures.

As described above, in certain embodiments (e.g. in the case of soluble F proteins), the proteins of the invention comprise a truncated F1 domain. As used herein a "truncated" F1 domain refers to a F1 domain that is not a full length F1 domain, i.e. wherein C-terminally one or more amino acid residues have been deleted. In certain embodiments, at least the transmembrane domain and cytoplasmic domain have been deleted to permit expression as a soluble ectodomain. Because the TM region is responsible for membrane anchoring and trimerization, the "anchorless" (i..e without TM and cytoplasmic domain) soluble F protein is

monomeric and shows low expression. In order to obtain soluble trimeric F protein in the stable pre-fusion conformation, the pre-fusion conformation thus needs to be stabilized. In order to promote trimerization it is known to replace the TM/CT region with a heterologous trimerization domain, such as a foldon domain.

According to the present invention, soluble trimeric HMPV pre-fusion F proteins are provided, without a heterologous trimerization domain. Thus, in certain embodiments, the proteins according to the invention comprise one or more stabilizing amino acid residues in the HR2 domain, said HR2 domain comprising the amino acids 453 to 484 of the HMPV F precursor (F0) protein. According to the invention, it has surprisingly been found that by the presence of the one or more of the stabilizing mutations in the HR2 domain, the trimer content is increased, even if no heterologous trimerization domain is present, as compared to HMPV F proteins without the one or more stabilizing mutations in the HR2 domain.

As indicated above, HMPV F protein typically is a homotrimer, i.e. a macromolecular complex formed by three, usually non-covalently bound, protein monomers (or protomers). In particular embodiments, the one or more stabilizing amino acids in the HR2 domain optimize the interprotomeric interactions between one or more amino acid residues in the HR2 domains of different HMPV F protomers in the trimer.

In a preferred embodiment, the amino acid at position 477 is I, V, L or F or M.

In certain embodiments, furthermore the amino acid residue at position 473 is I, F or W, and/or the amino acid residue at position 474 is or I, and/or the amino acid residue at position 475 is R, and/or the amino acid residue at position 476 is K, and/or the amino acid residue at position 478 is D, and/or the amino acid residue at position 479 is E, and/or the amino acid residue at position 480 is L, and/or the amino acid residue at position 484 is I, and/or the amino acid residue at position 488 is I.

In a preferred embodiment, the amino acid residue at position 473 W, the amino acid residue at position 477 is I, and the amino acid residue at position 484 is I.

In another preferred embodiment, the amino acid residue at position 473 is W, the amino acid residue at position 476 is K, the amino acid residue at position 477 is F, and the amino acid residue at position 484 is I.

In yet another particular embodiment, the amino acid at position 477 is I, V, L or F or M, the amino acid residue at position 473 is I, F or W, the amino acid residue at position 475 is R, the amino acid residue at position 476 is K, the amino acid residue at position 478 is D, the amino acid residue at position 479 is E, and the amino acid residue at position 484 is I.

In order to further stabilize the trimeric pre-fusion HMPV F proteins, in certain embodiments, the amino acid residue at position 112 is R, and/or the amino acid residue at position 209 is E, and/or the amino acid residue at position 453 is P or Q.

In further embodiments, the amino acid residue at position 149 is Y, and/or the amino acid residue at position 313 is W, and/or the amino acid residue at position 445 is Y.

In a preferred embodiment, the amino acid residue at position 112 is R, the amino acid residue at position 209 is E, and the amino acid residue at position 453 is P or Q.

According to the present invention, HMPV F proteins are stabilized (in the trimeric prefusion conformation) by introducing one or more modifications, such as the addition, deletion or substitution, of one or more amino acids. One such stabilizing modification is the addition of at least one non-native cleavage site in the amino acid sequence of the HMPV F0 protein.

Another stabilizing modification is the introduction of one or more stabilizing amino acids in the HR2 domain.

According to the present invention, stabilized trimeric HMPV proteins in the pre-fusion conformation thus are provided. The modifications according to the invention

preferably result in increased stabilization of the pre-fusion conformation of HMPV F trimers as compared to HMPV F proteins that do not comprise these modification(s). The modifications according to the invention preferably result in a stable "closed" pre-fusion F trimer, with a reduced binding to antibodies directed against the post-fusion conformation (such as DS7) and/or reduced binding to apex interface binding antibodies MPV458 and MPV465, as compared to HMPV F proteins that do not contain the modifications. In addition, or alternatively, the modifications according to the invention result in increased trimer content, melting temperature and/or trimer stability after storage at 4°C and/or 37°C for two weeks or after snap freezing cycles, as compared to HMPV F proteins that do not comprise these modification(s). In particular, the modifications according to the invention result in increased trimer content and trimer stability after storage at 4°C for at least 6 months as compared to HMPV F proteins that do not comprise these modification(s).

In addition, or alternatively, the modification(s) of the invention result in increased expression levels of the pre-fusion HMPV F trimers, as compared to HMPV F proteins that do not comprise these modification(s).

According to the invention it has thus been demonstrated that the presence of specific amino acids at the indicated positions increase the stability of the trimeric proteins in the pre-fusion conformation. According to the invention, the specific amino acids may be already present at the indicated position (e.g. in a naturally occurring variant of an HMPV F protein), or may be introduced by substitution (mutation) of the amino acid at that position into the specific amino acid residue according to the invention. According to the invention, the proteins comprise one or more mutations in their amino acid sequence, i.e. one or more naturally occurring amino acids at the indicated positions have been substituted with another amino acid.

Certain stabilizing amino acid residues that occur in naturally occurring HMPV F proteins include 231I, 404P, and 368N. Thus, in certain embodiments the amino acid residue at position 231 is I, the amino acid residue at position 404 is P, and/or the amino acid residue at position 368 is N.

The proteins according to the present invention may further comprise one or more additional stabilizing mutations, e.g. one or more of the stabilizing mutations that have been described in the co-pending patent application EP21215259. Thus, in certain embodiments, the amino acid residue at position 69 is Y or W, and/or the amino acid residue at position 73 is W, and/or the amino acid residue at position 185 is P, and/or the amino acid residue at position 191 is I, and/or the amino acid residue at position 116 is H, and/or the amino acid residue at position 342 is P.

In addition, or alternatively, the proteins may further comprise one or more non-native intra- or inter-protomer disulfide bonds, as described in EP21215259. In certain embodiments, the one or more disulfide bonds are selected from an intraprotomeric disulfide bond between the amino acid residues 140 and 147 and/or an intraprotomeric disulfide bond between the amino acid residues 141 or 161, and/or an intraprotomeric disulfide bond between the amino acid residues 360 and 459.

Any and/or all of the stabilizing modifications can be used individually and/or in combination with any of the other stabilizing modifications disclosed herein to produce a HMPV F protein according to the invention. In exemplary embodiments the HMPV F protein comprises at least one non-native cleavage site. In addition, or alternatively, the HMPV F proteins comprise one or more stabilizing mutations in the HR2 domain. In addition, or alternatively, the HMPV F proteins comprise an amino acid sequence wherein the amino acid residue at position 112 is R, and/or the amino acid residue at position 209 is E, and/or the

amino acid residue at position 453 is P or Q, and/or the amino acid residue at position 149 is Y, and/or the amino acid residue at position 313 is W, and/or the amino acid residue at position 445 is Y.

It is again noted that for the positions of the amino acid residues reference is made to SEQ ID NO: 1. A skilled person will be able to determine the corresponding amino acid residues in F proteins of other HMPV strains.

As described above, in certain embodiments (e.g. in the case of soluble F proteins), the proteins of the invention comprise a truncated F1 domain. As used herein a "truncated" F1 domain refers to a F1 domain that is not a full length F1 domain, i.e. wherein C-terminally one or more amino acid residues have been deleted. In certain embodiments, at least the transmembrane domain and cytoplasmic domain have been deleted to permit expression as a soluble ectodomain. In certain embodiments, the F1 domain is truncated after amino acid residue 481, 482, 483, 484, 485, 486, 487, 488 or 489.

In a preferred embodiment the truncated F1 domain comprises, or consists of, the amino acids 103-489 of the HMPV F protein.

In preferred embodiments, the soluble proteins according to the invention do not contain a heterologous trimerization domain. However, according to the invention, a heterologous trimerization domain may be linked to the truncated F1 domain, optionally through a linking sequence, if desired. In certain other embodiments, the heterologous trimerization domain is a foldon domain comprising the amino acid sequence GYIPEAPRDGQAYVRKDGEWVLLSTFL (SEQ ID NO: 136).

In preferred embodiments, the HMPV F0 proteins comprise an amino acid sequence selected from the group consisting of SEQ ID NO: 4-135, SEQ ID NO: 151-159, SEQ ID NO: 161-182 and SEQ ID NO: 184, or a fragment thereof. In a particularly preferred

embodiment, the HMPV F0 proteins comprise an amino acid sequence of SEQ ID NO: 30, 70, 81,82, 83, 96, 111, 119, 131, 132, 133, 134, 135, 155, 156, 159, 170, 180 and 184 or a fragment thereof. In a particularly preferred embodiment, the HMPV F0 sequence comprises the amino acid sequence of SEQ ID NO: 111, 159, 180 or 184, or a fragment thereof. In a preferred embodiment, the HMPV F0 sequence comprises the amino acid sequence of SEQ ID NO: 111, or a fragment thereof. In a preferred embodiment, the HMPV F0 sequence comprises the amino acid sequence of SEQ ID NO: 159, or a fragment thereof In another preferred embodiment, the HMPV F0 sequence comprises the amino acid sequence of SEQ ID NO: 180, or a fragment thereof. In another preferred embodiment, the HMPV F0 sequence comprises the amino acid sequence of SEQ ID NO: 184, or a fragment thereof. In a preferred embodiment, the amino acid sequence does not comprise any heterologous C-terminal tag sequences.

The present invention furthermore provides HMPV F proteins comprising at least one modification in the amino acid sequence of the F1 and/or F2 domain, wherein the protein has been cleaved at the one or more cleavage sites, resulting in an F2 and an F1 domain which are covalently linked by one or more native disulfide bridges, and wherein the protein is trimeric. As described above, the invention provides immature (or inactive) HMPV F0 proteins. After expression, in order to be activated, the proteins typically are cleaved (or processed) by proteases. The present invention also encompasses the processed HMPV F proteins, i.e. the HMPV F proteins after cleavage (or processing). Thus, the invention also provides processed HMPV F proteins, based on the HMPV F0 proteins as described above, wherein the processed proteins have been cleaved at one or more cleavage sites, resulting in HMPV F proteins comprising a (truncated) F2 domain and a (truncated) F1 domain which are covalently linked by one or more disulfide bridges, thus forming an F1-F2 dimers. Three F1-F2 dimers (each F1-F2 dimer being a protomer) then form a homotrimer. It will be

understood that in embodiments, wherein the inactive F0 proteins comprise a second non-native cleavage site, the spacer sequence between the first and second non-native site is removed by the cleavage.

It will also be understood that the mature HMPV F protein will not comprise a signal sequence.

In a preferred embodiment, the processed HMPV F protein according to the invention comprises an F1 domain, comprising the amino acids 103-481, preferably the amino acids 103-489 of the HMPV F0 protein, and an F2 domain comprising the amino acids 19-88 of the HMPV F0 protein. In a particularly preferred embodiment, the processed HMPV F protein according to the invention comprises an F1 domain, comprising the amino acids 103-489 of the HMPV F0 protein, and an F2 domain comprising the amino acids 19-89 of the HMPV F0 protein.

In a particular embodiment, the F1 domain consists of the amino acids 103-489 of the HMPV F0 protein, and the F2 domain consists of the amino acids 19-89 of the HMPV F0 protein. According to the invention, the amino acid sequence of the F1 and/or F2 domain contains one or more modifications as compared to the amino acid sequence of an F1 and/or F2 domain of a wild type HMPV F protein.

According to the invention, it has been found that when the F2 domain contains the amino acids 19-89, it is preferred that (after cleavage) the amino acid at position 91 is R (like it is in the native sequence). Thus, in certain embodiments, the amino acid at position 89+2 is R: the F2 domain has been truncated after the amino acid at position 89 but the addition of a non-native cleavage site (like RRRR) preferably results in preservation of an R at position 91.

In certain embodiments, the processed HMPV F proteins are derived from an HMPV F0 sequence selected from the group consisting of SEQ ID NO: 4-135, SEQ ID NO: 151-159,

SEQ ID NO: 161-182 and SEQ ID NO: 184, i.e. comprise an F1 and an F2 domain from an F0 sequence selected from the group consisting of SEQ ID NO: 4-135, SEQ ID NO: 151-159, SEQ ID NO: 161-182 and SEQ ID NO: 184.

In a particularly preferred embodiment, the processed HMPV F proteins comprise an F1 domain, comprising the amino acids 121-507, and an F2 domain comprising the amino acids 1-89, preferably the amino acids 19-89 of an amino acid sequence selected from the group consisting of SEQ ID NO: 30, 70, 81,82, 83, 96, 111, 119, 131, 132, 133, 134, 135, 155, 156, 159, 170, 180 and 184. In a particularly preferred embodiment, the HMPV F proteins comprise an F1 domain, comprising the amino acids 121-507, and an F2 domain comprising the amino acids 1-89, preferably 19-89 of the amino acid sequence of SEQ ID NO: 111, 159, 180 or 184.

As used throughout the present application nucleotide sequences are provided from 5' to 3' direction, and amino acid sequences from N-terminus to C-terminus, as custom in the art. It will be appreciated by a skilled person that the mutations can be made to the protein by routine molecular biology procedures.

As described above, according to the present invention, the HMPV F proteins are stabilized in the pre-fusion conformation, as measured by e.g. a decrease of binding to post-fusion specific HMPV F antibodies, such as DS7, and/or the decrease in binding of open apex interface binders, such as MPV458 and MPV465, as compared to HMPV F proteins without the modifications of the invention.

In addition, or alternatively, the trimer content of HMPV F proteins according to the invention is increased as compared to HMPV F proteins without the modifications of the invention, as measured by e.g. increased trimer content in supernatant detected by analytical SEC or trimer yield of purified protein.

In addition, or alternatively, the heat stability of the HMPV F proteins is increased as compared to HMPV F proteins without the modifications of the invention, as measured by e.g. trimer content after heat stress or melting temperatures in supernatant or melting temperatures of purified protein.

The present invention further provides nucleic acid molecules encoding the HMPV F proteins according to the invention. The nucleic acid molecules may be DNA or RNA polynucleotides. In preferred embodiments, the nucleic acid molecules encoding the proteins according to the invention are codon-optimized for expression in mammalian cells, preferably human cells. Methods of codon-optimization are known and have been described previously (e.g. WO 96/09378). A sequence is considered codon-optimized if at least one non-preferred codon as compared to a wild-type sequence is replaced by a codon that is more preferred. Herein, a non-preferred codon is a codon that is used less frequently in an organism than another codon coding for the same amino acid, and a codon that is more preferred is a codon that is used more frequently in an organism than a non-preferred codon. The frequency of codon usage for a specific organism can be found in codon frequency tables, such as in http://www.kazusa.or.jp/codon. Preferably more than one non-preferred codon, preferably most or all non-preferred codons, are replaced by codons that are more preferred. Preferably the most frequently used codons in an organism are used in a codon-optimized sequence. Replacement by preferred codons generally leads to higher expression.

It will be understood by a skilled person that numerous different polynucleotides and nucleic acid molecules can encode the same protein as a result of the degeneracy of the genetic code. It is also understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the protein sequence encoded by the nucleic acid molecules to reflect the codon usage of any particular host organism in which the proteins are to be expressed. Therefore, unless otherwise specified, a "nucleotide sequence or nucleic acid

molecule encoding an amino acid sequence" includes all nucleotide sequences or nucleic acid molecules that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may or may not include introns.

In certain embodiment, the nucleic acid molecules according to the invention encode a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4-135, SEQ ID NO: 151-159, SEQ ID NO: 161-182 and SEQ ID NO: 184 or a processed HMPV F protein derived therefrom or a fragment thereof. In a particularly preferred embodiment, the nucleic acid molecules encode a protein comprising an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 30, 70, 81,82, 83, 96, 111, 119, 131, 132, 133, 134, 135, 155, 156, 159, 170, 180 and 184, or a processed HMPV F protein derived therefrom, or a fragment thereof. In a particularly preferred embodiment, the nucleic acid encodes a protein comprising the amino acid sequence of SEQ ID NO: 111, 159, 180 or 184, or a processed HMPV F protein derived therefrom. In a preferred embodiment, the nucleic acid encodes a protein comprising the amino acid sequence of SEQ ID NO: 111, or a processed HMPV F protein derived therefrom. In a preferred embodiment, the nucleic acid encodes a protein comprising the amino acid sequence of SEQ ID NO: 159, or a processed HMPV F protein derived therefrom. In another preferred embodiment, the nucleic acid encodes a protein comprising the amino acid sequence of SEQ ID NO: 180, or a processed HMPV F protein derived therefrom. In another preferred embodiment, the nucleic acid encodes a protein comprising the amino acid sequence of SEQ ID NO: 184, or a processed HMPV F protein derived therefrom.

Nucleic acid sequences can be cloned using routine molecular biology techniques, or generated de novo by DNA synthesis, which can be performed using routine procedures by

service companies having business in the field of DNA synthesis and/or molecular cloning (e.g. GeneArt, GenScripts, Invitrogen, Eurofins).

As described above, the nucleic acid molecules as described herein may be RNA polynucleotides (or RNAs). The RNA may be mRNA, modified mRNA, self-replicating RNA, or circular mRNA.

Preferred RNAs are self-replicating. A self-replicating RNA molecule (replicon) can, when delivered to a vertebrate cell even without any proteins, lead to the production of multiple daughter RNAs by transcription from itself (via an antisense copy which it generates from itself). A self-replicating RNA molecule is typically a +-strand molecule which can be directly translated after delivery to a cell, and this translation provides a RNA-dependent RNA polymerase which then produces both antisense and sense transcripts from the delivered RNA. Thus the delivered RNA leads to the production of multiple daughter RNAs. These daughter RNAs, as well as collinear subgenomic transcripts, may be translated themselves to provide in situ expression of an encoded immunogen, or may be transcribed to provide further transcripts with the same sense as the delivered RNA which are translated to provide in situ expression of the immunogen. The overall result of this sequence of transcriptions is a huge amplification in the number of the introduced replicon RNAs and so the encoded immunogen becomes a major polypeptide product of the cells.

One suitable system for achieving self-replication is to use an alphavirus-based RNA replicon. These +-stranded replicons are translated after delivery to a cell to give of a replicase (or replicase- transcriptase). The replicase is translated as a polyprotein which auto-cleaves to provide a replication complex which creates genomic— strand copies of the +-strand delivered RNA. These— strand transcripts can themselves be transcribed to give further copies of the +-stranded parent RNA and also to give a subgenomic transcript which

encodes the immunogen. Translation of the subgenomic transcript thus leads to in situ expression of the immunogen by the infected cell. Suitable alphavirus replicons can use a replicase from a Sindbis virus, a Semliki forest virus, an eastern equine encephalitis virus, a Venezuelan equine encephalitis virus, etc. Mutant or wild-type viruses sequences can be used e.g. the attenuated TC83 mutant of VEEV has been used in replicons.

A preferred self-replicating RNA molecule thus encodes (i) a RNA-dependent RNA polymerase which can transcribe RNA from the self-replicating RNA molecule and (ii) an HPMV F protein according to the invention. The polymerase can be an alphavirus replicase e.g. comprising one or more of alphavirus proteins nsPl, nsP2, nsP3 and nsP4.

Whereas natural alphavirus genomes encode structural virion proteins in addition to the non-structural replicase polyprotein, it is preferred that a self-replicating RNA molecule of the invention does not encode alphavirus structural proteins. Thus, a preferred self-replicating RNA can lead to the production of genomic RNA copies of itself in a cell, but not to the production of RNA- containing virions. The inability to produce these virions means that, unlike a wild-type alphavirus, the self-replicating RNA molecule cannot perpetuate itself in infectious form. The alphavirus structural proteins which are necessary for perpetuation in wild-type viruses are absent from self-replicating RNAs of the invention and their place is taken by gene(s) encoding the immunogen of interest, such that the subgenomic transcript encodes the immunogen rather than the structural alphavirus virion proteins.

Thus, a self-replicating RNA molecule useful with the invention may have two open reading frames. The first (5') open reading frame encodes a replicase; the second (3') open reading frame encodes an immunogen. In some embodiments the RNA may have additional {e.g. downstream) open reading frames e.g. to encode further immunogens (see below) or to encode accessory polypeptides.

A self-replicating RNA molecule can have a 5' sequence which is compatible with the encoded replicase. Self-replicating RNA molecules can have various lengths but they are typically 5000-25000 nucleotides long e.g. 8000-15000 nucleotides, or 9000-12000 nucleotides.

A RNA molecule useful with the invention may have a 5' cap {e.g. a 7-methylguanosine). This cap can enhance in vivo translation of the RNA. The 5' nucleotide of a RNA molecule useful with the invention may have a 5' triphosphate group. In a capped RNA this may be linked to a 7-methylguanosine via a 5'-to-5' bridge.

A RNA molecule may have a 3' poly-A tail. It may also include a poly-A polymerase recognition sequence {e.g. AAUAAA) near its 3' end. A RNA molecule useful with the invention will typically be single-stranded. Single-stranded RNAs can generally initiate an adjuvant effect by binding to TLR7, TLR8, RNA helicases and/or PKR. RNA delivered in double-stranded form (dsRNA) can bind to TLR3, and this receptor can also be triggered by dsRNA which is formed either during replication of a single-stranded RNA or within the secondary structure of a single-stranded RNA.

A RNA molecule useful with the invention can conveniently be prepared by in vitro transcription (IVT). IVT can use a (cDNA) template created and propagated in plasmid form in bacteria, or created synthetically (for example by gene synthesis and/or polymerase chain-reaction engineering methods). For instance, a DNA-dependent RNA polymerase (such as the bacteriophage T7, T3 or SP6 RNA polymerases) can be used to transcribe the RNA from a DNA template. Appropriate capping and poly-A addition reactions can be used as required (although the replicon's poly-A is usually encoded within the DNA template). These RNA polymerases can have stringent requirements for the transcribed 5' nucleotide(s) and in some embodiments these requirements must be matched with the requirements of the encoded

replicase, to ensure that the IVT -transcribed RNA can function efficiently as a substrate for its self-encoded replicase.

The self-replicating RNA can include (in addition to any 5' cap structure) one or more nucleotides having a modified nucleobase. Thus the RNA can comprise m5C (5-methylcytidine), m5U (5-methyluridine), m6A (N6-methyladenosine), s2U (2-thiouridine), Um (2'- O-methyluridine), mlA (1-methyladenosine); m2A (2-methyladenosine); Am (2'-0-methyladenosine); ms2m6A (2-methylthio-N6-methyladenosine); i6A (N6-isopentenyladenosine); ms2i6A (2-methylthio-N6isopentenyladenosine); io6A (N6-(cis-hydroxyisopentenyl)adenosine); ms2io6A (2-methylthio-N6-(cis-hydroxyisopentenyl) adenosine); g6A (N6- glycinylcarbamoyladenosine); t6A (N6-threonyl carbamoyladenosine); ms2t6A (2-methylthio-N6- threonyl carbamoyladenosine); m6t6A (N6-methyl-N6-threonylcarbamoyladenosine); hn6A(N6.- hydroxynorvalylcarbamoyl adenosine); ms2hn6A (2-methylthio-N6-hydroxynorvalyl carbamoyladenosine); Ar(p) (2'-0-ribosyladenosine (phosphate)); I (inosine); mi l (1- methylinosine); m'lm (l,2'-0-dimethylinosine); m3C (3-methylcytidine); Cm (2T-0-methylcytidine); s2C (2-thiocytidine); ac4C (N4-acetylcytidine); f5C (5-fonnylcytidine); m5Cm (5,2-0- dimethylcytidine); ac4Cm (N4acetyl2TOmethylcytidine); k2C (lysidine); mlG (1-methylguanosine); m2G (N2-methylguanosine); m7G (7-methylguanosine); Gm (2'-0-methylguanosine); m22G (N2,N2-dimethylguanosine); m2Gm (N2,2'-0-dimethylguanosine); m22Gm (N2,N2,2'-0-trimethylguanosine); Gr(p) (2'-0-ribosylguanosine (phosphate)) ; yW (wybutosine); o2yW (peroxywybutosine); OHyW (hydroxywybutosine); OHyW* (undermodified hydroxywybutosine); imG (wyosine); mimG (methylguanosine); Q (queuosine); oQ (epoxyqueuosine); galQ (galtactosyl- queuosine); manQ (mannosyl-queuosine); preQo (7-cyano-7-deazaguanosine); preQi (7- aminomethyl-7-deazaguanosine); G* (archaeosine); D (dihydrouridine); m5Um (5,2'-0- dimethyluridine); s4U (4-thiouridine); m5s2U (5-methyl-2-

thiouridine); s2Um (2-thio-2'-0- methyluridine); acp3U (3-(3-amino-3-carboxypropyl)uridine); ho5U (5-hydroxyuridine); mo5U (5- methoxyuridine); cmo5U (uridine 5-oxyacetic acid); mcmo5U (uridine 5-oxyacetic acid methyl ester); chm5U (5-(carboxyhydroxymethyl)uridine)); mchm5U (5-(carboxyhydroxymethyl)uridine methyl ester); mcm5U (5-methoxycarbonyl methyluridine); mcm5Um (S-methoxycarbonylmethyl-2-O-methyluridine); mcm5s2U (5-methoxycarbonylmethyl-2-thiouridine); nm5s2U (5-aminomethyl-2- thiouridine); mnm5U (5-methylaminomethyluridine); mnm5s2U (5-methylaminomethyl-2- thiouridine); mnm5se2U (5-methylaminomethyl-2-selenouridine); ncm5U (5-carbamoylmethyl uridine); ncm5Um (5-carbamoylmethyl-2'-0-methyluridine); cmnm5U (5- carboxymethylaminomethyluridine); cnmm5Um (5-carboxymethylaminomethyl-2-L- Omethyluridine); cmnm5s2U (5-carboxymethylaminomethyl-2-thiouridine); m62A (N6,N6- dimethyladenosine); Tm (2'-0-methylinosine); m4C (N4-methylcytidine); m4Cm (N4,2-0- dimethylcytidine); hm5C (5-hydroxymethylcytidine); m3U (3-methyluridine); cm5U (5- carboxymethyluridine); m6Am (N6,T-0-dimethyladenosine); rn62Am (N6,N6,0-2- trimethyladenosine); m2'7G (N2,7-dimethylguanosine); m2'2'7G (N2,N2,7-trimethylguanosine); m3Um (3,2T-0-dimethyluridine); m5D (5-methyldihydrouridine); f5Cm (5-formyl-2'-0- methylcytidine); mlGm (l,2'-0-dimethylguanosine); m'Am (1,2-O-dimethyl adenosine) irinomethyluridine); tm5s2U (S-taurinomethyl-2-thiouridine)); imG-14 (4-demethyl guanosine); imG2 (isoguanosine); or ac6A (N6-acetyladenosine), hypoxanthine, inosine, 8-oxo-adenine, 7-substituted derivatives thereof, dihydrouracil, pseudouracil, 2-thiouracil, 4-thiouracil, 5-aminouracil, 5-(Cl-C6)-alkyluracil, 5-methyluracil, 5-(C2-C6)-alkenyluracil, 5-(C2-C6)-alkynyluracil, 5- (hydroxymethyl)uracil, 5-chlorouracil, 5-fluorouracil, 5-bromouracil, 5-hydroxycytosine, 5-(Cl-C6)-alkylcytosine, 5-methylcytosine, 5-(C2-C6)-alkenylcytosine, 5-(C2-C6)-alkynylcytosine, 5- chlorocytosine, 5-fluorocytosine, 5-bromocytosine, N2-

dimethylguanine, 7-deazaguanine, 8- azaguanine, 7-deaza-7-substituted guanine, 7-deaza-7-(C2-C6)alkynylguanine, 7-deaza-8-substituted guanine, 8-hydroxyguanine, 6-thioguanine, 8-oxoguanine, 2-aminopurine, 2-amino-6-chloropurine, 2,4-diaminopurine, 2,6-diaminopurine, 8-azapurine, substituted 7-deazapurine, 7-deaza-7-substituted purine, 7-deaza-8-substituted purine, or an abasic nucleotide. For instance, a self-replicating RNA can include one or more modified pyrimidine nucleobases, such as pseudouridine and/or 5-methylcytosine residues. In some embodiments, however, the RNA includes no modified nucleobases, and may include no modified nucleotides i.e. all of the nucleotides in the RNA are standard A, C, G and U ribonucleotides (except for any 5' cap structure, which may include a 7'-methylguanosine). In other embodiments, the RNA may include a 5' cap comprising a 7'-methylguanosine, and the first 1, 2 or 3 5' ribonucleotides may be methylated at the 2' position of the ribose. A RNA used with the invention ideally includes only phosphodiester linkages between nucleosides, but in some embodiments it can contain phosphoramidate, phosphorothioate, and/or methylphosphonate linkages. Ideally, a liposome includes fewer than 10 different species of RNA e.g. 5, 4, 3, or 2 different species; most preferably, a liposome includes a single RNA species i.e. all RNA molecules in the liposome have the same sequence and same length.

In certain embodiment, the nucleic acid molecules are RNA polynucleotides having an open reading frame encoding an HPMV F protein according to the invention, or a fragment thereof, and may be formulated in a cationic lipid nanoparticle, cationic nano-emulsion or polymer-based formulation or any combination or alternative formulation suitable to bring saRNA into human or animal cells in vitro or in vivo in different species including humans.

In some embodiments, an RNA replicon of the disclosure can be formulated using one or more liposomes, lipoplexes, and/or lipid nanoparticles. In some embodiments, liposome or lipid nanoparticle formulations described herein can comprise a polycationic composition. In

some embodiments, the formulations comprising a polycationic composition can be used for the delivery of the RNA replicon described herein in vivo and/or ex vitro.

The present invention thus further provides RNA replicons encoding a recombinant pre-fusion HMPV F protein or a fragment thereof, wherein the HMPV F protein comprises an amino acid sequence selected from SEQ ID NO: 180 or 184 or a fragment or variant thereof.

In certain aspects, the RNA replicon comprises, from the 5'- to 3' end:

(1) a 5' untranslated region (5'-UTR) required for nonstructural protein-mediated amplification of an RNA virus;

(2) a polynucleotide sequence encoding at least one, preferably all, of non-structural proteins of the RNA virus;

(3) a subgenomic promoter of the RNA virus;

(4) a polynucleotide sequence encoding the recombinant pre-fusion HMPV F protein or the fragment or variant thereof; and

(5) a 3' untranslated region (3'-UTR) required for nonstructural protein-mediated amplification of the RNA virus.

In certain aspects, the RNA replicon comprises, from the 5'- to 3'-end:

(1) an alphavirus 5' untranslated region (5'-UTR),

(2) a 5' replication sequence of an alphavirus non-structural gene nsp1,

(3) a downstream loop (DLP) motif of a virus species,

(4) a polynucleotide sequence encoding an autoprotease peptide,

(5) a polynucleotide sequence encoding alphavirus non-structural proteins nsp1, nsp2, nsp3 and nsp4,

(6) an alphavirus subgenomic promoter,

(7) the polynucleotide sequence encoding the recombinant pre-fusion HMPV F protein or the fragment thereof,

(8) an alphavirus 3′ untranslated region (3′ UTR), and

(9) optionally, a poly adenosine sequence.

In certain aspects, provided herein are RNA replicons, comprising, from the 5'- to 3'-end,

(1) a 5'-UTR having the polynucleotide sequence of SEQ ID NO:136,

(2) a 5' replication sequence having the polynucleotide sequence of SEQ ID NO:137,

(3) a DLP motif comprising the polynucleotide sequence of SEQ ID NO:138,

(4) a polynucleotide sequence encoding a P2A sequence of SEQ ID NO: 139,

(5) a polynucleotide sequence encoding alphavirus non-structural proteins nsp1, nsp2, nsp3 and nsp4 having the nucleic acid sequences of SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142 and SEQ ID NO: 143, respectively,

(6) a subgenomic promoter having polynucleotide sequence of SEQ ID NO: 144,

(7) a polynucleotide sequence encoding a pre-fusion HMPV F protein having the amino acid sequence selected from the group consisting of SEQ ID NOs: 180 and SEQ ID NO: 184, or a fragment or variant thereof, and

(8) a 3′ UTR having the polynucleotide sequence of SEQ ID NO: 145.

In certain aspects (a) the polynucleotide sequence encoding the P2A sequence comprises SEQ ID NO: 146, and the RNA replicon further comprises a poly adenosine

sequence, preferably the poly adenosine sequence has the SEQ ID NO: 147, at the 3'-end of the replicon.

In certain aspects, the RNA replicon comprises the polynucleotide sequence of SEQ ID NO: 187 or 188. In a preferred embodiment, the polynucleotide sequence 7972-9649 of SEQ ID NO: 187 is the coding sequence for the HMPV F protein (including stop codons), in particular the HMPV protein of SEQ ID NO: 184. In another preferred embodiment, the polynucleotide sequence 7972-9500 of SEQ ID NO: 188 is the coding sequence for the HMPV F protein (including stop codons), in particular the HMPV protein of SEQ ID NO: 184.

Also provided are nucleic acids comprising a DNA sequence encoding the RNA replicons described herein, preferably, the nucleic acid further comprises a T7 promoter operably linked to the 5'-end of the DNA sequence, more preferably, the T7 promoter comprises the nucleotide sequence of SEQ ID NO: 148.

The term "fragment" as used herein refers to a protein or (poly)peptide that has an amino-terminal and/or carboxy-terminal and/or internal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the sequence of a HMPV F protein, for example, the full-length sequence of an HMPV F protein. It will be appreciated that for inducing an immune response and in general for vaccination purposes, a protein does not need to be full length nor have all its wild type functions, and fragments of the protein are equally useful.

A fragment according to the invention is an immunologically active fragment, and typically comprises at least 15 amino acids, or at least 30 amino acids, of the HMPV F protein. In certain embodiments, it comprises at least 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, or 550 amino acids, of the HMPV F protein.

The person skilled in the art will also appreciate that changes can be made to a protein, e.g., by amino acid substitutions, deletions, additions, etc., e.g., using routine molecular biology procedures. Generally, conservative amino acid substitutions may be applied without loss of function or immunogenicity of a polypeptide. This can easily be checked according to routine procedures well known to the skilled person.

It is understood by a skilled person that numerous different nucleic acids can encode the same polypeptide or protein as a result of the degeneracy of the genetic code. It is also understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the amino acid sequence encoded by the nucleic acids, to reflect the codon usage of any particular host organism in which the polypeptides are to be expressed. Therefore, unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

Nucleic acid sequences can be cloned using routine molecular biology techniques, or generated de novo by DNA synthesis, which can be performed using routine procedures by service companies having business in the field of DNA synthesis and/or molecular cloning (e.g. GeneArt, GenScript, Invitrogen, Eurofins).

The invention also provides vectors comprising a nucleic acid molecule as described above. In certain embodiments, a nucleic acid molecule according to the invention, thus, is part of a vector. Such vectors can easily be manipulated by methods well known to the person skilled in the art and can for instance be designed for being capable of replication in prokaryotic and/or eukaryotic cells. In addition, many vectors can be used for transformation of eukaryotic cells and will integrate in whole or in part into the genome of such cells,

resulting in stable host cells comprising the desired nucleic acid in their genome. The vector used can be any vector that is suitable for cloning DNA and that can be used for transcription of a nucleic acid of interest.

Preferably, the vector is a self-replicating RNA replicon.

As used herein, "self-replicating RNA molecule," which is used interchangeably with "self-amplifying RNA molecule" or "RNA replicon" or "replicon RNA" or "saRNA," refers to an RNA molecule engineered from genomes of plus-strand RNA viruses that contains all of the genetic information required for directing its own amplification or self-replication within a permissive cell. A self-replicating RNA molecule resembles mRNA. It is single-stranded, 5′-capped, and 3′-poly-adenylated and is of positive orientation. To direct its own replication, the RNA molecule 1) encodes polymerase, replicase, or other proteins which can interact with viral or host cell-derived proteins, nucleic acids or ribonucleoproteins to catalyze the RNA amplification process; and 2) contain cis-acting RNA sequences required for replication and transcription of the subgenomic replicon-encoded RNA. Thus, the delivered RNA leads to the production of multiple daughter RNAs. These daughter RNAs, as well as collinear subgenomic transcripts, can be translated themselves to provide in situ expression of a gene of interest, or can be transcribed to provide further transcripts with the same sense as the delivered RNA which are translated to provide in situ expression of the gene of interest. The overall results of this sequence of transcriptions is a huge amplification in the number of the introduced replicon RNAs and so the encoded gene of interest becomes a major polypeptide product of the cells.

In certain embodiment, an RNA replicon of the application comprises, ordered from the 5'- to 3'-end: (1) a 5' untranslated region (5'-UTR) required for nonstructural protein-mediated amplification of an RNA virus; (2) a polynucleotide sequence encoding at least one,

preferably all, of non-structural proteins of the RNA virus; (3) a subgenomic promoter of the RNA virus; (4) a polynucleotide sequence encoding the recombinant pre-fusion HMPV F protein or the fragment or variant thereof; and (5) a 3' untranslated region (3'-UTR) required for nonstructural protein-mediated amplification of the RNA virus.

In certain embodiments, a self-replicating RNA molecule encodes an enzyme complex for self-amplification (replicase polyprotein) comprising an RNA-dependent RNA-polymerase function, helicase, capping, and poly-adenylating activity. The viral structural genes downstream of the replicase, which are under control of a subgenomic promoter, can be replaced by a pre-fusion HMPV F protein or the fragment or variant thereof described herein. Upon transfection, the replicase is translated immediately, interacts with the 5′ and 3′ termini of the genomic RNA, and synthesizes complementary genomic RNA copies. Those act as templates for the synthesis of novel positive-stranded, capped, and poly-adenylated genomic copies, and subgenomic transcripts. Amplification eventually leads to very high RNA copy numbers of up to 2 × 105 copies per cell. Thus, much lower amounts of saRNA compared to conventional mRNA suffice to achieve effective gene transfer and protective vaccination (Beissert et al., Hum Gene Ther. 2017, 28(12): 1138–1146).

Subgenomic RNA is an RNA molecule of a length or size which is smaller than the genomic RNA from which it was derived. The viral subgenomic RNA can be transcribed from an internal promoter, whose sequences reside within the genomic RNA or its complement. Transcription of a subgenomic RNA can be mediated by viral-encoded polymerase(s) associated with host cell-encoded proteins, ribonucleoprotein(s), or a combination thereof. Numerous RNA viruses generate subgenomic mRNAs (sgRNAs) for expression of their 3′-proximal genes.

In some embodiments of the present disclosure, a pre-fusion HMPV F protein or a fragment thereof described herein is expressed under the control of a subgenomic promoter. In certain embodiments, instead of the native subgenomic promoter, the subgenomic RNA can be placed under control of internal ribosome entry site (IRES) derived from encephalomyocarditis viruses (EMCV), Bovine Viral Diarrhea Viruses (BVDV), polioviruses, Foot-and-mouth disease viruses (FMD), enterovirus 71, or hepatitis C viruses. Subgenomic promoters range from 24 nucleotide (Sindbis virus) to over 100 nucleotides (Beet necrotic yellow vein virus) and are usually found upstream of the transcription start.

In some embodiments, the RNA replicon includes the coding sequence for at least one, at least two, at least three, or at least four nonstructural viral proteins (e.g., nsP1, nsP2, nsP3, nsP4). Alphavirus genomes encode non-structural proteins nsP1, nsP2, nsP3, and nsP4, which are produced as a single polyprotein precursor, sometimes designated P1234 (or nsP1-4 or nsP1234), and which is cleaved into the mature proteins through proteolytic processing. nsP1 can be about 60 kDa in size and may have methyltransferase activity and be involved in the viral capping reaction. nsP2 has a size of about 90 kDa and may have helicase and protease activity while nsP3 is about 60 kDa and contains three domains: a macrodomain, a central (or alphavirus unique) domain, and a hypervariable domain (HVD). nsP4 is about 70 kDa in size and contains the core RNA-dependent RNA polymerase (RdRp) catalytic domain. After infection the alphavirus genomic RNA is translated to yield a P1234 polyprotein, which is cleaved into the individual proteins. In disclosing the nucleic acid or polypeptide sequences herein, for example sequences of nsP1, nsP2, nsP3, nsP4, also disclosed are sequences considered to be based on or derived from the original sequence.

In some embodiments, RNA replicon includes the coding sequence for a portion of the at least one nonstructural viral protein. For example, the RNA replicon can include about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, or a range between any two

of these values, of the encoding sequence for the at least one nonstructural viral protein. In some embodiments, the RNA replicon can include the coding sequence for a substantial portion of the at least one nonstructural viral protein. As used herein, a "substantial portion" of a nucleic acid sequence encoding a nonstructural viral protein comprises enough of the nucleic acid sequence encoding the nonstructural viral protein to afford putative identification of that protein, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as BLAST (see, for example, in "Basic Local Alignment Search Tool"; Altschul S F et al., J. Mol. Biol. 215:403-410, 1993). In some embodiments, the RNA replicon can include the entire coding sequence for the at least one nonstructural protein. In some embodiments, the RNA replicon comprises substantially all the coding sequence for the native viral nonstructural proteins. In certain embodiments, the one or more nonstructural viral proteins are derived from the same virus. In other embodiments, the one or more nonstructural proteins are derived from different viruses.

The RNA replicon can be derived from any suitable plus-strand RNA viruses, such as alphaviruses or flaviviruses. Preferably, the RNA replicon is derived from alphaviruses. The term "alphavirus" describes enveloped single-stranded positive sense RNA viruses of the family Togaviridae. The genus alphavirus contains approximately 30 members, which can infect humans as well as other animals. Alphavirus particles typically have a 70 nm diameter, tend to be spherical or slightly pleomorphic, and have a 40 nm isometric nucleocapsid. The total genome length of alphaviruses ranges between 11,000 and 12,000 nucleotides and has a 5′cap and 3′ poly-A tail. There are two open reading frames (ORF's) in the genome, non-structural (ns) and structural. The ns ORF encodes proteins (nsP1-nsP4) necessary for transcription and replication of viral RNA. The structural ORF encodes three structural proteins: the core nucleocapsid protein C, and the envelope proteins P62 and El that associate

as a heterodimer. The viral membrane-anchored surface glycoproteins are responsible for receptor recognition and entry into target cells through membrane fusion. The four ns protein genes are encoded by genes in the 5′ two-thirds of the genome, while the three structural proteins are translated from a subgenomic mRNA colinear with the 3′ one-third of the genome.

In some embodiments, the self-replicating RNA useful for the invention is an RNA replicon derived from an alphavirus virus species. In some embodiments, the alphavirus RNA replicon is of an alphavirus belonging to the VEEV/EEEV group, or the SF group, or the SIN group. Non-limiting examples of SF group alphaviruses include Semliki Forest virus, O'Nyong-Nyong virus, Ross River virus, Middelburg virus, Chikungunya virus, Barmah Forest virus, Getah virus, Mayaro virus, Sagiyama virus, Bebaru virus, and Una virus. Non-limiting examples of SIN group alphaviruses include Sindbis virus, Girdwood S. A. virus, South African Arbovirus No. 86, Ockelbo virus, Aura virus, Babanki virus, Whataroa virus, and Kyzylagach virus. Non-limiting examples of VEEV/EEEV group alphaviruses include Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), Everglades virus (EVEV), Mucambo virus (MUCV), Pixuna virus (PIXV), Middleburg virus (MIDV), Chikungunya virus (CHIKV), O'Nyong-Nyong virus (ONNV), Ross River virus (RRV), Barmah Forest virus (BF), Getah virus (GET), Sagiyama virus (SAGV), Bebaru virus (BEBV), Mayaro virus (MAYV), and Una virus (UNAV).

Non-limiting examples of alphavirus species include Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), Everglades virus (EVEV), Mucambo virus (MUCV), Semliki forest virus (SFV), Pixuna virus (PIXV), Middleburg virus (MIDV), Chikungunya virus (CHIKV), O'Nyong-Nyong virus (ONNV), Ross River virus (RRV), Barmah Forest virus (BF), Getah virus (GET), Sagiyama virus (SAGV), Bebaru virus (BEBV), Mayaro virus (MAYV), Una virus (UNAV), Sindbis virus (SINV), Aura virus

(AURAV), Whataroa virus (WHAV), Babanki virus (BABV), Kyzylagach virus (KYZV), Western equine encephalitis virus (WEEV), Highland J virus (HJV), Fort Morgan virus (FMV), Ndumu (NDUV), and Buggy Creek virus. Virulent and avirulent alphavirus strains are both suitable. In some embodiments, the alphavirus RNA replicon is of a Sindbis virus (SIN), a Semliki Forest virus (SFV), a Ross River virus (RRV), a Venezuelan equine encephalitis virus (VEEV), or an Eastern equine encephalitis virus (EEEV). In some embodiments, the alphavirus RNA replicon is of a Venezuelan equine encephalitis virus (VEEV).

In certain embodiments, a self-replicating RNA molecule comprises a polynucleotide encoding one or more nonstructural proteins nsp1-4, a subgenomic promoter, such as 26S subgenomic promoter, and a gene of interest encoding a pre-fusion HMPV F protein or the fragment thereof described herein.

A self-replicating RNA molecule can have a 5' cap (e.g., a 7-methylguanosine). This cap can enhance in vivo translation of the RNA.

The 5' nucleotide of a self-replicating RNA molecule useful with the invention can have a 5' triphosphate group. In a capped RNA this can be linked to a 7-methylguanosine via a 5'-to-5' bridge. A 5' triphosphate can enhance RIG-I binding.

A self-replicating RNA molecule can have a 3' poly-A tail. It can also include a poly-A polymerase recognition sequence (e.g., AAUAAA) near its 3' end.

In any of the embodiments of the present disclosure, the RNA replicon can lack (or not contain) the coding sequence(s) of at least one (or all) of the structural viral proteins (e.g., nucleocapsid protein C, and envelope proteins P62, 6K, and E1). In these embodiments, the sequences encoding one or more structural genes can be substituted with one or more heterologous sequences such as, for example, a coding sequence for a pre-fusion HMPV F

protein or the fragment thereof described herein. In a preferred embodiment, the RNA replicon lacks (or does not contain) all coding sequence(s) for structural viral protein(s).

In certain embodiments, a self-replicating RNA vector of the application comprises one or more features to confer a resistance to the translation inhibition by the innate immune system or to otherwise increase the expression of the GOI (e.g., a pre-fusion HMPV F protein or the fragment or variant thereof described herein).

In certain embodiments, the RNA sequence can be codon optimized to improve translation efficiency. The RNA molecule can be modified by any method known in the art in view of the present disclosure to enhance stability and/or translation, such by adding a polyA tail, e.g., of at least 30 adenosine residues; and/or capping the 5-end with a modified ribonucleotide, e.g., 7-methylguanosine cap, which can be incorporated during RNA synthesis or enzymatically engineered after RNA transcription.

In certain embodiments, an RNA replicon of the application comprises, ordered from the 5'- to 3'-end, (1) an alphavirus 5' untranslated region (5'-UTR), (2) a 5' replication sequence of an alphavirus non-structural gene nsp1, (3) a downstream loop (DLP) motif of a virus species, (4) a polynucleotide sequence encoding an autoprotease peptide, (5) a polynucleotide sequence encoding alphavirus non-structural proteins nsp1, nsp2, nsp3 and nsp4, (6) an alphavirus subgenomic promoter, (7) the polynucleotide sequence encoding the recombinant pre-fusion HMPV F protein or the fragment or variant thereof, (8) an alphavirus 3′ untranslated region (3′ UTR), and (9) optionally, a poly adenosine sequence.

In certain embodiments, a self-replicating RNA vector of the application comprises a downstream loop (DLP) motif of a virus species. As used herein, a "downstream loop" or "DLP motif" refers to a polynucleotide sequence comprising at least one RNA stem-loop, which when placed downstream of a start codon of an open reading frame (ORF) provides

increased translation of the ORF compared to an otherwise identical construct without the DLP motif. As an example, members of the Alphavirus genus can resist the activation of antiviral RNA-activated protein kinase (PKR) by means of a prominent RNA structure present within in viral 26S transcripts, which allows an eIF2-independent translation initiation of these mRNAs. This structure, called the downstream loop (DLP), is located downstream from the AUG in SINV 26S mRNA. The DLP is also detected in Semliki Forest virus (SFV). Similar DLP structures have been reported to be present in at least 14 other members of the Alphavirus genus including New World (for example, MAYV, UNAV, EEEV (NA), EEEV (SA), AURAV) and Old World (SV, SFV, BEBV, RRV, SAG, GETV, MIDV, CHIKV, and ONNV) members. The predicted structures of these Alphavirus 26S mRNAs were constructed based on SHAPE (selective 2′-hydroxyl acylation and primer extension) data (Toribio et al., Nucleic Acids Res. May 19; 44(9):4368-80, 2016), the content of which is hereby incorporated by reference). Stable stem-loop structures were detected in all cases except for CHIKV and ONNV, whereas MAYV and EEEV showed DLPs of lower stability (Toribio et al., 2016 supra). In the case of Sindbis virus, the DLP motif is found in the first 150 nt of the Sindbis subgenomic RNA. The hairpin is located downstream of the Sindbis capsid AUG initiation codon (AUG is collated at nt 50 of the Sindbis subgenomic RNA). Previous studies of sequence comparisons and structural RNA analysis revealed the evolutionary conservation of DLP in SINV and predicted the existence of equivalent DLP structures in many members of the Alphavirus genus (see, e.g., Ventoso, J. Virol. 9484-9494, Vol. 86, September 2012). Examples of a self-replicating RNA vector comprising a DLP motif are described in US Patent Application Publication US2018/0171340 and the International Patent Application Publication WO2018106615, the content of which is incorporated herein by reference in its entirety. In some embodiments, a replicon RNA of the application comprises a DLP motif exhibiting at least 90%, at least 95%, at least 96%, at least

97%, at least 98%, at least 99%, or 100% sequence identity to the sequences set forth in SEQ ID NO: 138,

In one embodiment, the self-replicating RNA molecule also contains a coding sequence for an autoprotease peptide operably linked downstream of the DLP motif and upstream of the coding sequences of the nonstructural proteins (e.g., one or more of nsp1-4) or gene of interest (e.g., a pre-fusion HMPV F protein or the fragment thereof described herein). Examples of the autoprotease peptide include, but are not limited to, a peptide sequence selected from the group consisting of porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), and a combination thereof. In some embodiments, a replicon RNA of the application comprises a coding sequence for P2A having the amino acid sequence of SEQ ID NO: 139. Preferably, the coding sequence exhibits at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the sequences set forth in SEQ ID NO: 146.

Any of the replicons of the invention can also comprise a 5' and a 3' untranslated region (UTR). The UTRs can be wild type New World or Old World alphavirus UTR sequences, or a sequence derived from any of them. In various embodiments the 5' UTR can be of any suitable length, such as about 60 nt or 50-70 nt or 40-80 nt. In some embodiments the 5' UTR can also have conserved primary or secondary structures (e.g., one or more stem-loop(s)) and can participate in the replication of alphavirus or of replicon RNA. In some embodiments the 3' UTR can be up to several hundred nucleotides, for example it can be 50-900 or 100-900 or 50-800 or 100-700 or 200 nt-700 nt. The '3 UTR also can have secondary structures, e.g., a step loop, and can be followed by a polyadenylate tract or poly-A tail. In any of the embodiments of the invention the 5' and 3' untranslated regions can be operably

linked to any of the other sequences encoded by the replicon. The UTRs can be operably linked to a promoter and/or sequence encoding a heterologous protein or peptide by providing sequences and spacing necessary for recognition and transcription of the other encoded sequences. Any polyadenylation signal known to those skilled in the art in view of the present disclosure can be used. For example, the polyadenylation signal can be a SV40 polyadenylation signal, LTR polyadenylation signal, bovine growth hormone (bGH) polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human β-globin polyadenylation signal.

In another embodiment, a self-replicating RNA replicon of the application comprises a modified 5' untranslated region (5′-UTR), preferably the RNA replicon is devoid of at least a portion of a nucleic acid sequence encoding viral structural proteins. For example, the modified 5′-UTR can comprise one or more nucleotide substitutions at position 1, 2, 4, or a combination thereof. Preferably, the modified 5′-UTR comprises a nucleotide substitution at position 2, more preferably, the modified 5′-UTR has a U->G or U->A substitution at position 2. Examples of such self-replicating RNA molecules are described in US Patent Application Publication US2018/0104359 and the International Patent Application Publication WO2018075235, the content of which is incorporated herein by reference in its entirety. In some embodiments, a replicon RNA of the application comprises a 5′-UTR exhibiting at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the sequences set forth in SEQ ID NO: 136.

In some embodiments, an RNA replicon of the application comprises a polynucleotide sequence encoding a signal peptide sequence. Preferably, the polynucleotide sequence encoding the signal peptide sequence is located upstream of or at the 5'-end of the polynucleotide sequence encoding the pre-fusion HMPV F protein or the fragment thereof. Signal peptides typically direct localization of a protein, facilitate secretion of the protein

from the cell in which it is produced, and/or improve antigen expression and cross-presentation to antigen-presenting cells. A signal peptide can be present at the N-terminus of a pre-fusion HMPV F protein or fragment thereof when expressed from the replicon, but is cleaved off by signal peptidase, e.g., upon secretion from the cell. An expressed protein in which a signal peptide has been cleaved is often referred to as the "mature protein." Any signal peptide known in the art in view of the present disclosure can be used. For example, a signal peptide can be a cystatin S signal peptide; an immunoglobulin (Ig) secretion signal, such as the Ig heavy chain gamma signal peptide SPIgG, the Ig heavy chain epsilon signal peptide SPIgE, or the short leader peptide sequence of the HMPV. Exemplary nucleic acid sequence encoding a signal peptide is shown in SEQ ID NO: 149.

In various embodiments the RNA replicons disclosed herein can be engineered, synthetic, or recombinant RNA replicons. As non-limiting examples, an RNA replicon can be one or more of the following: 1) synthesized or modified in vitro, for example, using chemical or enzymatic techniques, for example, by use of chemical nucleic acid synthesis, or by use of enzymes for the replication, polymerization, exonucleolytic digestion, endonucleolytic digestion, ligation, reverse transcription, transcription, base modification (including, e.g., methylation), or recombination (including homologous and site-specific recombination) of nucleic acid molecules; 2) conjoined nucleotide sequences that are not conjoined in nature; 3) engineered using molecular cloning techniques such that it lacks one or more nucleotides with respect to the naturally occurring nucleotide sequence; and 4) manipulated using molecular cloning techniques such that it has one or more sequence changes or rearrangements with respect to the naturally occurring nucleotide sequence.

Any of the components or sequences of the RNA replicon can be operably linked to any other of the components or sequences. The components or sequences of the RNA replicon can be operably linked for the expression of the gene of interest in a host cell or

treated organism and/or for the ability of the replicon to self-replicate. As used herein, the term "operably linked" is to be taken in its broadest reasonable context and refers to a linkage of polynucleotide elements in a functional relationship. A polynucleotide is "operably linked" when it is placed into a functional relationship with another polynucleotide. For instance, a promoter or UTR operably linked to a coding sequence is capable of effecting the transcription and expression of the coding sequence when the proper enzymes are present. The promoter need not be contiguous with the coding sequence, so long as it functions to direct the expression thereof. Thus, an operable linkage between an RNA sequence encoding a heterologous protein or peptide and a regulatory sequence (for example, a promoter or UTR) is a functional link that allows for expression of the polynucleotide of interest. Operably linked can also refer to sequences such as the sequences encoding the RdRp (e.g., nsP4), nsP1-4, the UTRs, promoters, and other sequences encoding in the RNA replicon, are linked so that they enable transcription and translation of the pre-fusion HMPV F protein and/or replication of the replicon. The UTRs can be operably linked by providing sequences and spacing necessary for recognition and translation by a ribosome of other encoded sequences.

The immunogenicity of a pre-fusion HMPV F protein or a fragment or variant thereof expressed by an RNA replicon can be determined by a number of assays known to persons of ordinary skill in view of the present disclosure.

Another general aspect of the application relates to a nucleic acid comprising a DNA sequence encoding an RNA replicon of the application. The nucleic acid can be, for example, a DNA plasmid or a fragment of a linearized DNA plasmid. Preferably, the nucleic acid further comprises a promoter, such as a T7 promoter, operably linked to the 5'-end of the DNA sequence. More preferably, the T7 promoter comprises the nucleotide sequence of SEQ ID NO: 148. The nucleic acid can be used for the production of an RNA replicon of the

application using a method known in the art in view of the present disclosure. For example, an RNA replicon can be obtained by in vivo or in vitro transcription of the nucleic acid.

Host cells comprising a RNA replicon or a nucleic acid encoding the RNA replicon of the application also form part of the invention. The HMPV F proteins or fragments or variants thereof may be produced through recombinant DNA technology involving expression of the molecules in host cells, e.g., Chinese hamster ovary (CHO) cells, tumor cell lines, BHK cells, human cell lines such as HEK293 cells, PER.C6 cells, or yeast, fungi, insect cells, and the like, or transgenic animals or plants. In certain embodiments, the cells are from a multicellular organism, in certain embodiments they are of vertebrate or invertebrate origin. In certain embodiments, the cells are mammalian cells, such as human cells, or insect cells. In general, the production of a recombinant proteins, such the HMPV F proteins or fragments or variants thereof of the invention, in a host cell comprises the introduction of a heterologous nucleic acid molecule encoding the protein in expressible format into the host cell, culturing the cells under conditions conducive to expression of the nucleic acid molecule and allowing expression of the protein or fragment or variant thereof in said cell. The nucleic acid molecule encoding a protein in expressible format may be in the form of an expression cassette, and usually requires sequences capable of bringing about expression of the nucleic acid, such as enhancer(s), promoter, polyadenylation signal, and the like. The person skilled in the art is aware that various promoters can be used to obtain expression of a gene in host cells. Promoters can be constitutive or regulated, and can be obtained from various sources, including viruses, prokaryotic, or eukaryotic sources, or artificially designed.

In certain embodiments of the invention, the vector is an adenovirus vector. An adenovirus according to the invention belongs to the family of the Adenoviridae, and preferably is one that belongs to the genus Mastadenovirus. It can be a human adenovirus, but also an adenovirus that infects other species, including but not limited to a bovine adenovirus

(e.g., bovine adenovirus 3, BAdV3), a canine adenovirus (e.g., CAdV2), a porcine adenovirus (e.g., PAdV3 or 5), or a simian adenovirus (which includes a monkey adenovirus and an ape adenovirus, such as a chimpanzee adenovirus or a gorilla adenovirus). Preferably, the adenovirus is a human adenovirus (HAdV, or AdHu), or a simian adenovirus such as chimpanzee or gorilla adenovirus (ChAd, AdCh, or SAdV), or a rhesus monkey adenovirus (RhAd). In the invention, a human adenovirus is meant if referred to as Ad without indication of species, e.g., the brief notation "Ad26" means the same as HAdV26, which is human adenovirus serotype 26. Also as used herein, the notation "rAd" means recombinant adenovirus, e.g., "rAd26" refers to recombinant human adenovirus 26.

Most advanced studies have been performed using human adenoviruses, and human adenoviruses are preferred according to certain aspects of the invention. In certain preferred embodiments, a recombinant adenovirus according to the invention is based upon a human adenovirus. In preferred embodiments, the recombinant adenovirus is based upon a human adenovirus serotype 5, 11, 26, 34, 35, 48, 49, 50, 52, etc. According to a particularly preferred embodiment of the invention, an adenovirus is a human adenovirus of serotype 26. Advantages of these serotypes include a low seroprevalence and/or low pre-existing neutralizing antibody titers in the human population, and experience with use in human subjects in clinical trials. In some embodiments, the adenovirus is replication deficient, e.g., because it contains a deletion in the E1 region of the genome. For adenoviruses being derived from non-group C adenovirus, such as Ad26 or Ad35, it is typical to exchange the E4-orf6 coding sequence of the adenovirus with the E4-orf6 of an adenovirus of human subgroup C such as Ad5. This allows propagation of such adenoviruses in well-known complementing (or packaging) cell lines that express the E1 genes of Ad5, such as for example 293 cells, PER.C6 cells, and the like (see, e.g., Havenga, et al., 2006, J Gen Virol

87: 2135-43; WO 03/104467). However, such adenoviruses will not be capable of replicating in non-complementing cells that do not express the E1 genes of Ad5.

Host cells comprising the nucleic acid molecules encoding the pre-fusion HMPV F proteins form also part of the invention. The pre-fusion HMPV F proteins may be produced through recombinant DNA technology involving expression of the molecules in host cells, e.g. Chinese hamster ovary (CHO) cells, tumor cell lines, BHK cells, human cell lines such as HEK293 cells, PER.C6 cells, or yeast, fungi, insect cells, and the like, or transgenic animals or plants. In certain embodiments, the cells are from a multicellular organism, in certain embodiments they are of vertebrate or invertebrate origin. In certain embodiments, the cells are mammalian cells. In certain embodiments, the cells are human cells. In general, the production of a recombinant proteins, such the pre-fusion HMPV F proteins of the invention, in a host cell comprises the introduction of a heterologous nucleic acid molecule encoding the protein in expressible format into the host cell, culturing the cells under conditions conducive to expression of the nucleic acid molecule and allowing expression of the protein in said cell. The nucleic acid molecule encoding a protein in expressible format may be in the form of an expression cassette, and usually requires sequences capable of bringing about expression of the nucleic acid, such as enhancer(s), promoter, polyadenylation signal, and the like. The person skilled in the art is aware that various promoters can be used to obtain expression of a gene in host cells. Promoters can be constitutive or regulated, and can be obtained from various sources, including viruses, prokaryotic, or eukaryotic sources, or artificially designed.

Cell culture media are available from various vendors, and a suitable medium can be routinely chosen for a host cell to express the protein of interest, here the pre-fusion HMPV F proteins. The suitable medium may or may not contain serum.

A "heterologous nucleic acid molecule" (also referred to herein as 'transgene') is a nucleic acid molecule that is not naturally present in the host cell. It is introduced into for instance a vector by standard molecular biology techniques. A transgene is generally operably linked to expression control sequences. This can for instance be done by placing the nucleic acid encoding the transgene(s) under the control of a promoter. Further regulatory sequences may be added. Many promoters can be used for expression of a transgene(s), and are known to the skilled person, e.g. these may comprise viral, mammalian, synthetic promoters, and the like. A non-limiting example of a suitable promoter for obtaining expression in eukaryotic cells is a CMV-promoter (US 5,385,839), e.g. the CMV immediate early promoter, for instance comprising nt. –735 to +95 from the CMV immediate early gene enhancer/promoter. A polyadenylation signal, for example the bovine growth hormone polyA signal (US 5,122,458), may be present behind the transgene(s). Alternatively, several widely used expression vectors are available in the art and from commercial sources, e.g. the pcDNA and pEF vector series of Invitrogen, pMSCV and pTK-Hyg from BD Sciences, pCMV-Script from Stratagene, etc, which can be used to recombinantly express the protein of interest, or to obtain suitable promoters and/or transcription terminator sequences, polyA sequences, and the like.

The cell culture can be any type of cell culture, including adherent cell culture, e.g. cells attached to the surface of a culture vessel or to microcarriers, as well as suspension culture. Most large-scale suspension cultures are operated as batch or fed-batch processes because they are the most straightforward to operate and scale up. Nowadays, continuous processes based on perfusion principles are becoming more common and are also suitable. Suitable culture media are also well known to the skilled person and can generally be obtained from commercial sources in large quantities, or custom-made according to standard protocols. Culturing can be done for instance in dishes, roller bottles or in bioreactors, using

batch, fed-batch, continuous systems and the like. Suitable conditions for culturing cells are known (see e.g. Tissue Culture, Academic Press, Kruse and Paterson, editors (1973), and R.I. Freshney, Culture of animal cells: A manual of basic technique, fourth edition (Wiley-Liss Inc., 2000, ISBN 0-471-34889-9)).

The invention further provides pharmaceutical compositions comprising a pre-fusion HMPV F protein, and/or fragment thereof, and/or a nucleic acid molecule, and/or a vector, as described herein. The invention thus provides compositions comprising a pre-fusion HMPV F protein, or fragment thereof, that displays an epitope that is present in a pre-fusion conformation of the HMPV F protein but is absent in the post-fusion conformation. The invention also provides compositions comprising a nucleic acid molecule and/or a vector, encoding such pre-fusion HMPV F protein or fragment. The invention in particular provides pharmaceutical compositions, e.g. vaccine compositions, comprising a pre-fusion HMPV F protein, a HMPV F protein fragment, and/or a nucleic acid molecule, and/or a vector, as described above and one or more pharmaceutically acceptable excipients.

The invention also provides the use of a stabilized pre-fusion HMPV F protein (fragment), a nucleic acid molecule, and/or a vector, according to the invention, for vaccinating a subject against HMPV.

The invention also provides the use of a stabilized pre-fusion HMPV F protein (fragment), a nucleic acid molecule, and/or a vector, according to the invention inducing an immune response against HMPV F protein in a subject. Further provided are methods for inducing an immune response against HMPV F protein in a subject, comprising administering to the subject a pre-fusion HMPV F protein (fragment), and/or a nucleic acid molecule, and/or a vector, according to the invention. Further provided is the use of the pre-fusion HMPV F protein (fragments), and/or nucleic acid molecules, and/or vectors according

to the invention for the manufacture of a medicament for use in inducing an immune response against HMPV F protein in a subject. The invention in particular provides pre-fusion HMPV F protein (fragments), and/or nucleic acid molecules, and/or vectors according to the invention for use as a vaccine.

The pre-fusion HMPV F protein (fragments), nucleic acid molecules, or vectors of the invention may be used for prevention (prophylaxis) and/or treatment of HMPV infections. In certain embodiments, the prevention and/or treatment may be targeted at patient groups that are susceptible HMPV infection. Such patient groups include, but are not limited to e.g., the elderly (e.g. $\geq$ 50 years old, $\geq$ 60 years old, and preferably $\geq$ 65 years old), the young (e.g. $\leq$ 5 years old, $\leq$ 1 year old), pregnant women (for maternal immunization), and hospitalized patients and patients who have been treated with an antiviral compound but have shown an inadequate antiviral response.

The pre-fusion HMPV F proteins, fragments, nucleic acid molecules and/or vectors according to the invention may be used in stand-alone treatment and/or prophylaxis of a disease or condition caused by HMPV, or in combination with other prophylactic and/or therapeutic treatments, such as (existing or future) vaccines, antiviral agents and/or monoclonal antibodies.

The invention further provides methods for preventing and/or treating HMPV infection in a subject utilizing the pre-fusion HMPV F proteins or fragments thereof, nucleic acid molecules and/or vectors according to the invention. In a specific embodiment, a method for preventing and/or treating HMPV infection in a subject comprises administering to a subject in need thereof an effective amount of a pre-fusion HMPV F protein (fragment), nucleic acid molecule and/or a vector, as described above. A therapeutically effective amount refers to an amount of a protein, nucleic acid molecule or vector, that is effective for

preventing, ameliorating and/or treating a disease or condition resulting from infection by HMPV. Prevention encompasses inhibiting or reducing the spread of HMPV or inhibiting or reducing the onset, development or progression of one or more of the symptoms associated with infection by HMPV. Amelioration as used in herein may refer to the reduction of visible or perceptible disease symptoms, viremia, or any other measurable manifestation of HMPV infection.

For administering to subjects, such as humans, the invention may employ pharmaceutical compositions comprising a pre-fusion HMPV F protein (fragment), a nucleic acid molecule and/or a vector as described herein, and a pharmaceutically acceptable carrier or excipient. In the present context, the term "pharmaceutically acceptable" means that the carrier or excipient, at the dosages and concentrations employed, will not cause any unwanted or harmful effects in the subjects to which they are administered. Such pharmaceutically acceptable carriers and excipients are well known in the art (see Remington's Pharmaceutical Sciences, 18th edition, A. R. Gennaro, Ed., Mack Publishing Company [1990]; Pharmaceutical Formulation Development of Peptides and Proteins, S. Frokjaer and L. Hovgaard, Eds., Taylor & Francis [2000]; and Handbook of Pharmaceutical Excipients, 3rd edition, A. Kibbe, Ed., Pharmaceutical Press [2000]). The HMPV F proteins, or nucleic acid molecules, preferably are formulated and administered as a sterile solution although it may also be possible to utilize lyophilized preparations. Sterile solutions are prepared by sterile filtration or by other methods known per se in the art. The solutions are then lyophilized or filled into pharmaceutical dosage containers. The pH of the solution generally is in the range of pH 3.0 to 9.5, e.g. pH 5.0 to 7.5. The HMPV F proteins typically are in a solution having a suitable pharmaceutically acceptable buffer, and the composition may also contain a salt. Optionally stabilizing agent may be present, such as albumin. In certain embodiments,

detergent is added. In certain embodiments, the HMPV F proteins may be formulated into an injectable preparation.

In certain embodiments, a composition according to the invention further comprises one or more adjuvants. Adjuvants are known in the art to further increase the immune response to an applied antigenic determinant. The terms “adjuvant” and "immune stimulant" are used interchangeably herein and are defined as one or more substances that cause stimulation of the immune system. In this context, an adjuvant is used to enhance an immune response to the HMPV F proteins of the invention. Examples of suitable adjuvants include aluminium salts such as aluminium hydroxide and/or aluminium phosphate; oil-emulsion compositions (or oil-in-water compositions), including squalene-water emulsions, such as MF59 (see e.g. WO 90/14837); saponin formulations, such as for example QS21 and Immunostimulating Complexes (ISCOMS) (see e.g. US 5,057,540; WO 90/03184, WO 96/11711, WO 2004/004762, WO 2005/002620); bacterial or microbial derivatives, examples of which are monophosphoryl lipid A (MPL), 3-O-deacylated MPL (3dMPL), CpG-motif containing oligonucleotides, ADP-ribosylating bacterial toxins or mutants thereof, such as E. coli heat labile enterotoxin LT, cholera toxin CT, and the like; eukaryotic proteins (e.g. antibodies or fragments thereof (e.g. directed against the antigen itself or CD1a, CD3, CD7, CD80) and ligands to receptors (e.g. CD40L, GMCSF, GCSF, etc), which stimulate immune response upon interaction with recipient cells. In certain embodiments the compositions of the invention comprise aluminium as an adjuvant, e.g. in the form of aluminium hydroxide, aluminium phosphate, aluminium potassium phosphate, or combinations thereof, in concentrations of 0.05 – 5 mg, e.g. from 0.075-1.0 mg, of aluminium content per dose.

In other embodiments, the compositions do not comprise adjuvants.

In certain embodiments, the invention provides methods for making a vaccine against respiratory syncytial virus (HMPV), comprising providing an HMPV F protein (fragment), nucleic acid or vector according to the invention and formulating it into a pharmaceutically acceptable composition. The term "vaccine" refers to an agent or composition containing an active component effective to induce a certain degree of immunity in a subject against a certain pathogen or disease, which will result in at least a decrease (up to complete absence) of the severity, duration or other manifestation of symptoms associated with infection by the pathogen or the disease. In the present invention, the vaccine comprises an effective amount of a pre-fusion HMPV F protein (fragment) and/or a nucleic acid molecule encoding a pre-fusion HMPV F protein, and/or a vector comprising said nucleic acid molecule, which results in an effective immune response against HMPV. This provides a method of preventing serious lower respiratory tract disease leading to hospitalization and the decrease in frequency of complications such as pneumonia and bronchiolitis due to HMPV infection and replication in a subject. The term “vaccine” according to the invention implies that it is a pharmaceutical composition, and thus typically includes a pharmaceutically acceptable diluent, carrier or excipient. It may or may not comprise further active ingredients. In certain embodiments it may be a combination vaccine that further comprises other components that induce an immune response, e.g. against other proteins of HMPV and/or against other infectious agents, e.g. against RSV, HMPV and/or influenza. The administration of further active components may for instance be done by separate administration or by administering combination products of the vaccines of the invention and the further active components.

Administration of the compositions according to the invention can be performed using standard routes of administration. Non-limiting embodiments include intramuscular injection.

A subject as used herein preferably is a mammal, for instance a rodent, e.g. a mouse, a cotton rat, or a non-human-primate, or a human. Preferably, the subject is a human subject.

In addition, the proteins of the invention may be used as diagnostic tool, for example to test the immune status of an individual by establishing whether there are antibodies in the serum of such individual capable of binding to the protein of the invention. The invention thus also relates to an in vitro diagnostic method for detecting the presence of an HMPV infection in a patient said method comprising the steps of a) contacting a biological sample obtained from said patient with a protein according to the invention; and b) detecting the presence of antibody-protein complexes.

The invention is further illustrated in the following examples. The examples do not limit the invention in any way. They merely serve to clarify the invention.

Examples

EXAMPLE 1: *Introducing a non-native F2-F1 cleavage site*

A schematic structure of full length and soluble HMPV F (HMPV F ectodomain) is shown in Figure 1 a and b, respectively (for numbering see SEQ ID NO: 1). The F1 domain in the soluble variant is C-terminally truncated and (optionally) has a foldon trimerization domain (GYIPEAPRDGQAYVRKDGEWVLLSTFL; SEQ ID NO: 2) fused to the F ectodomain analogous to many other soluble trimeric viral fusion proteins.

Based on the F protein of the wild type HMPV strain TN/00/3-14 (SEQ ID NO: 1), DNA fragments coding for hMPV proteins were synthesized (Genscript, Piscataway, NJ) and cloned in the pcDNA2004 expression vector (modified pcDNA3 plasmid with an enhanced CMV promotor).

HEK293 cells were transfected with plasmids corresponding to the HMPV F ectodomain of strain TN/00/3-14 with C-terminal foldon domain and 4 stabilizing mutations

and a non-native disulfide bridge (T69Y, A116H, A140C, A147C, D185P and E453Q), as described in the co-pending application EP21215259 and the natural variant H368N (as found in TN/85/6-3; Genbank ID ACJ53577.1) with the F2 C-termini as defined in Figure 2A. Changing the native TMPRSS2 cleavage site (*RQSR*) to a non-native polybasic furin site (*RRRR*) and a truncation of the F2 C-terminus (after position 90) were applied to increase cleavage efficiency of the HMPV F0 protein. In addition, in some of the proteins a second non-native polybasic furin site (RRRR) cleavage site was introduced in the F2 domain, positioned N-terminal from the first cleavage site and separated by a spacer sequence of one or more amino acid residues from the F2 domain.

The expression platform used was the Expi293F expression system (Thermo Fisher Scientific, Waltham, USA) in 96-well format. HMPV F encoding plasmids were co-transfected with plasmids encoding furin in a 5:1 HMPV F - furin DNA ratio. The constructs were transfected in duplicate or quadruplicate.

After 3 days, supernatants were harvested and analyzed by SDS-PAGE 4–12% (w/v) Bis–Tris NuPAGE gels, 1 x MOPS (Life Technologies) under reducing conditions followed by Western blot to detect the efficacy of cleavage (Figure 2B). Western Blot analysis was performed as follows: Semi-dry blotting performed according to manufacturers' recommendations with iBlot2: program P0. Blocking for 1hr in Odyssey Blocking Buffer, 1st antibody (anti-hMPV-F A2/B2 polyclonal sera, as generated after peptide immunization of rabbits at Genscript, Piscataway, NJ) 1:10.000 in Odyssey blocking buffer incubation for 1 hour 2nd antibody (α-Rabbit IRDYE CW800 (Rockland Immunochemicals, Inc., Limerick, PA, US) 1:5000 in Odyssey blocking buffer) incubation for 1 hr. All incubations were performed on a roller platform at room temperature. After first and second antibody, the blots were washed 3x using 50 ml TBS/0.05% Tween20 for each wash, for 5 min, followed by a

final wash using 50 ml of 1x PBS. The blots were visualized by scanning on an Odyssey scanner, using both the 700CW and 800CW channel.

The harvested crude cell supernatants were also analyzed for trimer content and trimer stability after heat stress on analytical Size Exclusion Chromatography (SEC) in an Ultra High-Performance Liquid Chromatography (UHPLC) system using a Vanquish system (ThermoFisher Scientific, Waltham, USA) with a Sepax Unix-C SEC-300 4.6X150mm 1.8 µm column (Sepax (231300-4615), injection volume 20µL, flow 0.35mL/min). The heat stability is reported as percent trimer after heat stress relative to the 4°C control. The constructs transfected in duplo or quadruple were pooled for SEC analysis. The elution was monitored by a UV detector. The SEC profiles were analyzed by the Chromeleon software version (version 7.2.7, Thermo Fisher Scientific. Plots were plotted in GraphPad Prism (version 9.0.0, GraphPad Software). (**Figure 2C**).

The antigenicity of the HMPV F proteins in crude cell supernatant was also assessed using biolayer interferometry on the Octet (ForteBio, Portsmouth, UK) and using the monoclonal antibodies ADI-14448 (Gilman et al., Sci Immunol. 2016 Dec 16;1(6):eaaj1879. doi: 10.1126/sciimmunol.aaj1879. Epub 2016 Dec 9.) and DS7 (Wen et al., Nat Struct Mol Biol . 2012 Mar 4;19(4):461-3. doi: 10.1038/nsmb.2250.)) (**Figure 2D**). ADI-14448 has been described as cross neutralizing antibody to RSV and HMPV, it is pre-fusion specific and binds to the antigenic site III of the RSV preF. ADI-14448 binding thus is indicative of a pre-fusion conformation. An X-ray structure of MAb DS7 has been described in complex with HMPV fusion protein (Wen et al., Nat Struct Mol Biol. 2012 Mar 4;19(4):461-3. doi: 10.1038/nsmb.2250.). The complex of HMPV F + DS7 shows that the heptad repeat 2 (HR2), which is part of the refolding region 2 (RR2), is in a non-preF conformation since beta-sheet 22 is moved away which is more reminiscent of a post-fusion conformation than a prefusion

conformation. DS7 binding thus is indicative of a non-pre-fusion or post-fusion conformation.

For a preferred pre-fusion HPMV F protein according to the invention, DS7 binding thus is undesirable. For the Octet assay the antibodies were immobilized on anti-human Fc biosensors (ForteBio, Portsmouth, UK). After equilibration of the sensors in kinetic buffer (ForteBio, Portsmouth, UK) for 600s the sensors were transferred to kinetic buffer with 5 ug/ml of the desired antibody. Subsequently, another equilibration step was included in mock cell medium. Lastly the sensors were transferred to a solution of cell culture supernatant that contains the pre-fusion HMPV F proteins. The initial slope (also referred to as the association phase; curve fitting was performed on the initial 300 seconds) and binding at 300 seconds in nm are reported. The data analysis was done using the ForteBio Data Analysis 8.2 software (ForteBio, Portsmouth, UK). Bar plots were plotted in GraphPad Prism (version 9.0.0, GraphPad Software).

All proteins showed expression (Figure 2B) and were trimeric (Figure 2C) according to analytical SEC. The processing of all proteins is incomplete as detected by western blot (Figure 2B).

With the introduction of the stabilizing mutations and the change of the native TMPRSS2 like cleavage site into a non-native furin cleavage site by substituting QS to RR, the expression levels (Figure 2C, left histogram panel) and heat stability (Figure 2C, right histogram panel) of MPV210531 were increased compared to MPV201285 without stabilizing mutations. Expression levels for proteins with a shorter (truncated) F2-C terminus (MPV210502, MPV210500, MPV210507 and MPV210509) were reduced compared to MPV210531. Heat stability at 60°C of MPV210500, MPV210507 and MPV210509 with a short F2 C-terminus were increased compared to MPV210531 with a long F2 C-terminus.

In octet, proteins showed both anti pre-F (binding to ADI-14448) and anti non-pre-F binding (binding to DS7) indicating that the proteins were not fully in the prefusion trimeric conformation (Figure 2D). The anti non-pre-F binding was reduced for designs with a truncated F2 C-terminus and two non-native cleavage sites (MPV210500, MPV210507 and MPV210509).

Stabilized HMPV designs with truncated F2 C-terminus and with an additional furin cleavage site showed improved cleavage of F0 (Fig 2B), improved heat stability (Fig 2C) and lower binding to the postF-specific MAb DS7 (Fig. 2D).

EXAMPLE 2: *Optimized processing of F0*

Since improved processing and truncation of F2 C-terminus increased stability and quality (e.g. improved cleavage of F0, improved heat stability, lower binding to post-F specific antibody DS7) of HMPV prefusion F protein, the F2 C-terminus was systematically truncated from position 102 to 88. In addition, a p27 peptide (comprising a first non-native cleavage site) and a second non-native cleavage site were introduced between the (truncated) F2 domain and the F1 domain.

It was shown that using the p27 region of RSV F, i.e. the amino acid sequence ELPRFMNYTLNNAKKTNVTLSKKRKRR (SEQ ID NO: 2; cleavage site underlined) improved the processing of F0. The p27 peptide of RSV F is known to be cleaved very efficiently by furin-like proteases (Krarup et. al. Nat Commun. 2015 Sep 3;6:8143. doi: 10.1038/ncomms9143.; Gonzales-Reyes et al. 2001, Proc Natl Acad Sci USA 98:9859–9864).

According to the invention, both the furin site RRRR and RSV F p27 domain were introduced between the (truncated) F2 domain and the F1 domain according to the designs

listed in Figure 3A. HEK293 cells were transfected with plasmids corresponding to the HMPV F ectodomain as described in Example 1 and with the F2 truncations according to Figure 3A. Addition of the RSV p27 domain C-terminally to the introduced furin site showed a very efficient increase in cleavage of HMPV F according Western blot after reduced SDS-PAGE (Figure 3B). According to analytical SEC, heat stability at 60°C of MPV210498 and MPV210751 was highest as measured by the remaining trimer content after heat stress (Figure 3C right) and also the antigenic quality (i.e. pre-fusion conformation) for these two variants was highest since they showed the lowest binding with the postF-specific Mab DS7 in Octet (Figure 3D).

Conclusion: The introduction of a second furin cleavage site and the RSV-p27 domain (comprising a first non-native cleavage site) between F1 and F2 resulted in successful complete processing. In addition, truncation of the F2 C-terminus after residue 89 or 90 (in addition to the second furin cleavage site of 4 Arginines (R) and the RSV-p27 domain resulted in the most stable HMPV preF protein with the highest pre-fusion quality, as indicated by the low DS7 binding.

EXAMPLE 3: *Stabilizing the HR2 region*

It is known to trimerize the soluble HMPV prefusion F ectodomain with a heterologous trimerization domain, such as a foldon. However, adding a foldon introduces an additional heterologous protein domain with no additional benefit for a vaccine immunogen except for F trimerization. Since immunogens in other vaccines or other vaccine components may also use foldon for trimerization, a preferred vaccine component is solely based on the viral protein and does not contain any additional heterologous non-viral protein domains. The present invention provides trimeric conformation of soluble HMPV proteins, without a

foldon, by optimization of the interactions in the HR2 region of the pre-fusion stem of the F protein. Stabilization of the preF trimer by stabilization of the HR2 stem region was evaluated in a variant which had the optimized F2 truncation (after amino acid residue 89), an introduced furin cleavage site at the truncated F2 terminus and an RSV F p27 domain for optimal cleavage. Further, the F1 C-terminus was truncated after position 481 or 489. The proteins also had a linker and a C-tag for purification purposes. When the foldon domain was deleted (MPV211241), hardly any trimer was detected in analytical SEC (Figure 4A left histogram panel, 5A left histogram panel). Several different stabilizing strategies in HR2 (according to the mutations in Figure 4 and 5A), however, restored and even improved trimer expression without foldon. The trimers without foldon remained relatively stable after storage for 6 to 8 weeks at 4 ° C (Figure 4 left histogram panel) and heating at 58°C for 30 minutes (Figure 4 right histogram panel and Fig 5A right histogram panel).

To measure the antigenic quality, supernatants were tested using Octet with the anti-preF (ADI-14448), anti-postF (DS7) and with a MAb directed to the interface of the pre-fusion F apex that can only be recognized if the trimer is open or able to 'breath' (transiently open or partially open) (MPV458; Huang et al, PLoS Pathog. 2020 Oct 9;16(10):e1008942. doi: 10.1371/journal.ppat.1008942. eCollection 2020 Oct.). HMPV F variants with stabilized HR2 showed anti pre-F, anti-apex interface (MPV458) and little anti non-pre-F binding (binding to DS7) (**Figure 5B**).

Conclusion: The HR2 stabilizing mutations increase trimerization without foldon and even increase trimer expression.

EXAMPLE 4: *Substitutions that stabilize the HMPV prefusion conformation*

In this Example, alternative stabilizing mutations were evaluated in the backbone corresponding to the designs of MPV211287 and MPV211918 but without the stabilizing

substitutions T69Y, A116H, disulfide A140C+A147C and D185P (mutations as described in co-pending application EP21215259). The backbone has an F2 truncation after amino acid position 89, an introduced furin cleavage site, and p27 of RSV, also comprising a furin cleavage site. The protein further comprised H368N, the stabilizing mutation E453Q, and the HR2 stabilizing substitutions (L473W, D475R, Q476K, S477F, N478D, R479E, A484I) or HR2 stabilizing substitutions (L473W, S477I, A484I), a linker and C-tag. One stabilizing substitution (V231I) was obtained from strain B2/3817/04, (Genbank ID AGL74059.1). One novel stabilizing substitution (E453P) replaced the stabilizing substitution E453Q (described earlier in co-pending application EP21215259). Two other novel stabilizing substitutions were V112R and D209E. When the former stabilizing substitutions at position 69, 116, 140, 147 and 185 (described in co-pending application EP21215259) were mutated back to wildtype (MPV211940 in Figure 6A and MPV211942 (Figure 7A), preF trimer expression was lost. All single stabilizing substitutions except for Q453P increased trimer expression, especially V112R. Combinations of substitutions further increased expression and improved heat stability (Fig 6A, 7 A), especially addition of the D209E substitution. Octet analysis showed that the variants with the novel stabilizing substitutions had improved antigenic quality with relatively lower binding to DS7 compared to the reference MPV211940 and MPV211942 (Figures 6B, 7B). According to the present invention, improved antigenic quality refers to pre-fusion quality, as measured by increased binding of a pre-F specific antibody (e.g. ADI-14448) and decreased binding of post-F binding antibody (e.g. DS7) and of an anti-interface antibodies (e.g. MPV458). These last two antibodies have very low neutralizing activity.

Since some of the binding to DS7 or MPV458 may also be caused by impurities in the cell culture supernatant, several HMPV F proteins were produced at higher scale and purified for thorough further analysis (see Example 8).

EXAMPLE 5: *Additional substitutions that stabilize the HMPV prefusion conformation*

Additional stabilizing mutations T69W, S149Y, N313W, and S445Y, and the natural variant N404P, were evaluated by introduction in the MPV212033 backbone that only contained stabilizing mutation V112R, F2 truncation after amino acid position 89, furin site + p27 of RSV, wild type variant H368N, stabilizing mutation E453Q, HR2 stabilization (L473W, D475R, Q476K, S477F, N478D, R479E, A484I), and a linker and C-tag. Combinations of substitutions further increased expression and improved heat stability (Fig 8 A and C) (see Example 1 for methods). Differential scanning fluorimetry (DSF) of the cell culture supernatants showed that all additional substitutions except for N404P increased the melting temperature (Tm50) of HMPV F as measured by DSF (see Example 4 for method details). Octet analysis showed that the variants with additional substitutions S149Y, T69W, S445Y and N313W had improved antigenic quality with relatively lower binding to DS7 compared with the reference MPV212033 (Figure 8B).

Since some of the binding to DS7 may also be caused by impurities in the cell culture supernatant, several HMPV F proteins were produced at higher scale and purified for further analysis (see Example 8).

EXAMPLE 6: *HR2 variations in a backbone with V112R, D209E, V231I and E453P*

Further HR2 variants were evaluated in a backbone that had an F2 truncation after amino acid position 89, an introduced furin cleavage site, p27 of RSV, an F1 truncation after amino acid position 489 and no foldon. The proteins further comprised the H368N, V112R, D209E, V231I and E453P mutations.

All tested variants showed increased trimer expression compared to MPV210571 (Fig 9 A) (see Example 1 for methods). Compared to MPV212047 with HR2 stabilizing mutations

L473W, D475R, Q476K, S477F, N478D, R479E, A484I (see Example 3) trimer content of MPV212032 (L473W, S477I, A484I), MPV220115 (S477I), MPV220120 (L473Y, S477I, A484I) and MPV220121 (L473I, S477I, A484I) were in a similar range. Binding profiles in octet were comparable to MPV210751 and MPV212047 (Figure 9B). Differential scanning fluorimetry (DSF) of the cell culture supernatants showed that all HR2 designs (except MPV220123, where no Tm50 could be defined due to a low signal) had improved heat stability compared to MPV210571, described as melting temperature in DSF (see Example 4 for method details) in supernatant.

Conclusion:

HR2 stabilizing mutations in MPV212032 (L473W, S477I, A484I), MPV220115 (S477I), MPV220120 (L473Y, S477I, A484I) and MPV220121 (L473I, S477I, A484I) improved the trimer content and heat stability compared to MPV210751. Since some of the binding to DS7 may also be caused by impurities in the cell culture supernatant, several HMPV F proteins were produced at higher scale and purified for further analysis (see Example 8.

EXAMPLE 7: *Further stabilization of HR2 variant with S477I and A484I*

MPV220116 with HR2 stabilizing mutations S477I, A484I, and a F2 truncation after amino acid position 89, an introduced furin cleavage site, p27 of RSV, no foldon, as well as the mutations H368N, V112R, D209E, V231I and E453P (+ linker and C-tag) was selected to test additional stabilizing mutations T69W, S149Y, N313W, and S445Y, and the natural variant N404P.

Combinations of substitutions further increased expression (Fig 10A) (see Example 1 for methods). Octet analysis showed that the variants with additional substitutions S149Y,

T69W, S445Y and N313W had improved antigenic quality with relatively lower binding to DS7 compared to the reference MPV220116 (Figure 10B). Differential scanning fluorimetry (DSF) of the cell culture supernatants showed that all additional substitutions improved the melting temperature (Tm50) of HMPV F Heat stability, described as melting temperature in DSF (see Example 4 for method details) in supernatant (Figure 10C).

Conclusion:

The additional substitutions S149Y, T69W, S445Y and N313W improved the trimer content, antigenic quality and heat stability. Since some of the binding to DS7 may also be caused by impurities in the cell culture supernatant, several HMPV F proteins were produced at higher scale and purified for further analysis (see Example 8).

EXAMPLE 8: *Production and purification of selected proteins*

Production and purification

Several HMPV proteins according to Table 1 were produced and purified. Most selected HMPV F proteins were described in previous Examples: MPV210530 (Fig. 3), MPV210751 (Fig. 4), MPV211918 (Fig. 4), MPV212017, MPV212047 (Fig. 6), MPV212043 (Fig. 6), MPV212044 (Fig. 6), MPV212045 (Fig. 6), MPV212046 (Fig. 6), MPV220087 and MPV220092.

On 300ml-scale the cells were transiently transfected using ExpiFectamine 293 (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's instructions and cultured in a shaking incubator for 5 days at 37°C and 10% CO2. The culture supernatants were harvested, centrifuged for 10 min at 600rpm and filtered over a 0.22µm PVDF filter to remove cells and cellular debris.

The proteins were purified by means of a two- or three -step protocol. First, the harvested and clarified culture supernatant was loaded on a pre-packed C-tagXL 5-ml column (Thermo Fisher Scientific, cat# 494307205, Waltham, USA). This column was pre-packed with an affinity resin (Capture Select) that consists of a C-tag specific single domain antibody, immobilized on an Agarose based bead. This resin is highly specific for binding proteins with the C-tag. Elution of the C-tagged proteins was performed using a TRIS buffer containing 2M MgCl2. Based on the UV signal (A280) the eluted fractions were pooled and concentrated using Amicon Ultracel 50kDa MWCO centrifugal filter devices (Merck Millipore, cat# UFC805024, Darmstadt, Germany. The proteins were purified by means of a two- or three -step protocol. First, the harvested and clarified culture supernatant was loaded on a pre-packed CaptureSelect C-tagXL 5 ml column (Thermo Fisher Scientific, cat# 494307205, Waltham, USA). This column was pre-packed with an affinity resin (Capture Select) that consists of a C-tag specific single domain antibody, immobilized on an Agarose based bead. This resin is highly specific for binding proteins with the C-tag. Elution of the C-tagged proteins was performed using a TRIS buffer containing 2M MgCl2. Based on the UV signal (A280) the eluted fractions were pooled and concentrated using Amicon Ultracel 50kDa MWCO centrifugal filter devices (Merck Millipore, cat# UFC805024, Darmstadt, Germany). Subsequently, the concentrated collected elution peak was applied to a Superdex200 Increase 10/300 column (Cytiva, cat# 28-9909-44, Marlborough, Massachusetts, United States) equilibrated in running buffer (20mM Tris, 150mM NaCl, pH7.4) for polishing purpose, i.e. remove the minimal amount of multimeric and monomeric protein. Three step protocol included an additional run before Superdex200, on a Superose 6 Increase 10/300 column (Cytiva, cat#29-0915-96, Marlborough, Massachusetts, United States) on the C-tag pool (applied for MPV210751, MPV211918, MPV212017 and MPV212047 only). Quality after purification of the designs indicated that this step is not

needed due to lack of larger aggregates and therefore it was not applied for the other purifications.

In addition, a postF hMPV protein (SEQ ID NO: 3) as described by Mas et. al., PLoS Pathog. 2016 Sep 9;12(9):e1005859. doi: 10.1371/journal.ppat.1005859. eCollection 2016 Sep.) was produced and purified. Expression plasmid encoding the recombinant post-fusion hMPV F protein was prepared as described in in Example 2. On 300 ml-scale the cells were transiently transfected and subsequently purified by means of a two-step protocol (see details above). Subsequently, TEV cleavage was performed to remove the foldon and c-tag. For 15 µg of protein 1 µL of TEV (10 000 Units/mL) was used. The protein-TEV mixture was incubated overnight at 4°C. The TEV-His protease was removed from the protein sample by a Ni Sepharose excel beads (GE Healthcare, 17-3712-03) pull down. Ni Sepharose excel beads were added to the protein-TEV mixture and incubated for 2 hours at room temperature. Flow through was collected via a micro bio-spin column (Bio Rad, 7326204). The cleaved protein sample was heat-shocked for 30 minutes at 45°C (Hsieh et al, Nat Commun 2022 Mar 14;13(1):1299. doi: 10.1038/s41467-022-28931-3.). Subsequently, the protein sample was applied to a Superose 6 Increase 10/300 column (GE Healthcare, Chicago, USA) equilibrated in running buffer (20mM Tris, 150mM NaCl, pH7.4) for polishing purpose, i.e. remove the minimal amount of multimeric and monomeric protein.

Proteins were subsequently analyzed on Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) (Figure 11A) under reducing conditions. Proteins were visualized on the gel upon staining with Instant Blue.

Results and conclusion

Yields of the different variants are shown in Table 1. Truncation of the F2 C-terminus reduced the yield, HR2 stabilization and deletion of the foldon increased the yield.

Improvement in expression was obtained by introducing V112R, D209E, V231I and E453P (MPV212047). Further S149Y, N404P (MPV220087) and subsequently S445P, N313W (MPV220092) were introduced, resulting in increased expression for MPV220092. Main band on the reduced SDS-PAGE are corresponding to F1 domain with no residual F0 (**Figure 11A**) indicating fully processed proteins.

Table 1: Purified proteins of Example 8

		Amino acid position																								Relative trimer content (%)						Tm50 (°C)
		69	116	112	140	147	149	185	209	231	313	368	404	445	453	473	475	476	477	478	479	484										
		Wild type amino acid																														
ID	*F2 border	T	A	V	A	A	S	D	D	V	N	H	N	S	E	L	D	Q	S	N	R	A	foldon	Yield mg/L	MW (kDa)	Trimer (%)	4°C	37°C		Snap freezing			
																											7w	2w	8w	1x	5x	10x	
MPV210530	98	Y	H		C	C		P				N			Q								+	46	150	100	100**	56	NA	90	65	35	60.4
MPV210751	89	Y	H		C	C		P				N			Q								+	14	153	100	96	100	96	98	87	64	65.1
MPV211918	89	Y	H		C	C		P				N			Q	W	R	K	F	D	E	I	-	20	151	100	101	103	89	100	93	75	61.7
MPV212017	89	Y	H	R	C	C		P	E	I		N			P	W	R	K	F	D	E	I	-	63	148	100	101	102	96	103	98	96	71.7
MPV212047	89			R					E	I		N			P	W	R	K	F	D	E	I	-	43	147	100	101	101	95	103	91	86	71.8
MPV212043	89			R					E	I		N			Q	W	R	K	F	D	E	I	-	30	150	100	90	104	NA	100	85	73	70.4
MPV212044	89			R						I		N			P	W	R	K	F	D	E	I	-	24	150	100	~107#	114	NA	99	71	29	66.8
MPV212045	89			R					E			N			P	W	R	K	F	D	E	I	-	27	150	100	99	102	NA	98	80	65	71.7
MPV212046	89								E	I		N			P	W	R	K	F	D	E	I	-	14	148	100	106	101	NA	97	79	60	71.5
MPV220087	89			R			Y		E	I		N	P		P	W	R	K	F	D	E	I	-	41	145	100	98	103	NA	99	88	71	73.7
MPV220092	89			R			Y		E	I	W	N	P	Y	P	W	R	K	F	D	E	I	-	54	147	100	89	102	NA	101	90	78	78.3

* Residue at F2 C-terminus located N-terminal to furin site; NA=not available; **after 3 weeks, # contains also smaller molecular weight species

Trimer content

The purified proteins were assessed by analytical Size Exclusion Chromatography (SEC) after purification and after storage at 4 °C to study trimer content after purification (Figure 11B). SEC was performed with an Ultra High-Performance Liquid Chromatography (UHPLC) using a Vanquish system (ThermoFisher Scientific) with a Sepax Unix-C SEC-300

4.6X150mm 1.8 µm column (Sepax (231300-4615), injection volume 20µL, flow 0.3mL/min.). The elution was monitored by a UV detector (Thermo Fisher Scientific), a µDawn Light Scatter (LS) detector (Wyatt Technologies), a µT-rEx Refractive Index (RI) detector (Wyatt Technologies) and a Nanostar Dynamic Light Scattering (DLS) detector (Wyatt Technologies). The SEC profiles were analyzed by the Astra 7.3.2.19 software package (Wyatt Technology). Chromatograms were plotted in Graph Pad Prism (version 9.0.0, GraphPad Software).

Results and conclusion

On analytical SEC all purified proteins are highly trimeric after purification (Figure 11B) and have the expected molecular weight (Table 1) of a trimeric protein. After storage for 7 weeks the high trimer content is remained for all proteins. MPV212044 shows after 7 weeks at 37°C next to the trimer a peak, a peak for a species with a smaller molecular weight than the trimer.

In conclusion, all purified proteins are trimeric (Figure 11B and Table 1) and MPV212047 shows high trimer content after 7 weeks at 4 ° C.

Stability at 37 °C

Purified proteins were aliquoted, and aliquots were incubated at 37 ° C (control samples of all proteins and timepoints were kept at 4 ° C) and subsequently analyzed with SEC-MALS (for methods see section above). The relative trimer content for the samples stored at 37 °C compared to the control sample are listed in Table 1. Chromatograms were plotted in Graph Pad Prism (version 9.0.0, GraphPad Software).

Results and conclusion

Chromatograms of control samples and samples stored at 37 °C for 2 weeks are superposed for each purified protein (Figure 11C). MPV210530 shows, compared to the designs with truncated F2 after 89, decreased trimer stability after 2 weeks at 37 ° C indicated by the reduced trimer peak and an additional peak reflecting aggregates. All proteins with truncated F2 C-terminus after position 89 showed the same trimer content after 37 ° C storage compared to the control. All proteins were stable for at least 2 weeks at 37 ° C.

Freezing stability

Proteins were one, five or ten times snap frozen. After thawing the trimer content was compared to a non-frozen sample by SEC-MALS (for methods see section above). The relative trimer content for the stressed samples to the control sample are listed in Table 1. Chromatograms were plotted in Graph Pad Prism (version 9.0.0, GraphPad Software).

Results and conclusion

The proteins were tested in a buffer without cryo-protectant (buffer composition: 20mM Tris, 150mM NaCl, pH7.4) and after one snap freeze all proteins remained highly trimeric. After ten snap freeze-thaw cycles (Figure 11D) differentiation in freezing stability could be observed. Mutations that increase the freeze/thaw stability can be ranked in the following order D209E > V112R > V321I > Q453P. For MPV212044 some aggregates were detected. In conclusion, the HMPV F proteins of the invention show high freezing stability.

Thermostability of proteins

Thermo-stability of the purified pre-fusion HMPV F proteins was determined by Differential Scanning Fluorimetry (DSF) by monitoring the fluorescent emission of Sypro

Orange Dye (ThermoFisher Scientific) in a 96 well optical qPCR plate. 15µl of a 66.67µg/ml protein solution was used per well (buffer as described in Example 2) (Figure 11E). To each well, 5 µl of 20x Sypro orange solution was added. Upon gradual increase of the temperature, from 25°C to 95°C (0.015°C/s), the proteins unfold and the fluorescent dye binds to the exposed hydrophobic residues leading to a characteristic change in emission. The melting curves were measured using a ViiA7 real time PCR machine (Applied BioSystems). The 1st derivative of the fluorescent signal (a.u.) versus the temperature (°C) of three individual samples (technical triplicate), as well as the averaged melting curve, were plotted with Graphpad Prism software (Dan Diego, CA, US). From the averaged melting curve, the Tm50 was deducted (lowest point on the curve). The Tm50 values represent the temperature at which 50% of the protein is unfolded and thus are a measure for the temperature stability of the proteins.

Results and conclusion

The design with truncated F2 C-terminus at 89 (MPV210751) which further comprised a furin site, and RSV p27, and T69Y, A116H, A140C, A147C, D185P, H368N, E453Q, showed an increase in melting temperature of ~5°C, respectively, compared to the designs with long F2 C-terminus MPV210530 (Figure 11E). The removal of the foldon and introduction of the HR2 stabilizing mutations in MPV211918 reduced the melting temperature by about 3°C compared to MPV210751. Introduction of stabilizing substitutions V112R, D209E, V231I and 453P (MPV212017) increased the melting temperature by about 10°C compared to MPV211918. Disulfide bridge 140-147 and stabilizing substitutions T69Y, A116H and D185P had no additional stabilizing effect and removal did not reduce the melting temperature (compare MPV212047 with MPV212017).

Conclusion: HMPV F with truncated F2 C-terminus, in combination with stabilization of HR2 and addition of novel stabilizing mutations resulted in high yield of highly stable trimers in the absence of a foldon domain.

In vitro antigenicity

In vitro antigenic quality of selected purified proteins was tested using biolayer interferometry technology with octet (Figure 11F). In addition to the antibodies described in Example 2, also anti-apex interface binder MPV465 (Huang et al, PLoS Pathog. 2020 Oct 9;16(10):e1008942. doi: 10.1371/journal.ppat.1008942. eCollection 2020 Oct.) and antibody ADI-18992, recognizing the preF and postF conformation and described to bind to site IV, were used (Gilman et al Sci Immunol. 2016 Dec 16;1(6):eaaj1879. doi: 10.1126/sciimmunol.aaj1879. Epub 2016 Dec 9.). The antibodies were immobilized as described in Example 2. After equilibration of the sensors in kinetic buffer (ForteBio) for 600s the sensors were transferred to kinetic buffer with 5 ug/ml of the desired antibody. Subsequently another equilibration step was included in kinetic buffer. Lastly the sensors were transferred to a solution of the proteins (20 µg/mL in 1xKB). Analysis was performed as described in Example 2.

Results and conclusion

For the HMPV F variant in which the F2 C-terminus is not truncated (MPV210530), binding to non preF antibodies and anti-interface antibodies was higher compared to purified proteins with the F2 truncation. The stabilized prefusion F proteins with the novel stabilizing mutations MPV212017 and MPV212047 showed the most favorable binding and only bound the preF-specific antibody which confirms that the lower antigenic quality in supernatant for e.g. MPV212047 observed in Figure 5B was caused by impurities.

Structural characterization by nsTEM

The purified proteins were analyzed by negative stain Transmission Electron Microscopy (nsTEM) (Figure 11G).

Continuous carbon grids (copper, EMS) were glow discharged for 30 seconds in an easiglow plasma cleaner. Four microliters of the sample diluted (20mM Tris, 150mM NaCl, pH7.4) to concentrations ranging from 5 to 25 μg/ml were applied to glow-discharged grids and incubated for 1min. The sample solution was partially absorbed by gentle side blotting, and the grid was immediately stained with by depositing it on top of a 40 μl drop of a 2% (w/v) uranyl acetate solution for a total of 1 min. After staining, the grid was blotted dry and stored at room temperature prior to imaging. The prepared grids were imaged in a Talos L120C TEM (Thermo Fisher Scientific) equipped with a Ceta camera. Resulting pixel ranged from 2.4 to 2.8 ang per pixel depending on imaging conditions. The parameters of the Contrast Transfer Function (CTF) were estimated on each micrograph using CTFFIND4 and the rest of the processing (picking and 2D classification) was done in RELION (version 3 or 4).

Result and conclusion

Two-dimensional (2D) class averages obtained by an electron microscopy (EM) analysis of the negative-stained samples, using an acidic stain, of the purified MPV212047 revealed regular homogenous closed trimers (Figure 11G)

Structural characterization of MPV212047 by Cryo Electron Microscopy

The Cryo Electron Microscopy (Cryo-EM) structure of HMPV F design MPV212047 was solved to confirm the correct, trimeric prefusion conformation this protein (Figure 12). To this end, 3.5 μL of 0.8–1.0 mg/ml purified preF protein (MPV212047) complex was applied to the plasma-cleaned (Gatan Solarus) Quantifoil 1.2/1.3 holey gold grid, and subsequently

vitrified using a Vitrobot Mark IV (FEI Company). Cryo grids were loaded into a Glacios transmission electron microscope (ThermoFisher Scientific) operating in nanoprobe at 200 keV with a Falcon IV direct electron detector. Images were recorded with EPU in counting mode with a pixel size of 0.948 Å and a nominal defocus range of -1.8 to -1.2 μm. Images were recorded with a 5.7 s exposure in EER format corresponding to a total dose of ~ 40 electrons per $Å^2$. The movies were subjected to beam-induced motion correction, contrast transfer function (CTF) parameters estimation, automated reference particle picking, particle extraction with a box size of 280 pixels, and two-dimensional (2D) classification in CryoSPARC live during the data acquisition. Particle images with clear HA features were merged and subjected to Ab initio 3D reconstruction and followed by 3D heterogeneous refinement with C3 symmetry in CryoSPARC. The particles were then refined using Non-uniform (UN) refinement within CryoSPARC with C3 symmetry. Prior to visualization, all density maps were sharpened by applying different negative temperature factors using automated procedures, along with the half maps, were used for model building. Local resolution was determined using ResMap. The initial template of the HMPV F trimer was derived from SWISS-MODEL. The model was docked into the EM density map using Chimera and followed by manually adjustment using COOT. Model geometry was further improved using Rosetta. The geometry parameters of the final models were validated in COOT and using MolProbity and EMRinger. These refinements were performed iteratively until no further improvements were observed. Model overfitting was evaluated through its refinement against one cryo-EM half map. FSC curves were calculated between the resulting model and the working half map as well as between the resulting model and the free half and full maps for cross-validation. Figures were produced using PyMOL (Figure 12). One protomer is depicted as cartoon representation and two protomers as surface representation.

In corroboration with earlier SEC-MALS, BLI, and nsTEM data (Figure 11), Cryo-EM analysis of the MPV212047 design confirmed the presence of a prefusion trimer with the apex (distal to HR2) present in a closed conformation.

EXAMPLE 9: *Immunogenicity of closed prefusion HMPV F protein MPV212047.*

Adjuvanted HMPV prefusion F protein is more immunogenic and efficacious in naïve cotton rats than postfusion HMPV F.

The immunogenicity of prefusion HMPV F (preF) MPV212047 was compared to postfusion HMPV F (postF) MPV190470 by immunizing naïve cotton rats intramuscularly with either 16, 3 or 0.6 µg preF or with 10 µg postF protein, adjuvanted with 50 µL AS01B per animal (n=8 per dose). Proteins were purified as described in Example 8. As controls, cotton rats were intramuscularly injected with either phosphate buffered saline (PBS) (negative control, n=8) or with 10^4 plaque forming units (PFU) HMPV A2 (positive control, n=6). Animals were immunized twice with indicated doses at day 0 and day 21. The positive control group was immunized once at day 0. Animals were challenged with 10^5 PFU HMPV A2 on day 42 and sacrificed on day 46. ELISA binding antibody titers were determined against HMPV preF (site-specific biotinylated MPV212047: MPV220554) in serum isolated at day 42 (Figure 13A). Titers are displayed as the log10 of the relative potency. The assay is exploratory, LLoD is based on a 99% quantile limit using all expected negative samples. Every dot depicts the value of an individual animal, and the horizontal line indicates the median response of the group. Neutralizing antibody titers were determined against HMPV A2 (TN/94-49) in serum isolated at day 42 by plaque reduction neutralization test (PRNT) (Figure 13B). Titers are displayed as the log2 of the 50% inhibitory concentrations (IC50). Every dot depicts the value of an individual animal, and the horizontal line indicates the median response of the group.

HMPV A2 viral load at day 4 post infection (day 46) was measured in nose homogenates by plaque assay and expressed as log10 pfu per gram of tissue (Figure 13C). Every dot depicts an individual animal and median responses per group are indicated with a horizontal line. Open symbols indicate animals without detectable levels of preF binding antibodies in day 42 sera.

The level of HMPV preF binding antibodies was significantly higher in animals immunized with 3 μg preF protein compared to the 10 μg postF protein group, corresponding with significantly higher levels of neutralizing antibodies (Figure 13A,B). In addition, protection against HMPV A2 virus in the nose was significantly lower if animals are immunized with postF protein compared to PreF protein (Figure 13C).

In summary, adjuvanted HMPV preF protein is more immunogenic and efficacious than HMPV postF protein in naïve cotton rats.

Adjuvanted closed prefusion HMPV F protein is more immunogenic than open prefusion HMPV F protein.

The *in vivo* immunogenicity of closed prefusion HMPV F MPV212047 (closed F) was compared to an HMPV F protein with an open conformation (MPV220215; open F). To this end, MPV220215 HMPV F trimer was purified by C-tag affinity purification and SEC and HMPV F trimer was characterized as described in Example 8. MPV220215 was designed as a single-chain construct carrying a foldon trimerization domain and stabilizing substitutions V112R, S149Y, V231I, and E453P. The trimeric conformation was confirmed by SEC-MALS analysis, eluting at approximately 4 minutes retention time with a size of 161 kDa (Figure 14A). The open prefusion conformation of MPV220215 was confirmed in BLI by the detection of ADI-14448 prefusion-specific antibody binding and by enhanced MPV458 and MPV465 apex-interface binding compared to closed MPV212047 HMPV F (Figure 14B).

The immunogenicity of purified open MPV220215 and closed MPV212047 HMPV prefusion F trimers was assessed by intramuscular immunization of female Balb/C mice (n=5) at day 0 and day 28 with 1.5, 5, or 15 μg protein, adjuvanted with 10 μL AS01B per animal, or PBS-immunized negative control group (n=3). ELISA binding antibody titers were determined against HMPV preF as described above in serum isolated 2 weeks post second immunization (day 42) (Figure 14C). A dose-dependent increase in the level of HMPV preF binding antibodies was observed upon immunization with both proteins and reached significantly higher levels for the closed MPV212047 protein than the open MPV220215 variant. Moreover, significantly higher HMPV A2 neutralizing titers were detected upon immunization with closed prefusion HMPV F, in comparison with open MPV220215 (Figure 14D).

These results demonstrate that the closed prefusion HMPV F conformation induces more potent neutralizing antibodies in mice than an HMPV F protein in an open conformation.

Immunogenicity of HMPV prefusion F in pre-exposed mice.

The immunogenicity of HMPV prefusion F (MPV212047) was assessed in a HMPV A2 pre-exposed setting by intramuscular immunization of female Balb/C mice (n=7) with 15 μg unadjuvanted MPV212047 protein, purified as described in Example 8. Mice were pre-exposed with at least $1x10^3$ up till $3x10^5$ PFU HMPV HMPV A2 on day 0 and immunized with MPV212047 at week 12. ELISA binding antibody titers were determined against HMPV preF as described above in Example 9 in serum isolated 2- and 4-weeks post immunization (week 14 and 16) (Figure 15). The level of HMPV preF binding antibodies was enhanced dramatically by immunization with MPV212047 with a 186-fold geomean increase in titer, demonstrating the *in vivo* immunogenicity of unadjuvanted HMPV prefusion F in a pre-exposed setting.

EXAMPLE 10: *Transfer of stabilizing substitutions to HMPV F A2, B1, and B2 strain variants.*

Prefusion HMPV subtype A2 (2000) F protein with minimal amino acid substitutions.

To create an HMPV prefusion F protein with minimal HR2 stabilizing substitutions, several HR2 amino acids were reverted to wildtype in stabilized backbone MPV212047. The resulting construct MPV220558 did carry HR2 substitutions L473W, Q476K, S477F and A484I, but had wildtype amino acids D475, N478, and R479. Additionally, from variant MPV220558, the surface-exposed H368N substitution was reverted to wildtype, creating MPV220647. In summary, MPV220647 was based on HMPV A2 strain TN/00/3-14 (A2 2000), carrying a p27 peptide, F2 domain was truncated at amino acid 89, and containing the following set of stabilizing mutations: V112R, D209E, V231I and E453P, in combination with the four stabilizing HR2 mutations L473W, Q476K, S477F, and A484I.

HMPV F trimer expression was assessed upon transient expression of MPV212047, MPV220558, and MPV220647, as described in Example 1. The melting temperature (Tm50) of HMPV F trimers in supernatant was determined by differential scanning fluorimetry (DSF). To this end, the fluorescent emission of Sypro Orange Dye (ThermoFisher Scientific) added to HMPV F protein in solution was monitored. The measurement was performed with a starting temperature of 25 °C and a final temperature of 95 °C (54 °C increase per hour). Melting curves were measured using a ViiA7 real-time PCR machine (Applied Biosystems), and Tm50 values were derived from the negative first derivative as described previously (Rutten et al. (2020) Cell Rep 30:4540-4550).

An impact on trimer peak height was observed by subsequent reversal of three HR2 stabilizing substitutions and by H368N reversal to wildtype (Figure 16A, black bars). However, the melting temperature of the three HMPV F proteins was not negatively affected as

demonstrated by an average Tm50 of 72.3°C, 73.2°C, and 73.5°C for MPV212047, MPV220558, and MPV220647, respectively (Figure 16A, grey bars).

Transfer of stabilizing substitutions to HMPV F strain variants.

Next, transfer of stabilizing substitutions in MPV220647 to a more recent A2 strain as well as to recent subtype B1 and B2 strains was evaluated as described above (Figure 16B). The stabilizing impact of these mutations was demonstrated by comparison of wildtype B2 (MPV23362) to stabilized B2 variant MPV220641. Wildtype HMPV F B2 eluted at the expected monomer retention time in analytical SEC (Figure 16C, grey line), while stabilized HMPV F B2 MPV220641 expressed at considerably higher levels and eluted as a trimer at around approximately 4.5 minutes retention time (Figure 16C, black line). In concordance, the average melting temperature of wildtype HMPV F B2 increased from 57.9°C to 72.7°C for stabilized MPV220641, as determined by DSF (Figure 16D). Finally, stabilization of prefusion HMPV F was confirmed in biolayer interferometry (BLI) measurements using quantitative Octet with HMPV F prefusion (ADI-61026), apex interface (MPV458), and non-prefusion (DS7) antibodies as described in Example 1.

Compared to wildtype HMPV F B2, stabilized HMPV F B2 MPV220641 showed improved binding to prefusion-specific ADI-61026 and reduced binding to apex interface MPV458 and non-prefusion DS7 antibodies (Figure 16E).

A trimeric expression pattern was also observed for stabilized HMPV F A2 2019 (MPV220639) and HMPV F B1 2020 (MPV220640), albeit at slightly reduced expression levels compared to A2 2000 (MPV220647). The melting temperature of recent HMPV F A2 and B1 strains was comparable or higher than MPV220639 (73.6°C and 75.8°C for MPV220640 and MPV22647, respectively) (Figure 16D). All stabilized HMPV F proteins

were expressed in a prefusion conformation as confirmed by ADI-61026 prefusion antibody binding, in combination with low MPV458 and DS7 binding in Octet (Figure 16E).

In conclusion, the stabilizing HMPV F substitutions that were discovered in an HMPV A2 (2000) backbone were successfully transferred to other, more recent HMPV subtypes, yielding stable, trimeric prefusion HMPV F proteins.

EXAMPLE 11. *Stabilization, purification, and immunogenicity of HMPV subtype A2 (2019) prefusion F protein.*

Improved trimer expression of further stabilized tag-free HMPV F variant MPV221190.

Stabilized HMPV subtype A2 (2019) prefusion F variant MPV220639 from Example 10 was created without a C-tag purification tag, yielding variant MPV220759. In this backbone, additional substitutions S149Y and N404P were introduced, yielding variant MPV221190, and their effect on trimer expression was evaluated following transient transfection in Expi293F cells as described in Example 1. Analytical SEC of cell-culture supernatant demonstrated increased trimer expression for MPV221190, carrying both S149Y and N404P, over backbone MPV220759 (Figure 17A).

Purification and characterization of tag-free MPV220759 and MPV221190 HMPV F trimers.

Next, both MPV220759 and MPV221190 HMPV F variants were transiently transfected in Expi293F cells using ExpiFectamine (Life Technologies) according to the manufacturer's instructions and cultured for 5 days at 37°C and 10% CO2. The culture supernatant was harvested and spun for 10 minutes at 600 g to remove cells and cellular debris, then sterile-filtered using a 0.22 μm vacuum filter. HMPV F proteins were purified using a two-step purification protocol including ion exchange (Cation) purification at pH 5.0 and

polishing via size exclusion chromatography using a Superdex 200 16/600 pg column. The trimeric fraction was pooled and further characterized by analytical SEC-MALS using an ultra-high-performance liquid chromatography system (Vanquish, Thermo Scientific) and μDAWN TREOS instrument (Wyatt) coupled to an Optilab μT-rEX Refractive Index Detector (Wyatt), in combination with an in-line Nanostar DLS reader (Wyatt). Protein was loaded onto a Unix-C SEC-300 15 cm column (Sepax Technologies) with the corresponding guard column (Sepax Technologies) equilibrated in running buffer (150 mM sodium phosphate, 50 mM NaCl, pH 7.0) at 0.35 mL/min. Analytical SEC data was analyzed using Chromeleon 7.2.8.0 software package, and molecular weight of HMPV F trimers was calculated by Astra software and compared to the calculated weight, confirming a trimeric conformation (Figure 17B, C).

Melting temperature (Tm50) of purified HMPV F trimers was determined by DSF as described in Example 10. MPV221190 carrying substitutions S149Y and N404P had a higher melting temperature of 75.5°C compared to the Tm50 of 73.5 °C of MPV220759 without these substitutions (Figure 17D).

The prefusion conformation of purified HMPV F proteins was confirmed in biolayer interferometry (BLI) measurements using quantitative Octet with HMPV F prefusion (ADI-14448 and ADI-61026), apex interface (MPV458), and non-prefusion (DS7) antibodies. Antibodies were immobilized to anti-human IgG sensors at a concentration of 5 μg/ml, and initial binding rate of HMPV F at 20 μg/ml for 300 seconds association was plotted as average +SD of 3-6 individual measurements (Figure 17E). Binding of both HMPV F proteins to prefusion-specific ADI-14448 and ADI-61026 antibodies was confirmed as well as the absence of binding to apex interface binding antibody MPV458 and to non-prefusion DS7.

The stress resilience of both purified HMPV F proteins MPV220759 and MPV221190 to repeated snap freezing (SF) cycles in liquid nitrogen, and to supercooling stress towards -20°C was assessed by measuring the relative trimer content in analytical SEC compared to non-

treated protein (Figure 17F). Incremental SF stress reduced trimer content for both proteins but was less pronounced for MPV221190 (Figure 17F, filled bars). In line with this, the average reduction in trimer content of five supercooling stress measurements was 23% for MPV220759, and 11% for MPV221190 (Figure 17F, open bars).

In conclusion, two stable trimeric HMPV prefusion F proteins based on A2 (2019) sequence were produced without a trimerization domain or purification tag. Variant MPV221190 with additional stabilizing substitutions S149Y and N404P displayed higher trimer expression and improved temperature stress resilience compared to MPV220759.

Immunogenicity of HMPV prefusion F MPV220759 and MPV221190.

The *in vivo* immunogenicity of purified MPV220759 and MPV221190 HMPV prefusion F trimer was assessed by intramuscular immunization of female Balb/C mice (n=8) at day 0 and day 28 with 1.5, 5, or 15 µg protein adjuvanted with 10 µL AS01B per animal and compared to 15 µg AS01B-adjuvanted MPV212047 (n=8) or PBS-immunized control group (n=3). ELISA binding antibody titers were determined against HMPV preF as described in Example 9 in serum isolated 2 weeks post second immunization (day 43) (Figure 17G). A dose-dependent increase in the level of HMPV preF binding antibodies was observed upon immunization with MPV220759 and MPV221190 and reached comparable levels to MPV212047 at the 15-µg protein dose, illustrating the immunogenicity of these HMPV prefusion F proteins in mice.

EXAMPLE 12. In vitro *comparison of differently stabilized HMPV prefusion F proteins.*

A prefusion-stabilized HMPV F protein was described by Hsieh *et al.* (Nat Commun. 2022 Mar 14;13(1):1299). The expression of this so-called DS-CavEs2 design (MPV220552)

was compared with MPV221190 following transient transfection in Expi293F cells as described in Example 1. DS-CavEs2 (MPV220552) has a foldon trimerization domain and eluted after approximately 4 minutes retention time, while the foldon-less MPV221190 eluted later at approximately 4.5 minutes retention time and demonstrated strongly increased trimer expression (Figure 18A).

Next, MPV220552 trimer was purified following transient expression in Expi293F cells as described in Example 11 by using StrepTag affinity chromatography (GE Healthcare, 28-9075) followed by polishing via size exclusion chromatography using a Superose 6 (GE Healthcare) column. The trimeric fraction was pooled and further characterized by SEC-MALS, DSF, and Octet, as described in Example 11. Trimeric conformation of the DS-CavEs2 design was confirmed with a melting temperature of 71.1°C, comparable to earlier reports (Hsieh *et al.*, 2022), but lower than alternatively stabilized HMPV F MPV221190 (Figure 17D, 18B,C). Both proteins bound ADI-14448 and ADI-61026 prefusion antibodies and did not bind non-prefusion DS7. However, MPV220552 demonstrated high binding to the apex interface antibody MPV458, indicative of a more open apex structure compared to MPV221190 (Figure 18D).

The stability of both purified proteins was assessed by prolonged incubation at either 4°C or 37°C, followed by analytical SEC. Examination of the HMPV F elution pattern demonstrated a stable trimeric peak for MPV221190, while a reduced trimer peak was observed for MPV220552, concomitant with the appearance of smaller species eluting between 4.5-5 minutes retention time upon 2 weeks storage at 37°C (Figure 18E).

In summary, MPV221190 HMPV prefusion F stabilized trimer has higher expression level, a more closed trimeric structure as determined by BLI, and enhanced stability as determined by DSF and by stress resilience compared to previously published DS-CavEs2 (MPV220552) HMPV prefusion F.

EXAMPLE 13. *Stabilization of HMPV prefusion F trimers without a heterologous trimerization domain and without stabilization of the HR2 region.*

Trimer expression of HMPV prefusion F protein was evaluated in a backbone without a heterologous trimerization domain and without stabilizing substitutions in the HR2 region, to investigate the requirement of HR2 stabilization in the presence of multiple alternative stabilizing substitutions in the head domain. This backbone was comparable to MPV212047, except for the introduction of seven HR2 substitutions. In short, backbone MPV220847 had an F2 truncation after amino acid position 89, an introduced furin cleavage site and p27 of RSV, also comprising a furin cleavage site and a C-terminal truncation at position 489. The variant further comprised substitutions V112R, D209E, V231I, H368N, E453P, a linker and C-tag.

Trimer expression of MPV220847 was compared to two variants carrying either a S477I (MPV220115) or S477L (MPV220851) substitution in the HR2 region, which were shown previously to improve trimer content of HMPV F (Figure 4). Upon transient expression in Expi293F cells, as described in Example 1, all three HMPV F variants showed a trimeric peak eluting at approximately 4.5 minutes retention time (Figure 19A), indicating that HR2 stabilization is not required when sufficient alternative substitutions are introduced. However, although a single melting event for all three HMPV F variants was measured around approximately 70°C, the stabilizing effect of S447I and S477L substitutions was still apparent by the increased melting temperature of MPV220115 and MPV220851, compared with backbone MPV220847 in DSF (Figure 19B). The prefusion conformation of all three HMPV F variants was confirmed in BLI, by binding of prefusion-specific antibody ADI-14448 and the absence of binding to non-prefusion antibody DS7 (Figure 19C) (DSF and BLI carried out as described in Example 11).

In conclusion, trimeric HMPV prefusion F protein can be stabilized without the requirement of a heterologous trimerization domain and without the need for stabilization of the HR2 region, although the latter provides clear benefit in terms of temperature stability of the HMPV F protein.

EXAMPLE 14. *Stabilization of HMPV prefusion F trimers without a heterologous trimerization domain through stabilization of the HR2 region at position 477.*

Stabilization of prefusion HMPV F through HR2 stem region optimization identified position S477 as a suitable target for stabilization (Figure 4). The impact of hydrophobic amino acid substitutions was systemically evaluated in backbone MPV211241, which was previously demonstrated to hardly express trimeric HMPV F (Figure 4A left histogram panel, 5A left histogram panel). To this end, HMPV F variants carrying either wildtype S477, or S477I, S477L, S477F, S477V, S477M, S477Y or S477W were expressed in Expi293F cells and analyzed as described in Example 1. To assess the stability of HMPV F trimer expression, the culture supernatants were subjected to 30-minute incubation at 58°C and evaluated in analytical SEC. As previously demonstrated, backbone MPV211241 carrying wildtype S477 did not express HMPV F trimer but eluted with a retention time between 4.5-5 minutes, corresponding to HMPV F monomer (Figure 20, black line). Upon heat stress, aggregates appeared with a retention time of approximately 3 minutes (Figure 20, grey line). Substitutions S477I, S477L, S477F, S477V, S477M (MPV211247, MPV211249, MPV23278, MPV211248, MPV23279, respectively) were successful at restoring HMPV F trimer expression, and substitutions S477Y and S477W (MPV23280 and MPV23281) partially restored HMPV F trimer expression, expressing as a mix of trimer and monomer (Figure 20, black lines). Upon 58°C heat stress, HMPV F trimers expressed from variants carrying either S477I or S477L substitutions

remained trimeric (MPV211247 and MPV211249, Figure 20, grey lines; Figure 4A), while all other S477 variants were impacted, having reduced trimer content and showing the presence of aggregates (Figure 20, grey lines).

In summary, the introduction of a hydrophobic amino acid residue at position 477 in the HR2 region of HMPV F was demonstrated to enhance trimer expression, with S477I and S477L substitutions yielding the most stable trimers.

EXAMPLE 15. *Stabilization of full-length HMPV prefusion F trimers.*

The impact of stabilizing substitutions in the head domain (with 'head domain' being defined here as that part of the mature, processed protein that is N-terminal of the HR2 stem region) and in the HR2 region of full-length HMPV F was assessed by flow cytometry. To this end, full-length wildtype HMPV A2 (2019) F with an F2 truncation after amino acid position 89, an introduced furin cleavage site and p27 of RSV, also comprising a furin cleavage site was designed (MPV221364). This wildtype backbone was compared to MPV221376, which additionally carried four HR2 substitutions, L473W, Q476K, S477F, and A484I. Likewise, the wildtype backbone was compared to MPV221371, which carried head domain substitutions V112R, D209E, V231I and E453P, but no HR2 stabilization. Finally, variant MPV221377 carried both head and HR2 stabilizations as described above.

HMPV F encoding plasmids were co-transfected in Expi293F cells at a 3:2:5 ratio HMPV F: furin: GFP plasmid DNA. Two days post-transfection, cells were collected, washed, stained, and fixed before being subjected to flow cytometry (FACS Canto II, Becton Dickinson). Staining steps included live/dead violet stain (ThermoFisher) and HMPV F antibodies AD-61026 and DS7, followed by staining with Alexa Fluor 647-labeld anti-human IgG detection antibody. Median fluorescence intensity (MFI) of HMPV F antibody signal was

determined by applying a single cell, live, GFP-positive cell gate. HMPV prefusion F-specific antibody ADI-61026 was detected at comparable levels for all four HMPV F variants, demonstrating that full-length membrane-expressed processed HMPV F is present in a prefusion conformation on the surface of transfected Expi293F cells (Figure 21). However, the presence of HMPV non-prefusion F was also confirmed in these cells, with the highest DS7 binding detected for the wildtype backbone MPV221364. Introduction of HR2 substitutions reduced DS7 binding, as did the introduction of head domain substitutions. The combination of both head domain and HR2 region substitutions yielded the strongest reduction in DS7 binding, confirming the stabilizing effect of substitutions in both HMPV F protein regions in full-length HMPV F (Figure 21).

EXAMPLE 16. *Alternative p27 sequences improve HMPV F processing without the need for exogenous furin co-expression.*

The introduction of a second furin cleavage site and the RSV p27 domain between F1 and F2 of in HMPV resulted in complete processing of F0 when co-transfected with furin (Example 2). To achieve full processing while negating the requirement for exogenous furin co-transfection, the RSV p27 sequence was optimized.

Variations of RSV p27 included a sequence based on either a representative RSV A (MPV23259) or B (MPV23260) sequence, which was introduced in stabilized backbone MPV221190 (Figure 22A). HMPV F encoding plasmids were co-transfected as described in Example 1 with a plasmid encoding furin in a 5:1 HMPV F:furin DNA ratio (20% furin), or with a plasmid encoding a carrier plasmid in a 5:1 HMPV F:carrier DNA ratio (0% furin), and trimer expression was evaluated by analytical SEC as described in Example 1. Backbone MPV221190 as well as p27 RSV A and RSV B variants (MPV23259 and MPV232660,

respectively) eluted as a trimer at approximately 4.5 minutes retention time when co-transfected with 20% furin (Figure 22B, grey lines). However, without furin co-transfection, the elution pattern shifted to a shorter retention time (Figure 22B, black lines), indicative of non-complete processed HMPV F. The latter was confirmed by detection of HMPV F1 and F2 by Western Blot analysis of cell lysates under reducing conditions, as described in Example 1. Without furin co-transfection, an additional band was detected between F1 and F2 fragments, which likely corresponded to F2 with a p27 fragment attached. This additional band was absent in HMPV F cell lysates that were co-transfected with 20% furin (Figure 22C).

Additional variations on the RSV p27 fragment were based on backbone MPV23259, having a p27 RSV A sequence, and included systematic deletion(s) of glycosylation sites in the p27 sequence (MPV23261-MPV23267), and p27 sequence deletions (MPV23268, MPV23269) as indicated in Figure 22A. These variants were evaluated as described above, showing a comparable trimer elution pattern when co-transfected with 20% furin (Figure 22B, grey lines), but different elution profiles when transfected without additional furin (Figure 22C, black lines). Expression of p27 variants without furin co-transfection resulted in non-complete processing of HMPV F for variants with one or two glycan deletions (MPV23261-MPV23266) (Figure 22B,C). However, removal of all three glycosylation sites in MPV23267 yielded the same trimer retention time whether or not this variant was co-transfected with furin (Figure 22B) and showed complete HMPV F processing on Western Blot (Figure 22C) without the requirement for exogenous furin expression. A comparable effect was observed by removal of 'NNTKNTNVTLS' from the p27 sequence in variant MPV23269, while removal of 'LPRFMNYTL' in MPV23268 was not successful.

In conclusion, complete HMPV F processing could be achieved without the requirement of exogenous furin expression by optimization of the RSV p27 sequence that is inserted between F1 and F2 of HMPV F.

Table 2. Standard amino acids, abbreviations and properties

Amino Acid	3-Letter	1-Letter	Side chain polarity	Side chain charge (pH 7.4)
alanine	Ala	A	non-polar	Neutral
arginine	Arg	R	polar	Positive
asparagine	Asn	N	polar	Neutral
aspartic acid	Asp	D	polar	Negative
cysteine	Cys	C	non-polar	Neutral
glutamic acid	Glu	E	polar	Negative
glutamine	Gln	Q	polar	Neutral
glycine	Gly	G	non-polar	Neutral
histidine	His	H	polar	positive(10%) neutral(90%)
isoleucine	Ile	I	non-polar	Neutral
leucine	Leu	L	non-polar	Neutral
lysine	Lys	K	polar	Positive
methionine	Met	M	non-polar	Neutral
phenylalanine	Phe	F	non-polar	Neutral
proline	Pro	P	non-polar	Neutral
serine	Ser	S	polar	Neutral
threonine	Thr	T	polar	Neutral
tryptophan	Trp	W	non-polar	Neutral
tyrosine	Tyr	Y	polar	Neutral
valine	Val	V	non-polar	Neutral

Sequences

SEQ ID NO: 1 hMPV A2 fusion protein (TN/00/3-14) full length (Signal peptide bold; wild type cleavage site region italic, F2 domain underlined)

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALRE
LKTVSADQLAREEQIENP*RQSR*FVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNALKTTNEAVSTLGNGVRVL
ATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNM
PTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWY
CQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLGALVACYK
GVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPIKFPEDQFNVALDQVF
ENIENSQALVDQSNRILSSAEKGNTGFIIVIILIAVLGSSMILVSIFIIIKKTKKPTGAPPELSGVTNNGFIPHS

SEQ ID NO: 2: p27 RSV F

ELPRFMNYTLNNAKKTNVTLSKKRKRR

SEQ ID NO: 3 MPV190470 (postF); optimized furin cleavage site bold; linkers italic; TEV CS bold italic; foldon bold underlined; Strep tag underlined italic; C-tag underlined

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LRTVSADQLAREEQIENPRQS**KKRKRR**VATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRVLATA
VRELKDFVSKNLTRAINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTS
AGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKENYACLLREDQGWYCQN
AGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLGALVACYKGVSC
SIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPVKFPEDQFNVALDQVFESIE
NSQALVDQSNRILSSAEKGNT*GG**RENLYFQ**GGGGS***GYIPEAPRDGQAYVRKDGEWVLLSTF****AAWSHPQFEKGA*
*A*EPEA

SEQ ID NO 4: MPV201285 (F1 truncated after 481, linker italic; foldon underlined; C-tag bold)

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL
KTVSADQLAREEQIENPRRRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNALKTTNEAVSTLGNGVRVLA
TAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMP
TSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWYC
QNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLGALVACYKG
VSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPIKFPEDQFNVALDQVFE
NIENSQALVDQSNRIL*SGGG*GYIPEAPRDGQAYVRKDGEWVLLSTFL*GGS***EPEA**

SEQ ID NO 5: MPV210498

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLARARRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKN
CLKTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPA
ISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCS
EKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRN
PISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSF
DPIKFPQDQFNVALDQVFENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEPEA

SEQ ID NO 6: MPV210500

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLARRRREEQIENPRRRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCLKTTNECVSTLGNGVR
VLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSN
MPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGW
YCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPISMVALSPLGALVACYK
GVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPIKFPQDQFNVALDQV
FENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEPEA

SEQ ID NO 7: MPV210502

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLARRRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCLKTTNECVSTLGNGVRVLATAVRELKD
FVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKL
MLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWYCQNAGSTV
YYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPISMVALSPLGALVACYKGVSCSIGSN
RVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPIKFPQDQFNVALDQVFENIENSQA
LVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEPEA

SEQ ID NO 8: MPV210505

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLARRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNC
LKTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAI
SLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCS
EKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRN
PISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSF
DPIKFPQDQFNVALDQVFENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEPEA

SEQ ID NO 9: MPV210507

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLARRRRIENPRRRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCLKTTNECVSTLGNGVRVLA
TAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMP
TSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWYC
QNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPISMVALSPLGALVACYKG
VSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPIKFPQDQFNVALDQVFE
NIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEPEA

SEQ ID NO 10: MPV210509

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQRRRREQIENPRRRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCLKTTNECVSTLGNGVRVLA
TAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMP
TSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWYC
QNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPISMVALSPLGALVACYKG
VSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPIKFPQDQFNVALDQVFE
NIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEPEA

SEQ ID NO 11: MPV210530

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KTVSADQLAREEQIENPRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLE
SEVTAIKNCLKTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFS
DNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWI
VKAAPSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYP
CKVSTGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIK
GRPVSSSFDPIKFPQDQFNVALDQVFENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGG
SEPEA

SEQ ID NO 12: MPV210531

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLAREEQIENPRRRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCLKTTNECVSTLGNGVRVLA
TAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMP
TSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWYC
QNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPISMVALSPLGALVACYKG

VSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPIKFPQDQFNVALDQVFE

NIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEPEA

SEQ ID NO 13: MPV210742

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLAREEQIENPRQSRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTI

RLESEVTAIKNCLKTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVR

QFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTP

CWIVKAAPSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTT

NYPCKVSTGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQ

HVIKGRPVSSSFDPIKFPQDQFNVALDQVFENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLST

FLGGSEPEA

SEQ ID NO 14: MPV210743

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLAREEQIENPRQRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIR

LESEVTAIKNCLKTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQ

FSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPC

WIVKAAPSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTN

YPCKVSTGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQH

VIKGRPVSSSFDPIKFPQDQFNVALDQVFENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFL

GGSEPEA

SEQ ID NO 15: MPV210744

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLAREEQIENPRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLES

EVTAIKNCLKTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSD

NAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIV
KAAPSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPC
KVSTGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKG
RPVSSSFDPIKFPQDQFNVALDQVFENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGS
EPEA

SEQ ID NO 16: MPV210745

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLAREEQIENRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESE
VTAIKNCLKTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDN
AGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVK
AAPSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCK
VSTGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGR
PVSSSFDPIKFPQDQFNVALDQVFENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSE
PEA

SEQ ID NO 17: MPV210746

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLAREEQIERRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEV
TAIKNCLKTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNA
GITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKA
APSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVS
TGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRP
VSSSFDPIKFPQDQFNVALDQVFENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEP
EA

SEQ ID NO 18: MPV210747

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLAREEQIRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVT
AIKNCLKTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNA
GITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKA
APSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVS
TGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRP
VSSSFDPIKFPQDQFNVALDQVFENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEP
EA

SEQ ID NO 19: MPV210748

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLAREEQRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVT
AIKNCLKTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNA
GITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKA
APSCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVS
TGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRP
VSSSFDPIKFPQDQFNVALDQVFENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEP
EA

SEQ ID NO 20: MPV210749

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLAREERRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAI
KNCLKTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGIT
PAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPS
CSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTG

RNPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSS

SFDPIKFPQDQFNVALDQVFENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEPEA

SEQ ID NO 21: MPV210750

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLARERRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIK

NCLKTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITP

AISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSC

SEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGR

NPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSS

FDPIKFPQDQFNVALDQVFENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEPEA

SEQ ID NO 22: MPV210751

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

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KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEPEA

SEQ ID NO 23: MPV210752

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCLK

TTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISL

DLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

KFPQDQFNVALDQVFENIENSQALVDQSNRILSGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEPEA

SEQ ID NO 24: MPV211241 (F1 cut after 489, italic linker, C-tag bold)

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQSNRILSSAEKGNT*GGS***EPEA**

SEQ ID NO 25: MPV211242

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAIVDQSNRILSSAEKGNTGGSEPEA

SEQ ID NO 26: MPV211243

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAFVDQSNRILSSAEKGNTGGSEPEA

SEQ ID NO 27: MPV211244

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALIDQSNRILSSAEKGNTGGSEPEA

SEQ ID NO 28: MPV211245

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALLDQSNRILSSAEKGNTGGSEPEA

SEQ ID NO 29: MPV211246

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALFDQSNRILSSAEKGNTGGSEPEA

SEQ ID NO 30: MPV211247

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQINRILSSAEKGNTGGSEPEA

SEQ ID NO 31: MPV211248

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQVNRILSSAEKGNTGGSEPEA

SEQ ID NO 32: MPV211249

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQLNRILSSAEKGNTGGSEPEA

SEQ ID NO 33: MPV211250

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQSNRVLSSAEKGNTGGSEPEA

SEQ ID NO 34: MPV211251

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQSNRLLSSAEKGNTGGSEPEA

SEQ ID NO 35: MPV211252

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQSNRILSSIEKGNTGGSEPEA

SEQ ID NO 36: MPV211253

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQSNRILSSVEKGNTGGSEPEA

SEQ ID NO 37: MPV211254

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQSNRILSSLEKGNTGGSEPEA

SEQ ID NO 38: MPV211255

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQSNRILSSAEKGITGGSEPEA

SEQ ID NO 39: MPV211256

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQSNRILSSAEKGVTGGSEPEA

SEQ ID NO 40: MPV211257

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQSNRILSSAEKGLTGGSEPEA

SEQ ID NO 41: MPV211258

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAIIDQSNRILSSAEKGNTGGSEPEA

SEQ ID NO 42: MPV211259

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAIVDQINRILSSAEKGNTGGSEPEA

SEQ ID NO 43: MPV211260

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAIVDQSNRILSSIEKGNTGGSEPEA

SEQ ID NO 44: MPV211261

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAIVDQSNRILSSAEKGITGGSEPEA

SEQ ID NO 45: MPV211262

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALIDQINRILSSAEKGNTGGSEPEA

SEQ ID NO 46: MPV211263

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALIDQSNRILSSIEKGNTGGSEPEA

SEQ ID NO 47: MPV211264

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALIDQSNRILSSAEKGITGGSEPEA

SEQ ID NO 48: MPV211265

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 49: MPV211266

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQINRILSSAEKGITGGSEPEA

SEQ ID NO 50: MPV211267

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQSNRILSSIEKGITGGSEPEA

SEQ ID NO 51: MPV211268

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAIIDQINRILSSAEKGNTGGSEPEA

SEQ ID NO 52: MPV211269

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAIIDQSNRILSSIEKGTGGSEPEA

SEQ ID NO 53: MPV211270

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAIIDQSNRILSSAEKGITGGSEPEA

SEQ ID NO 54: MPV211271

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALIDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 55: MPV211272

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALIDQINRILSSAEKGITGGSEPEA

SEQ ID NO 56: MPV211273

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQALVDQINRILSSIEKGITGGSEPEA

SEQ ID NO 57: MPV211274

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAIIDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 58: MPV211275

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAIIDQINRILSSAEKGITGGSEPEA

SEQ ID NO 59: MPV211276

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAIIDQINRILSSIEKGITGGSEPEA

SEQ ID NO 60: MPV211277

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVDQINRILSSAEKGNTGGSEPEA

SEQ ID NO 61: MPV211278

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVDQINRLLSSAEKGNTGGSEPEA

SEQ ID NO 62: MPV211279

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVDQINRLLSSIEKGNTGGSEPEA

SEQ ID NO 63: MPV211280

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVDQINRLLSSIEKGITGGSEPEA

SEQ ID NO 64: MPV211281

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWIDQINRLLSSIEKGITGGSEPEA

SEQ ID NO 65: MPV211282

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAFVDQINRILSSAEKGNTGGSEPEA

SEQ ID NO 66: MPV211283

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL
KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS
LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP
ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD
PIKFPQDQFNVALDQVFENIENSQAFVDQINRLLSSAEKGNTGGSEPEA

SEQ ID NO 67: MPV211284

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL
KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS
LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP
ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD
PIKFPQDQFNVALDQVFENIENSQAFVDQINRLLSSIEKGNTGGSEPEA

SEQ ID NO 68: MPV211285

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL
KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS
LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAFVDQINRLLSSIEKGITGGSEPEA

SEQ ID NO 69: MPV211286

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KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAFIDQINRLLSSIEKGITGGSEPEA

SEQ ID NO 70: MPV211287

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 71: MPV211288

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVDQINRILSSIEKGITGGSEPEA

SEQ ID NO 72: MPV211289

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWIDQINRILSSIEKGITGGSEPEA

SEQ ID NO 73: MPV211290

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAFVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 74: MPV211291

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAFVDQINRILSSIEKGITGGSEPEA

SEQ ID NO 75: MPV211292

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAFIDQINRILSSIEKGITGGSEPEA

SEQ ID NO 76: MPV211917

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNTGGSEPEA

SEQ ID NO 77: MPV211918

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVRKFDEILSSIEKGNTGGSEPEA

SEQ ID NO 78: MPV211919

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVDKFNRILGGSEPEA

SEQ ID NO 79: MPV211940

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KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 80: MPV211942

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KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVRKFDEILSSIEKGNTGGSEPEA

SEQ ID NO 81: MPV212017

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAHAVTAGVAIAKTIRLESEVTAIKNCL

KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSENAGITPAISL

DLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKK

GNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

KFPPDQFNVALDQVFENIENSQAWVRKFDEILSSIEKGNTGGSEPEA

SEQ ID NO 82: MPV212018

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KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 83: MPV212019

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 84: MPV212020

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNAL
KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS
LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK
KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS
MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI
KFPQDQFNVALDQVFENIENSQAWVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 85: MPV212021

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNAL
KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS
LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP
ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD
PIKFPPDQFNVALDQVFENIENSQAWVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 86: MPV212022

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL
KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS
LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 87: MPV212023

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

KFPQDQFNVALDQVFENIENSQAWVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 88: MPV212024

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

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KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPPDQFNVALDQVFENIENSQAWVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 89: MPV212025

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KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 90: MPV212026

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPPDQFNVALDQVFENIENSQAWVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 91: MPV212027

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

KFPPDQFNVALDQVFENIENSQAWVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 92: MPV212028

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 93: MPV212029

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

KFPPDQFNVALDQVFENIENSQAWVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 94: MPV212030

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPPDQFNVALDQVFENIENSQAWVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 95: MPV212031

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 96: MPV212032

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KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 97: MPV212033

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVRKFDEILSSIEKGNTGGSEPEA

SEQ ID NO 98: MPV212034

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KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVRKFDEILSSIEKGNTGGSEPEA

SEQ ID NO 99: MPV212035

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KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 100: MPV212036

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KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPPDQFNVALDQVFENIENSQAWVRKFDEILSSIEKGNTGGSEPEA

SEQ ID NO 101: MPV212037

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPQDQFNVALDQVFENIENSQAWVRKFDEILSSIEKGNTGGSEPEA

SEQ ID NO 102: MPV212038

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KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

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KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 103: MPV212039

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KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

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SEQ ID NO 104: MPV212040

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LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 105: MPV212041

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LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

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SEQ ID NO 106: MPV212042

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LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 107: MPV212043

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KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 108: MPV212044

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KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 109: MPV212045

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LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

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SEQ ID NO 110: MPV212046

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LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 111: MPV212047

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LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 112: MPV220078

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LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

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SEQ ID NO 113: MPV220079

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LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

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SEQ ID NO 114: MPV220081

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LKTTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAI

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PISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSF

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SEQ ID NO 115: MPV220083

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KTTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSYFD

PIKFPQDQFNVALDQVFENIENSQAWVRKFDEILSSIEKGNTGGSEPEA

SEQ ID NO 116: MPV220084

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KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS

LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQWAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSYFD

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SEQ ID NO 117: MPV220087

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KTTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

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KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 118: MPV220092

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LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQWAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPI

SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSYFDP

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SEQ ID NO 119: MPV220115

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LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 120: MPV220116

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LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 121: MPV220117

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LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 122: MPV220118

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LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 123: MPV220119

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LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 124: MPV220120

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LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 125: MPV220121

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LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 126: MPV220122

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LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 127: MPV220123

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KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

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SEQ ID NO 128: MPV220124

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KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

KFPPDQFNVALDQVFENIENSQALVDQLNRILSSIEKGNTGGSEPEA

SEQ ID NO 129: MPV220125

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

KFPPDQFNVALDQVFENIENSQALVDQINRILSSLEKGNTGGSEPEA

SEQ ID NO 130: MPV220126

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

KFPPDQFNVALDQVFENIENSQALVDQLNRILSSLEKGNTGGSEPEA

SEQ ID NO 131: MPV220127

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

KFPPDQFNVALDQVFENIENSQALVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 132: MPV220128

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI

KFPPDQFNVALDQVFENIENSQALVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 133: MPV220129

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKWELDLTKSALRE

LKTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA

LKTTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI

SLDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE

KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP

ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD

PIKFPPDQFNVALDQVFENIENSQALVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 134: MPV220130

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS

MVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSYFDPI

KFPPDQFNVALDQVFENIENSQALVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO 135: MPV220131

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL

KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL

KTTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS

LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK

KGNYACLLREDQGWYCQWAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPI

SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSYFDP

IKFPPDQFNVALDQVFENIENSQALVDQINRILSSIEKGNTGGSEPEA

SEQ ID NO: 136: 5’UTR

ATAGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAA

SEQ ID NO: 137: Alpha 5' replication seq from nsP1

TAGGAGAAAGTTCACGTTGACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGGAGCTTCCCGCAG

TTTGAGGTAGAAGCCAAGCAGGTCACTGATAATGACCATGCTAATGCCAGAGCGTTTTCGCATCTGGCTTCAA

AACTGATCGAAACGGAGGTGGACCCATCCGACACGATCCTTGACATTGGA

SEQ ID NO: 138: Gdlp

ATAGTCAGCATAGTACATTTCATCTGACTAATACTACAACACCACCACCATGAATAGAGGATTCTTTAACATGC

TCGGCCGCCGCCCCTTCCCGGCCCCCACTGCCATGTGGAGGCCGCGGAGAAGGAGGCAGGCGGCCCCG

SEQ ID NO: 139: P2A

GSGATNFSLLKQAGDVEENPGP

SEQ ID NO: 140: nsp1 coding sequence

GAGAAAGTTCACGTTGACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGGAGCTTCCCGCAGTTTG
AGGTAGAAGCCAAGCAGGTCACTGATAATGACCATGCTAATGCCAGAGCGTTTTCGCATCTGGCTTCAAAACT
GATCGAAACGGAGGTGGACCCATCCGACACGATCCTTGACATTGGAAGTGCGCCCGCCCGCAGAATGTATTC
TAAGCACAAGTATCATTGTATCTGTCCGATGAGATGTGCGGAAGATCCGGACAGATTGTATAAGTATGCAACT
AAGCTGAAGAAAAACTGTAAGGAAATAACTGATAAGGAATTGGACAAGAAAATGAAGGAGCTCGCCGCCGT
CATGAGCGACCCTGACCTGGAAACTGAGACTATGTGCCTCCACGACGACGAGTCGTGTCGCTACGAAGGGCA
AGTCGCTGTTTACCAGGATGTATACGCGGTTGACGGACCGACAAGTCTCTATCACCAAGCCAATAAGGGAGTT
AGAGTCGCCTACTGGATAGGCTTTGACACCACCCCTTTTATGTTTAAGAACTTGGCTGGAGCATATCCATCATA
CTCTACCAACTGGGCCGACGAAACCGTGTTAACGGCTCGTAACATAGGCCTATGCAGCTCTGACGTTATGGAG
CGGTCACGTAGAGGGATGTCCATTCTTAGAAAGAAGTATTTGAAACCATCCAACAATGTTCTATTCTCTGTTGG
CTCGACCATCTACCACGAGAAGAGGGACTTACTGAGGAGCTGGCACCTGCCGTCTGTATTTCACTTACGTGGC
AAGCAAAATTACACATGTCGGTGTGAGACTATAGTTAGTTGCGACGGGTACGTCGTTAAAAGAATAGCTATCA
GTCCAGGCCTGTATGGGAAGCCTTCAGGCTATGCTGCTACGATGCACCGCGAGGGATTCTTGTGCTGCAAAGT
GACAGACACATTGAACGGGGAGAGGGTCTCTTTTCCCGTGTGCACGTATGTGCCAGCTACATTGTGTGACCAA
ATGACTGGCATACTGGCAACAGATGTCAGTGCGGACGACGCGCAAAAACTGCTGGTTGGGCTCAACCAGCGT
ATAGTCGTCAACGGTCGCACCCAGAGAAACACCAATACCATGAAAAATTACCTTTTGCCCGTAGTGGCCCAGG
CATTTGCTAGGTGGGCAAAGGAATATAAGGAAGATCAAGAAGATGAAAGGCCACTAGGACTACGAGATAGA
CAGTTAGTCATGGGGTGTTGTTGGGCTTTTAGAAGGCACAAGATAACATCTATTTATAAGCGCCCGGATACCC
AAACCATCATCAAAGTGAACAGCGATTTCCACTCATTCGTGCTGCCCAGGATAGGCAGTAACACATTGGAGAT

CGGGCTGAGAACAAGAATCAGGAAAATGTTAGAGGAGCACAAGGAGCCGTCACCTCTCATTACCGCCGAGG
ACGTACAAGAAGCTAAGTGCGCAGCCGATGAGGCTAAGGAGGTGCGTGAAGCCGAGGAGTTGCGCGCAGCT
CTACCACCTTTGGCAGCTGATGTTGAGGAGCCCACTCTGGAAGCCGATGTCGACTTGATGTTACAAGAGGCTG
GGGCC

SEQ ID NO: 141: nsp2 coding sequence

GGCTCAGTGGAGACACCTCGTGGCTTGATAAAGGTTACCAGCTACGATGGCGAGGACAAGATCGGCTCTTAC
GCTGTGCTTTCTCCGCAGGCTGTACTCAAGAGTGAAAAATTATCTTGCATCCACCCTCTCGCTGAACAAGTCAT
AGTGATAACACACTCTGGCCGAAAAGGGCGTTATGCCGTGGAACCATACCATGGTAAAGTAGTGGTGCCAGA
GGGACATGCAATACCCGTCCAGGACTTTCAAGCTCTGAGTGAAAGTGCCACCATTGTGTACAACGAACGTGA
GTTCGTAAACAGGTACCTGCACCATATTGCCACACATGGAGGAGCGCTGAACACTGATGAAGAATATTACAAA
ACTGTCAAGCCCAGCGAGCACGACGGCGAATACCTGTACGACATCGACAGGAAACAGTGCGTCAAGAAAGA
ACTAGTCACTGGGCTAGGGCTCACAGGCGAGCTGGTGGATCCTCCCTTCCATGAATTCGCCTACGAGAGTCTG
AGAACACGACCAGCCGCTCCTTACCAAGTACCAACCATAGGGGTGTATGGCGTGCCAGGATCAGGCAAGTCT
GGCATCATTAAAAGCGCAGTCACCAAAAAAGATCTAGTGGTGAGCGCCAAGAAAGAAAACTGTGCAGAAATT
ATAAGGGACGTCAAGAAAATGAAAGGGCTGGACGTCAATGCCAGAACTGTGGACTCAGTGCTCTTGAATGGA
TGCAAACACCCCGTAGAGACCCTGTATATTGACGAAGCTTTTGCTTGTCATGCAGGTACTCTCAGAGCGCTCAT
AGCCATTATAAGACCTAAAAAGGCAGTGCTCTGCGGGGATCCCAAACAGTGCGGTTTTTTTAACATGATGTGC
CTGAAAGTGCATTTTAACCACGAGATTTGCACACAAGTCTTCCACAAAAGCATCTCTCGCCGTTGCACTAAATC
TGTGACTTCGGTCGTCTCAACCTTGTTTTACGACAAAAAAATGAGAACGACGAATCCGAAAGAGACTAAGATT
GTGATTGACACTACCGGCAGTACCAAACCTAAGCAGGACGATCTCATTCTCACTTGTTTCAGAGGGTGGGTGA
AGCAGTTGCAAATAGATTACAAAGGCAACGAAATAATGACGGCAGCTGCCTCTCAAGGGCTGACCCGTAAAG
GTGTGTATGCCGTTCGGTACAAGGTGAATGAAAATCCTCTGTACGCACCCACCTCTGAACATGTGAACGTCCT
ACTGACCCGCACGGAGGACCGCATCGTGTGGAAAACACTAGCCGGCGACCCATGGATAAAAACACTGACTGC
CAAGTACCCTGGGAATTTCACTGCCACGATAGAGGAGTGGCAAGCAGAGCATGATGCCATCATGAGGCACAT

CTTGGAGAGACCGGACCCTACCGACGTCTTCCAGAATAAGGCAAACGTGTGTTGGGCCAAGGCTTTAGTGCC

GGTGCTGAAGACCGCTGGCATAGACATGACCACTGAACAATGGAACACTGTGGATTATTTTGAAACGGACAA

AGCTCACTCAGCAGAGATAGTATTGAACCAACTATGCGTGAGGTTCTTTGGACTCGATCTGGACTCCGGTCTA

TTTTCTGCACCCACTGTTCCGTTATCCATTAGGAATAATCACTGGGATAACTCCCCGTCGCCTAACATGTACGG

GCTGAATAAAGAAGTGGTCCGTCAGCTCTCTCGCAGGTACCCACAACTGCCTCGGGCAGTTGCCACTGGAAG

AGTCTATGACATGAACACTGGTACACTGCGCAATTATGATCCGCGCATAAACCTAGTACCTGTAAACAGAAGA

CTGCCTCATGCTTTAGTCCTCCACCATAATGAACACCCACAGAGTGACTTTTCTTCATTCGTCAGCAAATTGAAG

GGCAGAACTGTCCTGGTGGTCGGGGAAAAGTTGTCCGTCCCAGGCAAAATGGTTGACTGGTTGTCAGACCGG

CCTGAGGCTACCTTCAGAGCTCGGCTGGATTTAGGCATCCCAGGTGATGTGCCCAAATATGACATAATATTTG

TTAATGTGAGGACCCCATATAAATACCATCACTATCAGCAGTGTGAAGACCATGCCATTAAGCTTAGCATGTTG

ACCAAGAAAGCTTGTCTGCATCTGAATCCCGGCGGAACCTGTGTCAGCATAGGTTATGGTTACGCTGACAGGG

CCAGCGAAAGCATCATTGGTGCTATAGCGCGGCAGTTCAAGTTTTCCCGGGTATGCAAACCGAAATCCTCACT

TGAAGAGACGGAAGTTCTGTTTGTATTCATTGGGTACGATCGCAAGGCCCGTACGCACAATCCTTACAAGCTT

TCATCAACCTTGACCAACATTTATACAGGTTCCAGACTCCACGAAGCCGGATGT

SEQ ID NO: 142: nsp3 coding sequence

GCACCCTCATATCATGTGGTGCGAGGGGATATTGCCACGGCCACCGAAGGAGTGATTATAAATGCTGCTAAC

AGCAAAGGACAACCTGGCGGAGGGGTGTGCGGAGCGCTGTATAAGAAATTCCCGGAAAGCTTCGATTTACA

GCCGATCGAAGTAGGAAAAGCGCGACTGGTCAAAGGTGCAGCTAAACATATCATTCATGCCGTAGGACCAAA

CTTCAACAAAGTTTCGGAGGTTGAAGGTGACAAACAGTTGGCAGAGGCTTATGAGTCCATCGCTAAGATTGTC

AACGATAACAATTACAAGTCAGTAGCGATTCCACTGTTGTCCACCGGCATCTTTTCCGGGAACAAAGATCGAC

TAACCCAATCATTGAACCATTTGCTGACAGCTTTAGACACCACTGATGCAGATGTAGCCATATACTGCAGGGA

CAAGAAATGGGAAATGACTCTCAAGGAAGCAGTGGCTAGGAGAGAAGCAGTGGAGGAGATATGCATATCCG

ACGACTCTTCAGTGACAGAACCTGATGCAGAGCTGGTGAGGGTGCATCCGAAGAGTTCTTTGGCTGGAAGGA

AGGGCTACAGCACAAGCGATGGCAAAACTTTCTCATATTTGGAAGGGACCAAGTTTCACCAGGCGGCCAAGG

ATATAGCAGAAATTAATGCCATGTGGCCCGTTGCAACGGAGGCCAATGAGCAGGTATGCATGTATATCCTCG

GAGAAAGCATGAGCAGTATTAGGTCGAAATGCCCCGTCGAAGAGTCGGAAGCCTCCACACCACCTAGCACGC

TGCCTTGCTTGTGCATCCATGCCATGACTCCAGAAAGAGTACAGCGCCTAAAAGCCTCACGTCCAGAACAAAT

TACTGTGTGCTCATCCTTTCCATTGCCGAAGTATAGAATCACTGGTGTGCAGAAGATCCAATGCTCCCAGCCTA

TATTGTTCTCACCGAAAGTGCCTGCGTATATTCATCCAAGGAAGTATCTCGTGGAAACACCACCGGTAGACGA

GACTCCGGAGCCATCGGCAGAGAACCAATCCACAGAGGGGACACCTGAACAACCACCACTTATAACCGAGGA

TGAGACCAGGACTAGAACGCCTGAGCCGATCATCATCGAAGAGGAAGAAGAGGATAGCATAAGTTTGCTGTC

AGATGGCCCGACCCACCAGGTGCTGCAAGTCGAGGCAGACATTCACGGGCCGCCCTCTGTATCTAGCTCATCC

TGGTCCATTCCTCATGCATCCGACTTTGATGTGGACAGTTTATCCATACTTGACACCCTGGAGGGAGCTAGCGT

GACCAGCGGGGCAACGTCAGCCGAGACTAACTCTTACTTCGCAAAGAGTATGGAGTTTCTGGCGCGACCGGT

GCCTGCGCCTCGAACAGTATTCAGGAACCCTCCACATCCCGCTCCGCGCACAAGAACACCGTCACTTGCACCC

AGCAGGGCCTGCTCGAGAACCAGCCTAGTTTCCACCCCGCCAGGCGTGAATAGGGTGATCACTAGAGAGGAG

CTCGAGGCGCTTACCCCGTCACGCACTCCTAGCAGGTCGGTCTCGAGAACCAGCCTGGTCTCCAACCCGCCAG

GCGTAAATAGGGTGATTACAAGAGAGGAGTTTGAGGCGTTCGTAGCACAACAACAATGACGGTTTGATGCGG

GTGCA

SEQ ID NO: 143: nsp4 coding sequence

TACATCTTTTCCTCCGACACCGGTCAAGGGCATTTACAACAAAAATCAGTAAGGCAAACGGTGCTATCCGAAG

TGGTGTTGGAGAGGACCGAATTGGAGATTTCGTATGCCCCGCGCCTCGACCAAGAAAAAGAAGAATTACTAC

GCAAGAAATTACAGTTAAATCCCACACCTGCTAACAGAAGCAGATACCAGTCCAGGAAGGTGGAGAACATGA

AAGCCATAACAGCTAGACGTATTCTGCAAGGCCTAGGGCATTATTTGAAGGCAGAAGGAAAAGTGGAGTGCT

ACCGAACCCTGCATCCTGTTCCTTTGTATTCATCTAGTGTGAACCGTGCCTTTTCAAGCCCCAAGGTCGCAGTG

GAAGCCTGTAACGCCATGTTGAAAGAGAACTTTCCGACTGTGGCTTCTTACTGTATTATTCCAGAGTACGATGC

CTATTTGGACATGGTTGACGGAGCTTCATGCTGCTTAGACACTGCCAGTTTTTGCCCTGCAAAGCTGCGCAGCT

TTCCAAAGAAACACTCCTATTTGGAACCCACAATACGATCGGCAGTGCCTTCAGCGATCCAGAACACGCTCCA

GAACGTCCTGGCAGCTGCCACAAAAAGAAATTGCAATGTCACGCAAATGAGAGAATTGCCCGTATTGGATTC

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CCCATCAGGCTTACTGAAGAAAACGTGGTAAATTACATTACCAAATTAAAAGGACCAAAAGCTGCTGCTCTTTT

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GCTAGCAACAGCGTATCTGTGCGGAATCCACCGAGAGCTGGTTAGGAGATTAAATGCGGTCCTGCTTCCGAA

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GTGTTCTGGAAACTGACATCGCGTCGTTTGATAAAAGTGAGGACGACGCCATGGCTCTGACCGCGTTAATGAT

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CATTTGCCCACTAAAACTAAATTTAAATTCGGAGCCATGATGAAATCTGGAATGTTCCTCACACTGTTTGTGAA

CACAGTCATTAACATTGTAATCGCAAGCAGAGTGTTGAGAGAACGGCTAACCGGATCACCATGTGCAGCATTC

ATTGGAGATGACAATATCGTGAAAGGAGTCAAATCGGACAAATTAATGGCAGACAGGTGCGCCACCTGGTTG

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GGCAGCAGACGATGAACATGATGATGACAGGAGAAGGGCATTGCATGAAGAGTCAACACGCTGGAACCGAG

TGGGTATTCTTTCAGAGCTGTGCAAGGCAGTAGAATCAAGGTATGAAACCGTAGGAACTTCCATCATAGTTAT

GGCCATGACTACTCTAGCTAGCAGTGTTAAATCATTCAGCTACCTGAGAGGGGCCCCTATAACTCTCTACGGC

SEQ ID NO: 144: 26S minimal promoter

CTCTCTACGGCTAACCTGAATGGA

SEQ ID NO: 145: 3'-UTR

ATACAGCAGCAATTGGCAAGCTGCTTACATAGAACTCGCGGCGATTGGCATGCCGCTTTAAAATTTTTATTTTA

TTTTTCTTTTCTTTTCCGAATCGGATTTTGTTTTTAATATTTC

SEQ ID NO: 146: P2A

GGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCT

SEQ ID NO: 147: polyA_site

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 148: T7 promotor

TAATACGACTCACTATAG

SEQ ID NO: 149: SIGNAL PEPTIDE HMPV.

MSWKVVIIFSLLITPQHG

SEQ ID NO: 150: sequence of p27 peptide from an RSV B

EAPQYMNYTINTTKNLNVSISKKRKRR

SEQ ID NO: 151: MPV220554

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL
KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS
LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK
KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS
MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI
KFPPDQFNVALDQVFENIENSQAWVRKFDEILSSIEKGNTAAALPETGGGSEPEA

SEQ ID NO: 152: MPV220558

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL
KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS
LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK
KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS
MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI
KFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNTGGSEPEA

SEQ ID NO: 153: MPV220647

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL
KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS
LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK
KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPIS
MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI
KFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNTGGSEPEA

SEQ ID NO: 154: MPV220639

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA
LKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
SLDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPI
SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP
VKFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNTGGSEPEA

SEQ ID NO: 155: MPV220640

MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTDGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGIAIAKTIRLESEVNAIKGA
LKQTNEAVSTLGNGVRVLATAVRELKEFVSKNLTSAINRNKCDIADLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
SLDLMTDAELARAISYMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIIKAAPSCSEK
NGNYACLLREDQGWYCKNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSRECNINISTTNYPCKVSTGRHPIS
MVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI
RFPPDQFNVALDQVFESIENSQAWVDKFNRILSSIEKGNTGGSEPEA

SEQ ID NO: 156: MPV220641

MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTDGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGIAIAKTIRLESEVNAIKGA
LKTTNEAVSTLGNGVRVLATAVRELKEFVSKNLTSAINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS
LDLMNDAELARAISYMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVINTPCWIIKAAPSCSEK
DGNYACLLREDQGWYCKNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSRECNINISTTNYPCKVSTGRHPIS
MVALSPLGALVACYKGVSCSTGSNQVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSNSFDP
IRFPPDQFNVALDQVFESIENSQAWVDKFNRILSSIEKGNTGGSEPEA

SEQ ID NO: 157: MPV23362 (WILD TYPE B)

MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTDGPSLIKTELDLTKSALRE
LKTVSADQLAREEQIENPRQSRFVLGAIALGVATAAAVTAGIAIAKTIRLESEVNAIKGALKTTNEAVSTLGNGVRVL
ATAVRELKEFVSKNLTSAINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMNDAELARAVSYM
PTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVINTPCWIIKAAPSCSEKDGNYACLLREDQGWYC
KNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSRECNINISTTNYPCKVSTGRHPISMVALSPLGALVACYKG
VSCSTGSNQVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSNSFDPIRFPEDQFNVALDQVF
ESIENSQALVDQSNRILSSAEKGNTGGSEPEA

SEQ ID NO: 158: MPV220759

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA
LKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
SLDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPI
SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP
VKFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNT

SEQ ID NO: 159: MPV221190

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA
LKKTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
SLDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPI
SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP
VKFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNT

SEQ ID NO: 160: MPV220552

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL
KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS
LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK
KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS
MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI
KFPPDQFNVALDQVFENIENSQAWVRKFDEILSSIEKGNTAAALPETGGGSEPEA

SEQ ID NO: 161: MPV220847

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL
KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS
LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK
KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS
MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI
KFPPDQFNVALDQVFENIENSQALVDQSNRILSSAEKGNTGGSEPEA

SEQ ID NO: 162: MPV220115

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL
KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS
LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK
KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS
MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI
KFPPDQFNVALDQVFENIENSQALVDQINRILSSAEKGNTGGSEPEA

SEQ ID NO: 163: MPV220851

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL
KTTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS
LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK
KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPIS
MVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPI
KFPPDQFNVALDQVFENIENSQALVDQLNRILSSAEKGNTGGSEPEA

SEQ ID NO: 164: MPV23278

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL
KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS
LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP
ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD
PIKFPQDQFNVALDQVFENIENSQALVDQFNRILSSAEKGNTGGSEPEA

SEQ ID NO: 165: MPV23279

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL
KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS
LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP
ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD
PIKFPQDQFNVALDQVFENIENSQALVDQMNRILSSAEKGNTGGSEPEA

SEQ ID NO: 166: MPV23280

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL
KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS
LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP
ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD
PIKFPQDQFNVALDQVFENIENSQALVDQYNRILSSAEKGNTGGSEPEA

SEQ ID NO: 167: MPV23281

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKYELDLTKSALREL
KTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAHAVTAGVAIAKTIRLESEVTAIKNCL
KTTNECVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAIS
LDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNP
ISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFD
PIKFPQDQFNVALDQVFENIENSQALVDQWNRILSSAEKGNTGGSEPEA

SEQ ID NO: 168: MPV221364

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNA
LKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAI
SLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCS
EKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRH
PISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSF
DPVKFPEDQFNVALDQVFENIENSQALVDQSNRILSSAEKGNTGFIIVIILIAVLGSSMILVSIFIIIKKTRKPTGAPPELS
GVTNNGFIPHS

SEQ ID NO: 169: MPV221376

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNA
LKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAI
SLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCS
EKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRH
PISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSF

DPVKFPEDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNTGFIIVIILIAVLGSSMILVSIFIIIKKTRKPTGAPPELS
GVTNNGFIPHS

SEQ ID NO: 170: MPV221371

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA
LKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
SLDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPI
SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP
VKFPPDQFNVALDQVFENIENSQALVDQSNRILSSAEKGNTGFIIVIILIAVLGSSMILVSIFIIIKKTRKPTGAPPELSGV
TNNGFIPHS

SEQ ID NO: 171: MPV221377

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA
LKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
SLDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPI
SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP
VKFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNTGFIIVIILIAVLGSSMILVSIFIIIKKTRKPTGAPPELSGV
TNNGFIPHS

SEQ ID NO: 172: MPV23259

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMNYTLNNTKNTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA

LKKTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
SLDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPI
SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP
VKFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNT

SEQ ID NO: 173: MPV23260

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRREAPQYMNYTINTTKNLNVSISKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNAL
KKTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAIS
LDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK
KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPIS
MVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV
KFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNT

SEQ ID NO: 174: MPV23261

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMQYTLNNTKNTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA
LKKTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
SLDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPI
SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP
VKFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNT

SEQ ID NO: 175: MPV23262

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMNYTLQNTKNTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA

LKKTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
SLDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPI
SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP
VKFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNT

SEQ ID NO: 176: MPV23263

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMNYTLNNTKNTQVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA
LKKTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
SLDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPI
SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP
VKFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNT

SEQ ID NO: 177: MPV23264

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMQYTLQNTKNTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA
LKKTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
SLDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPI
SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP
VKFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNT

SEQ ID NO: 178: MPV23265

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMNYTLQNTKNTQVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA

LKKTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
SLDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPI
SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP
VKFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNT

SEQ ID NO: 179: MPV23266

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMQYTLNNTKNTQVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA
LKKTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
SLDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPI
SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP
VKFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNT

SEQ ID NO: 180: MPV23267

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMQYTLQNTKNTQVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA
LKKTNEAVYTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
SLDLMTDAELARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSE
KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPI
SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP
VKFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNT

SEQ ID NO: 181: MPV23268

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRENNTKNTNVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVY

TLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAISLDLMTDAE
LARAISNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLL
REDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPL
GALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPVKFPPDQF
NVALDQVFENIENSQAWVDKFNRILSSIEKGNT

SEQ ID NO: 182: MPV23269

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMNYTLKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVYTL
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ALVACYKGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPVKFPPDQFN
VALDQVFENIENSQAWVDKFNRILSSIEKGNT

SEQ ID NO: 183: MPV220215

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCSDGPSLIKTELDLTKSALREL
KTVSADQLAREEQIENPRQSQSVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNALKTTNEAVYTLGNGVRVLA
TAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAISNMPT
SAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWYCQ
NAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRNPISMVALSPLGALVACYKGVS
CSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPIKFPPDQFNVALDQVFENI
ENSQALVDQSNRILSSAEKGNTGGGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGSEPEA

SEQ ID NO: 184: MPV23324

MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALRE
LKTVSADQLRRRRELPRFMQYTLQNTKNTQVTLSKKRKRRFVLGAIALGRATAAAVTAGVAIAKTIRLESEVTAIKNA

LKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSFSQFNRRFLNVVRQFSENAGITPAI
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KKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPI
SMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP
VKFPPDQFNVALDQVFENIENSQAWVDKFNRILSSIEKGNTGFIIVIILIAVLGSSMILVSIFIIIKKTRKPTGAPPELSGV
TNNGFIPHS

SEQ ID NO: 185: OPTIMIZED RSV A P27 SEQUENCE

ELPRFMQYTLQNTKNTQVTLSKKRKRR

SEQ ID NO: 186: OPTIMIZED RSV B P27 SEQUENCE

EAPQYMQYTIQTTKNLQVSISKKRKRR

SEQ ID NO: 187: pSMARRT_MPV23324_RNA Restriction Cloning ligation product.

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SEQ ID NO: 188: pSMARRT_MPV23267_(soluble)_RNA Restriction Cloning ligation product.

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AAAAAAAAAAA

Claims

1. Pre-fusion human pneumovirus (HMPV) F precursor (F0) protein, comprising an F1 and an F2 domain, and comprising at least one modification in the amino acid sequence of the F1 and/or F2 domain.

2. Protein according to claim 1, wherein the at least one modification stabilizes the pre-fusion conformation and/or increases trimer formation.

3. Protein according to claim 1 or 2, wherein the at least one modification is the introduction of at least one non-native cleavage site.

4. Protein according to claim 3, wherein the protein further comprises a second non-native cleavage site in the F2 domain positioned N-terminally from the first cleavage site, wherein a spacer sequence is present between the first and second non-native cleavage sites.

5. Protein according to claim 3 or 4, wherein the first and/or second non-native cleavage site comprises an amino acid sequence RXXR.

6. Protein according to claim 3, 4 or 5, wherein the first and/or second non-native cleavage comprises a sequence RX[K/R]R.

7. Protein according to any one of the claims 1-6, comprising a p27 peptide of an RSV F protein between the F1 and the F2 domain.

8. Protein according to claim 7, wherein the p27 peptide comprises the amino acid sequence ELPRFMNYTLNNAKKTNVTLSKKRKRR (SEQ ID NO: 2), or an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 2.

9. Protein according to claim 7, wherein the p27 peptide comprises an amino sequence of an RSV B p27 peptide comprising the amino acid sequence EAPQYMNYTINTTKNLNVSISKKRKRR (SEQ ID NO: 150), or an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 150.

10. Protein according to any one of claims 7-9, wherein one or more glycosylation sites in the p27 peptide sequence have been deleted.

11. Protein according to claim 10, wherein all glycosylation sites in the p27 sequence have been deleted.

12. Protein according to any one of claims 7-11, wherein the p27 peptide sequence comprises a deletion of 1-11 amino acid sequences from the p27 peptide sequence.

13. Protein according to any one of claims 7-12 wherein the p27 peptide comprises an amino acid sequence selected from SEQ ID NO: 185 and SEQ ID NO: 186.

14. Protein according to any one of the preceding claims 1-13, comprising an F2 domain which is C-terminally truncated.

15. Protein according to claim 14, wherein the F2 domain is truncated after the amino acid at position 89.

16. Protein according to any one of the preceding claims, comprising an F1 domain which is C-terminally truncated.

17. Protein according to claim 16, wherein the F1 domain is truncated after the amino acid residue at position 489 of the HMPV F precursor (F0) protein.

18. Protein according to any one of the preceding claims, comprising one or more stabilizing amino acid residues in the HR2 domain, said HR2 domain comprising the amino acids 453 to 484 of the HMPV F precursor (F0) protein.

19. Protein according to claim 18, wherein the one or more stabilizing amino acids optimize the interprotomeric interactions between one or more amino acid residues in the HR2 domains of different HMPV F protomers.

20. Protein according to claim 18 or 19, wherein the amino acid at position 477 is I, V, L or F or M.

21. Protein according to claim 18, 19 or 20, wherein furthermore the amino acid residue at position 473 is I, F or W, and/or the amino acid residue at position 474 is or I, and/or the amino acid residue at position 475 is R, and/or the amino acid residue at position 476 is K, and/or the amino acid residue at position 478 is D, and/or the amino acid residue at position 479 is E, and/or the amino acid residue at position 480 is L, and/or the amino acid residue at position 484 is I, and/or the amino acid residue at position 488 is I.

22. Protein according to any one of the claims 18-21, wherein the amino acid residue at position 473 W, the amino acid residue at position 477 is I, and the amino acid residue at position 484 is I.

23. Protein according to any one of the claims 18-21, wherein the amino acid residue at position 473 is W, the amino acid residue at position 476 is K, the amino acid residue at position 477 is F, and the amino acid residue at position 484 is I.

24. Protein according to any one of the claims 18-21, wherein the amino acid residue at position 473 is W, the amino acid residue at position 475 is R, the amino acid residue at position 476 is K, the amino acid at position 477 is I, the amino acid residue at position 478 is D, the amino acid residue at position 479 is E, the amino acid residue at position 484 is I.

25. Protein according to any one of the preceding claims, wherein the amino acid residue at position 112 is R, and/or the amino acid residue at position 209 is E, and/or the amino acid residue at position 453 is P or Q.

26. Protein according to any one of the preceding claims, wherein the amino acid residue at position 149 is Y, and/or the amino acid residue at position 313 is W, and/or the amino acid residue at position 445 is Y.

27. Protein according to claim 26, wherein the amino acid residue at position 112 is R, the amino acid residue at position 209 is E, and the amino acid residue at position 453 is P or Q.

28. Protein according to any one of the preceding claims, wherein the amino acid residue at position 231 is I.

29. Protein according to any one of the preceding claims, wherein the amino acid residue at position 404 is P.

30. Protein according to any one of the preceding claims, wherein the amino acid residue at position 368 is N.

31. Protein according to any one of the preceding claims, wherein the amino acid residue at position 69 is Y or W, and/or the amino acid residue at position 73 is W, and/or the amino acid residue at position 185 is P, and/or the amino acid residue at position 191 is I, and/or the amino acid residue at position 116 is H, and/or the amino acid residue at position 342 is P.

32. Protein according to any one of the preceding claims, further comprising one or more non-native intra- or inter-protomer disulfide bonds.

33. Protein according to claim 32, wherein the one or more disulfide bonds are selected from an intraprotomeric disulfide bond between the amino acid residues 140 and 147 and/or an intraprotomeric disulfide bond between the amino acid residues 141 or 161, and/or an intraprotomeric disulfide bond between the amino acid residues 360 and 459.

34. Protein according to any of the preceding claims, comprising an amino acid sequence selected from the group consisting of SEQ ID NO: SEQ ID NO: 4-135, SEQ ID NO: 151-159, SEQ ID NO: 161-182 and SEQ ID NO: 184, or a fragment thereof.

35. Protein according to claim 34, comprising the amino acid sequence of SEQ ID NO: 111, SEQ ID NO: 159, SEQ ID NO: 180 or SEQ ID NO: 184, or a fragment thereof.

36. Protein according to any one of the claims 1-35, wherein the protein has been cleaved at the one or more cleavage sites, resulting in an F2 and an F1 domain which are covalently linked by one or more native disulfide bridges, and wherein the protein is trimeric.

37. Protein according to claim 36, wherein the F1 domain comprises the amino acids 103-489 of the HMPV F0 protein, and the F2 domain comprises the amino acids 19-88 of the HMPV F0 protein.

38. Nucleic acid molecule encoding a protein according to any one of the preceding claims 1-37.

39. Nucleic acid according to claim 38, wherein the nucleic acid molecule is DNA or RNA.

40. Nucleic acid according to claim 38 or 39, encoding a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: SEQ ID NO: 4-135, SEQ ID NO: 151-159, SEQ ID NO: 161-182 and SEQ ID NO: 184, or a fragment thereof.

41. Nucleic acid according to claim 40, encoding a protein comprising the amino acid sequence of SEQ ID NO: 111, the amino acid sequence of SEQ ID NO: 159, the amino acid sequence of SEQ ID NO: 180 or the amino acid sequence of SEQ ID NO: 184.

42. Vector comprising a nucleic acid according to any one of the claims 38-41.

43. Pharmaceutical composition comprising a protein according to any one of the claims 1-37, a nucleic acid according to any one of the claims 38-41 and/or vector according to claim 42.

44. A method for vaccinating a subject against HMPV, the method comprising administering to the subject a pharmaceutical composition according to claim 43.

45. A method for preventing infection and/or replication of HMPV in a subject, comprising administering to the subject a pharmaceutical composition according to claim 43.

46. An isolated host cell comprising a nucleic acid according to any one of the claims 38-41.

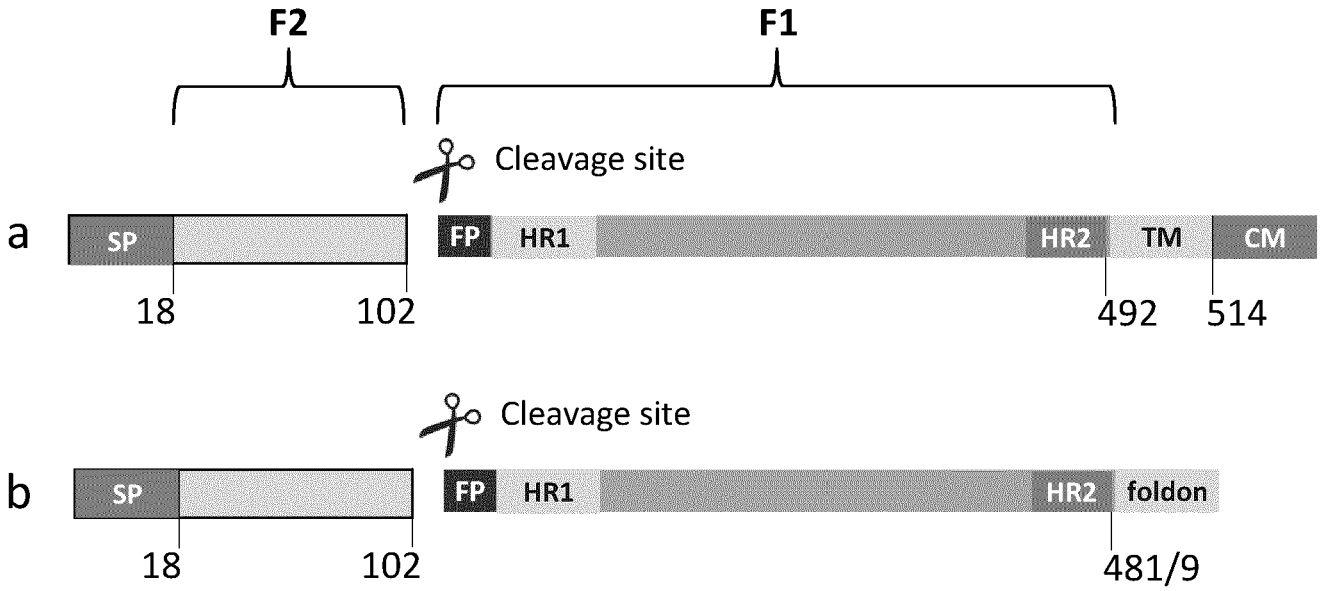

F2
F1
Cleavage site
a
SP
FP
HR1
HR2
TM
CM
18
102
492
514
Cleavage site
b
SP
FP
HR1
HR2
foldon
18
102
481/9

Fig. 1

A

MPV201285	*DQLARE*	*EQIENP*$_{98}$*RRRR*$_{102}$	**F$_{103}$V**
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MPV210502	*DQLA*$_{90}$--	------*RRRR*	**F$_{103}$V**
MPV210500	*DQLA*$_{90}$*RRRR*	-*I*$_{95}$*ENP*$_{98}$*RRRR*	**F$_{103}$V**
MPV210507	*DQ*$_{88}$*RRRR*	*E*$_{93}$*QIENP*$_{98}$*RRRR*	**F$_{103}$V**
MPV210509	*DQLA*$_{90}$*RRRR*	*E*$_{92}$*EQIENPRRRR*	**F$_{103}$V**

F2 domain F1 domain

B

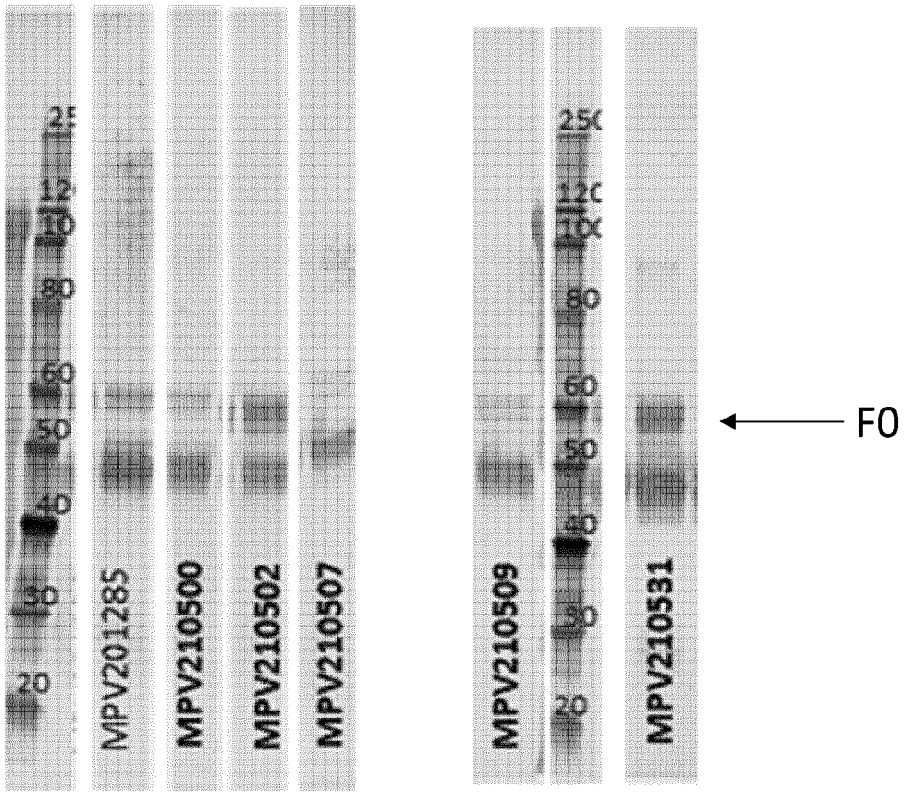


Fig. 2

C

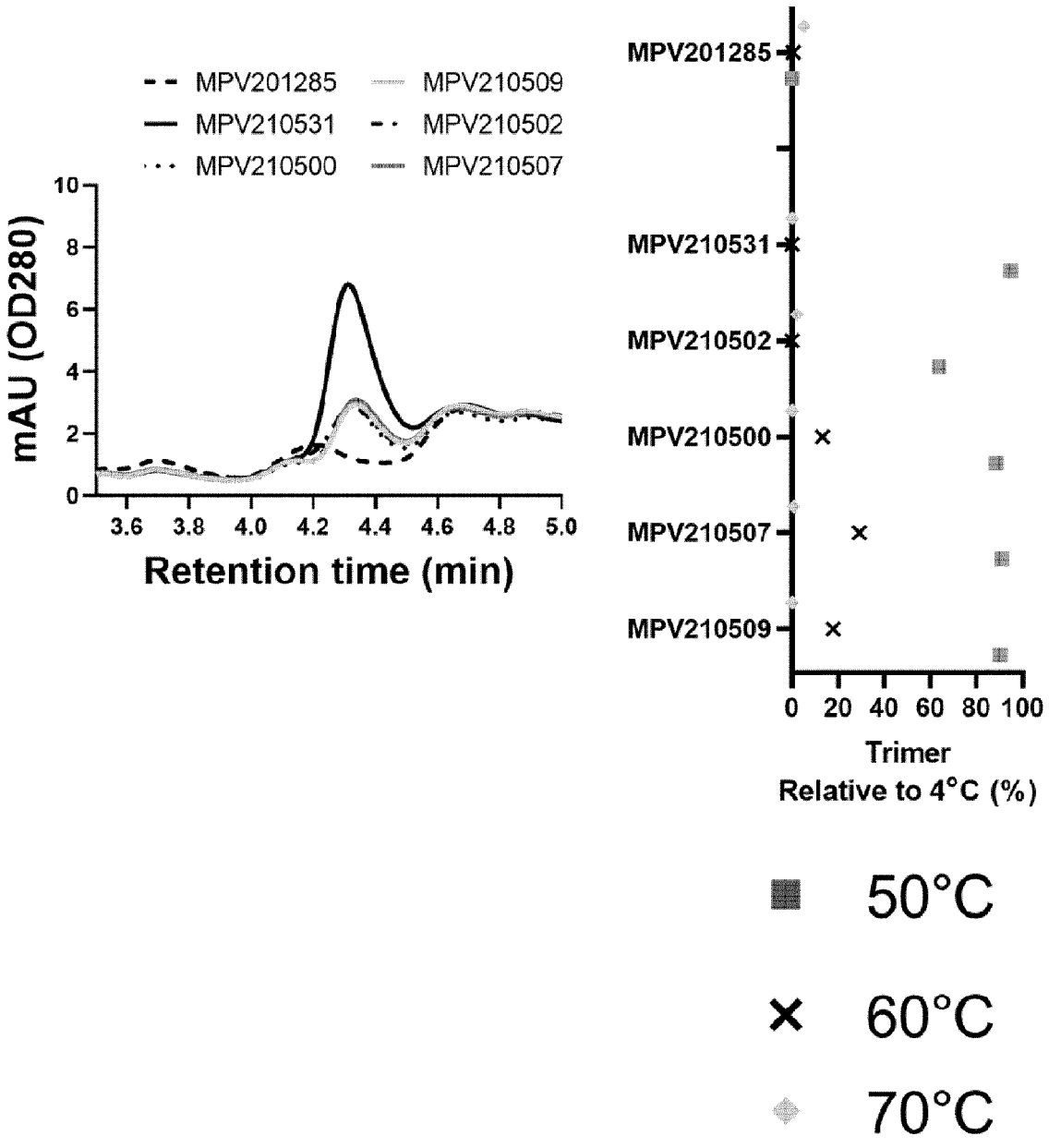


Fig. 2 continued

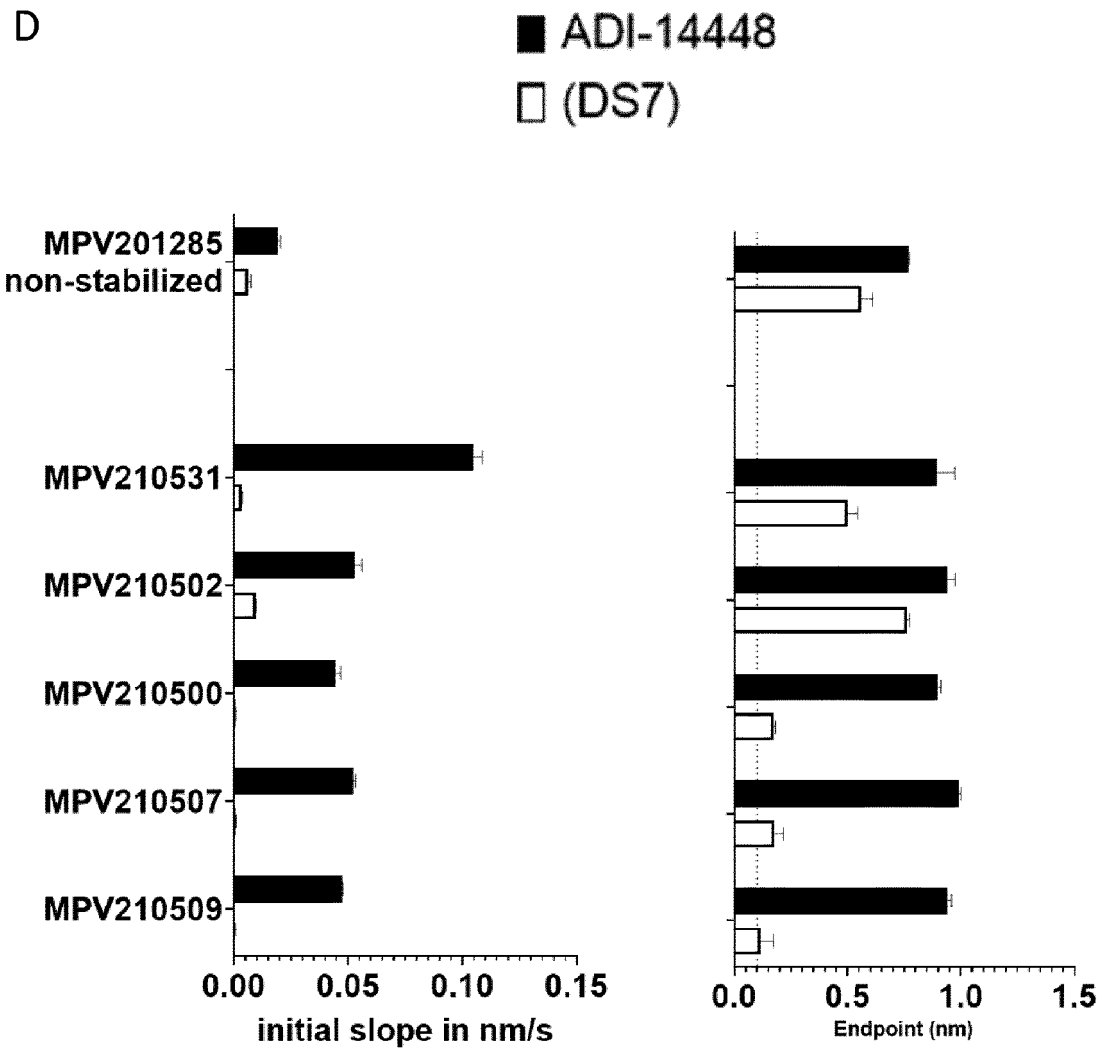


Fig. 2 continued

A

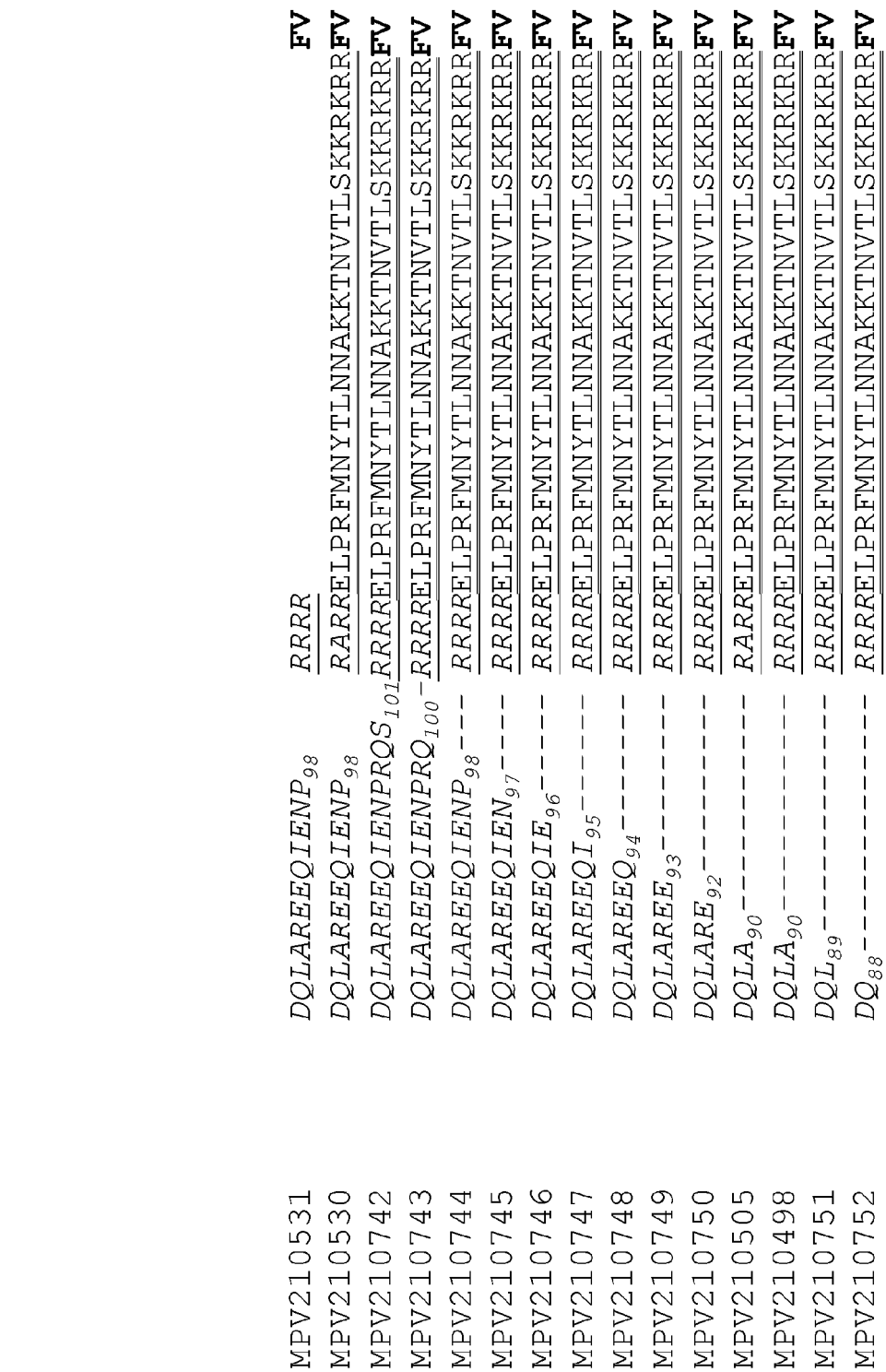
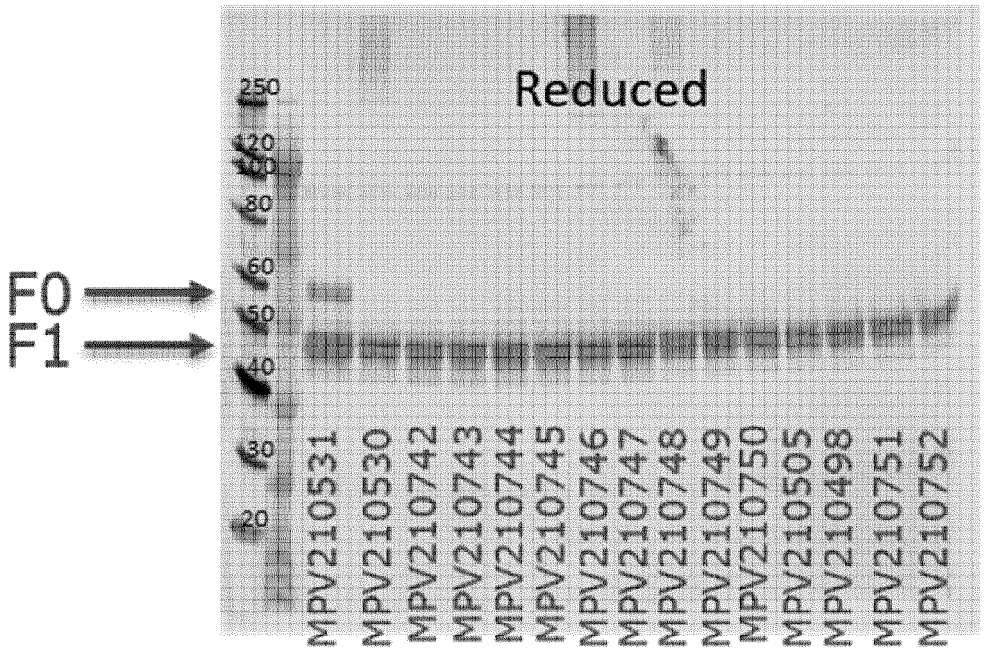

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MPV210751 DQL89----------- RRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFV
MPV210752 DQ88------------ RRRRELPRFMNYTLNNAKKTNVTLSKKRKRRFV

Fig. 3

B



C

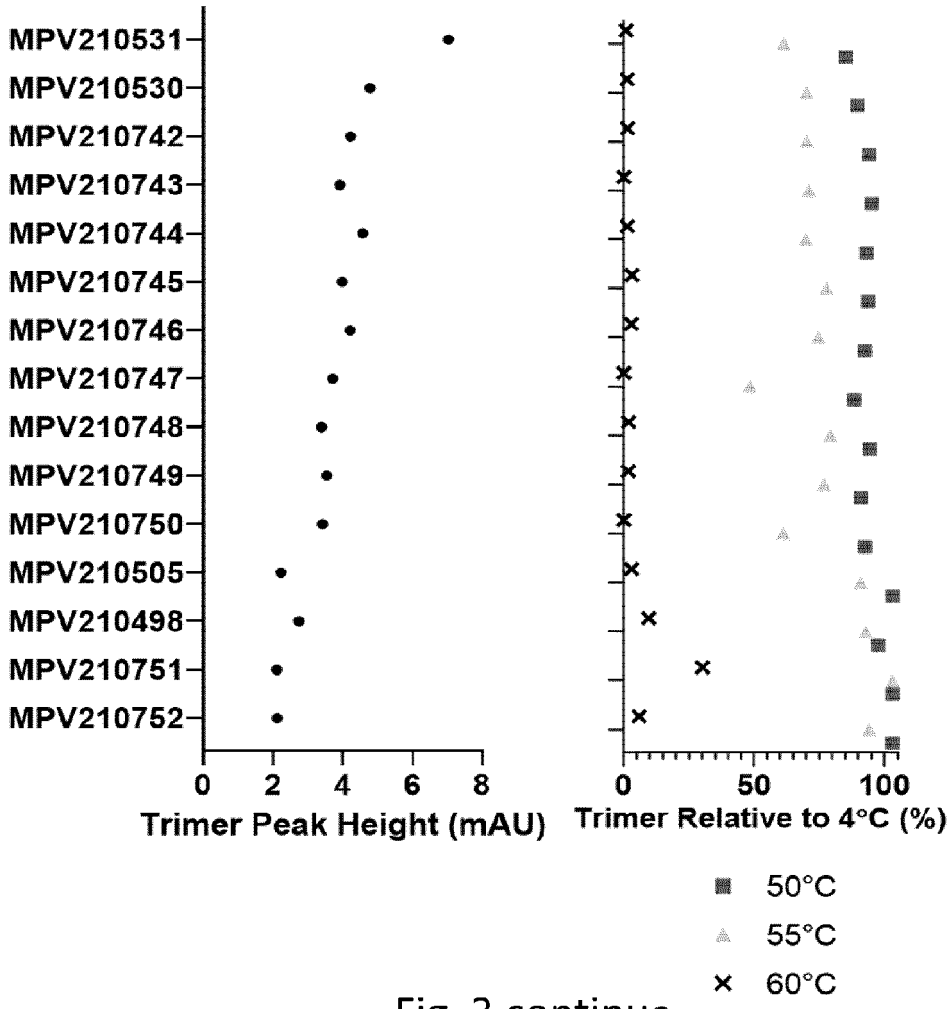


Fig. 3 continue

D

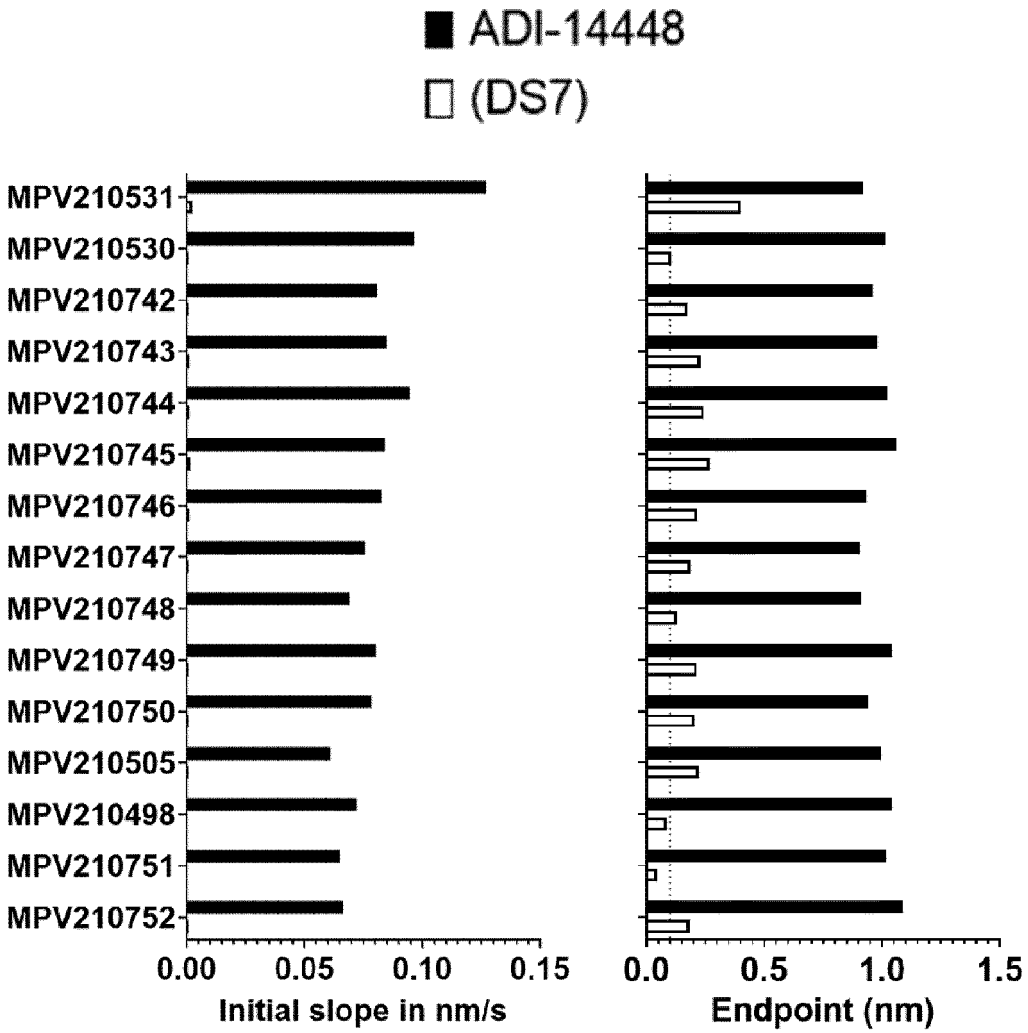


Fig. 3 continued

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MPV211242	I						489	No
MPV211243	F						489	No
MPV211244		I					489	No
MPV211245		L					489	No
MPV211246		F					489	No
MPV211247			I				489	No
MPV211248			V				489	No
MPV211249			L				489	No
MPV211250				V			489	No
MPV211251				L			489	No
MPV211252					I		489	No
MPV211253					V		489	No
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MPV211256						V	489	No
MPV211257						L	489	No

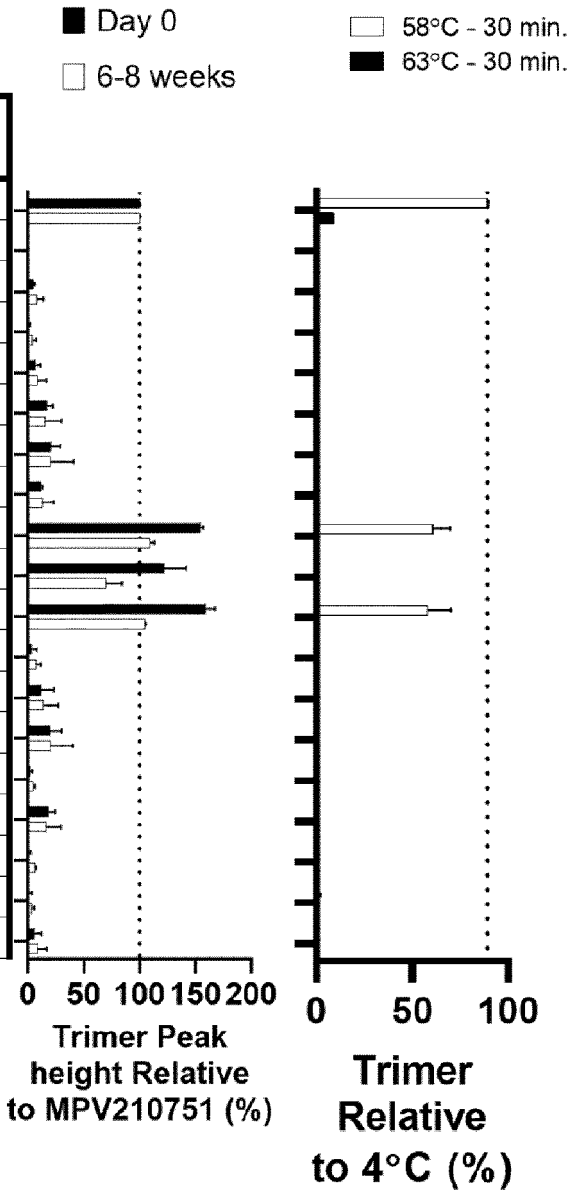
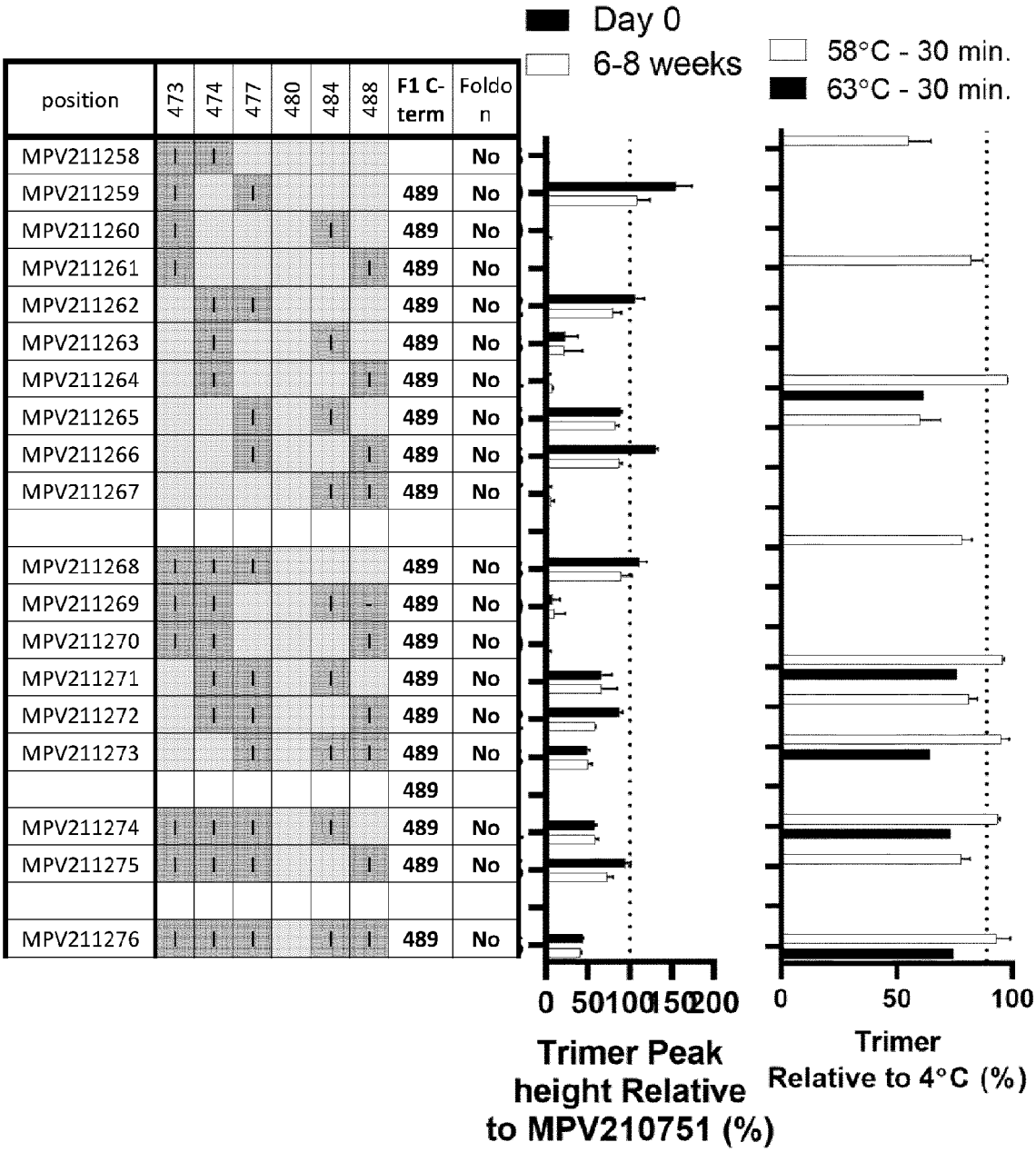
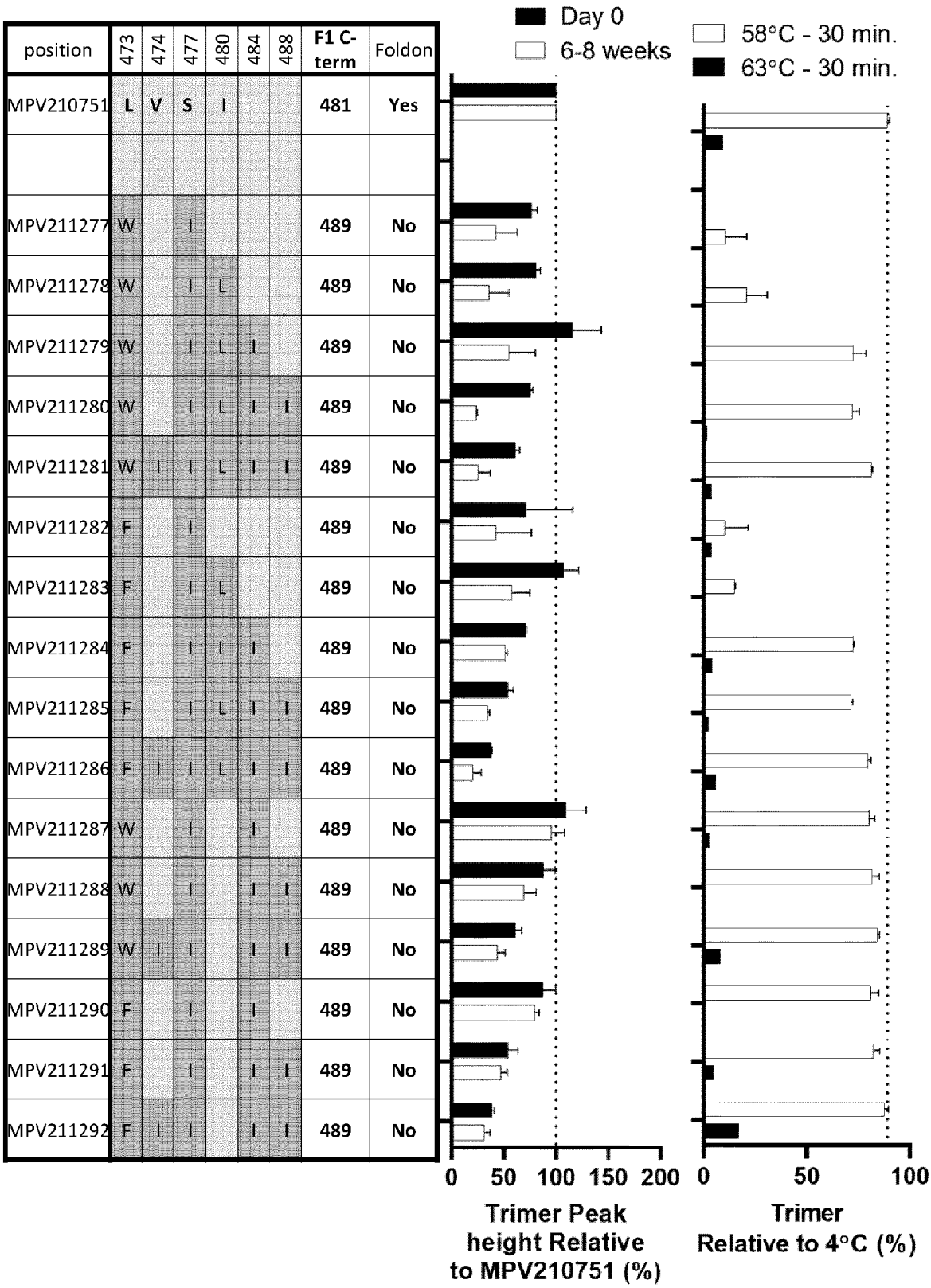


Fig. 4



position	473	474	477	480	484	488	F1 C-term	Foldon
MPV211258	I	I						No
MPV211259	I		I				489	No
MPV211260	I				I		489	No
MPV211261	I					I	489	No
MPV211262		I	I				489	No
MPV211263		I			I		489	No
MPV211264		I				I	489	No
MPV211265			I		I		489	No
MPV211266			I			I	489	No
MPV211267					I	I	489	No
MPV211268	I	I	I				489	No
MPV211269	I	I			I	-	489	No
MPV211270	I	I				I	489	No
MPV211271		I	I		I		489	No
MPV211272		I	I			I	489	No
MPV211273			I		I	I	489	No
							489	
MPV211274	I	I	I		I		489	No
MPV211275	I	I	I			I	489	No
MPV211276	I	I	I		I	I	489	No

Fig. 4 continued

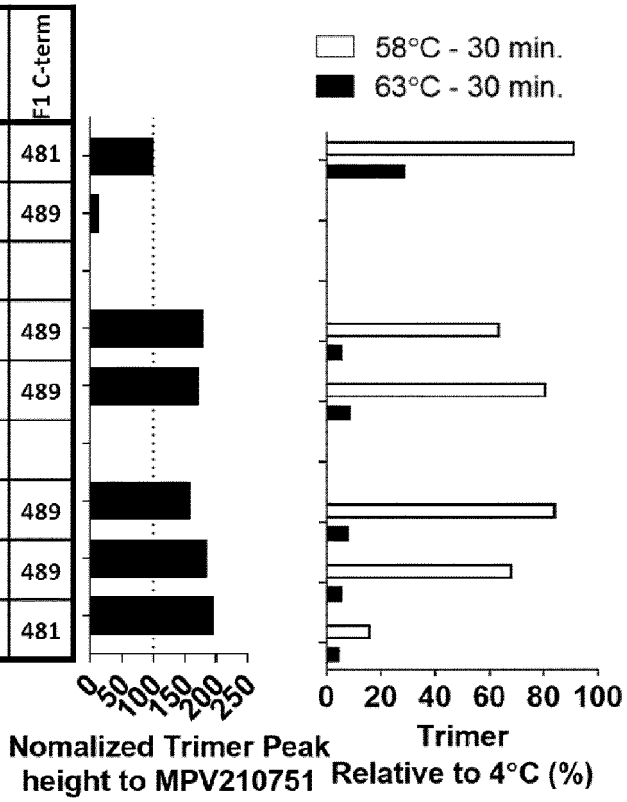


position	473	474	477	480	484	488	F1 C-term	Foldon
MPV210751	L	V	S	I			481	Yes
MPV211277	W		I				489	No
MPV211278	W		I	L			489	No
MPV211279	W		I	L	I		489	No
MPV211280	W		I	L	I	I	489	No
MPV211281	W	I	I	L	I	I	489	No
MPV211282	F		I				489	No
MPV211283	F		I	L			489	No
MPV211284	F		I	L	I		489	No
MPV211285	F		I	L	I	I	489	No
MPV211286	F	I	I	L	I	I	489	No
MPV211287	W		I		I		489	No
MPV211288	W		I		I	I	489	No
MPV211289	W	I	I		I	I	489	No
MPV211290	F		I		I		489	No
MPV211291	F		I		I	I	489	No
MPV211292	F	I	I		I	I	489	No

Fig. 4 continued

A

position	473	475	476	477	478	479	484	Foldon	F1 C-term
MPV210751	L	D	Q	S	N	R		yes	481
MPV211241	L	D	Q	S	N	R	A	no	489
MPV211247				I				no	489
MPV211287	W			I			I	no	489
MPV211917	W		K	F			I	no	489
MPV211918	W	R	K	F	D	E	I	no	489
MPV211919	W		K	F				no	481



B

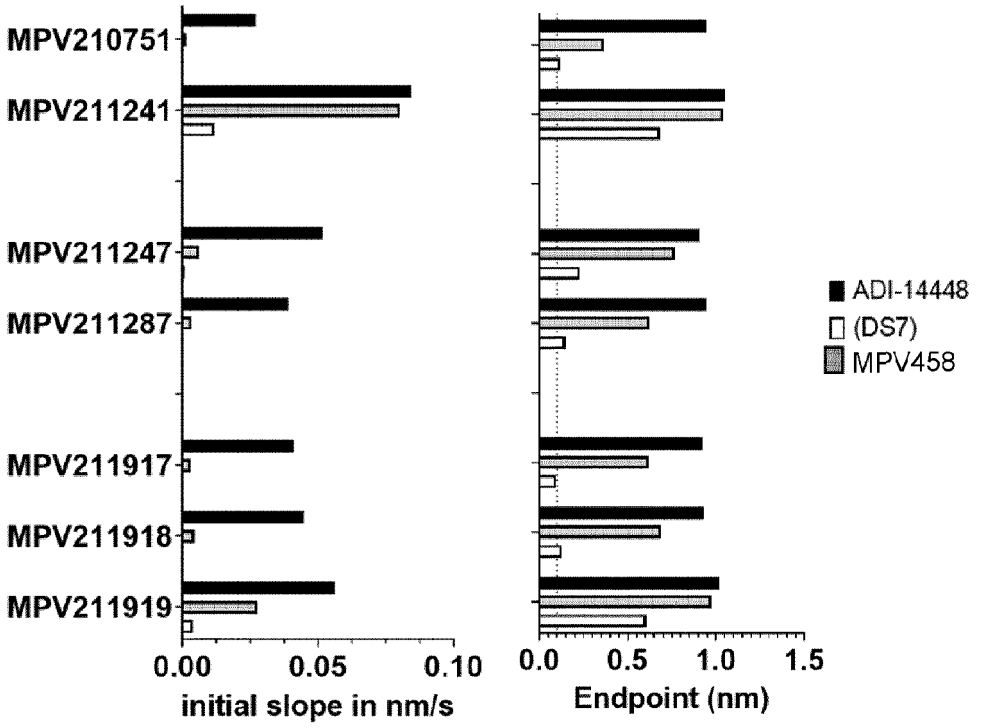
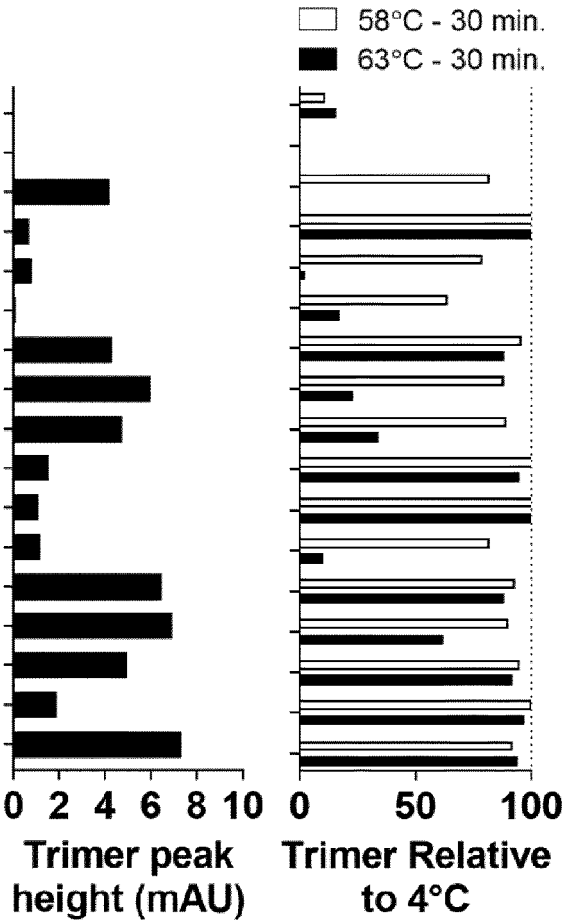


Fig. 5

A

	V112	D209	V231	E453
MPV211940	-	-	-	Q
MPV212018	R	-	-	Q
MPV212019	-	E	-	Q
MPV212020	-	-	I	Q
MPV212021	-	-	-	P
MPV212022	R	E	-	Q
MPV212023	R	-	I	Q
MPV212024	R	-	-	P
MPV212025	-	E	I	Q
MPV212026	-	E	-	P
MPV212027	-	-	I	P
MPV212028	R	E	I	Q
MPV212029	R	-	I	P
MPV212030	R	E	-	P
MPV212031	-	E	I	P
MPV212032	R	E	I	P



B

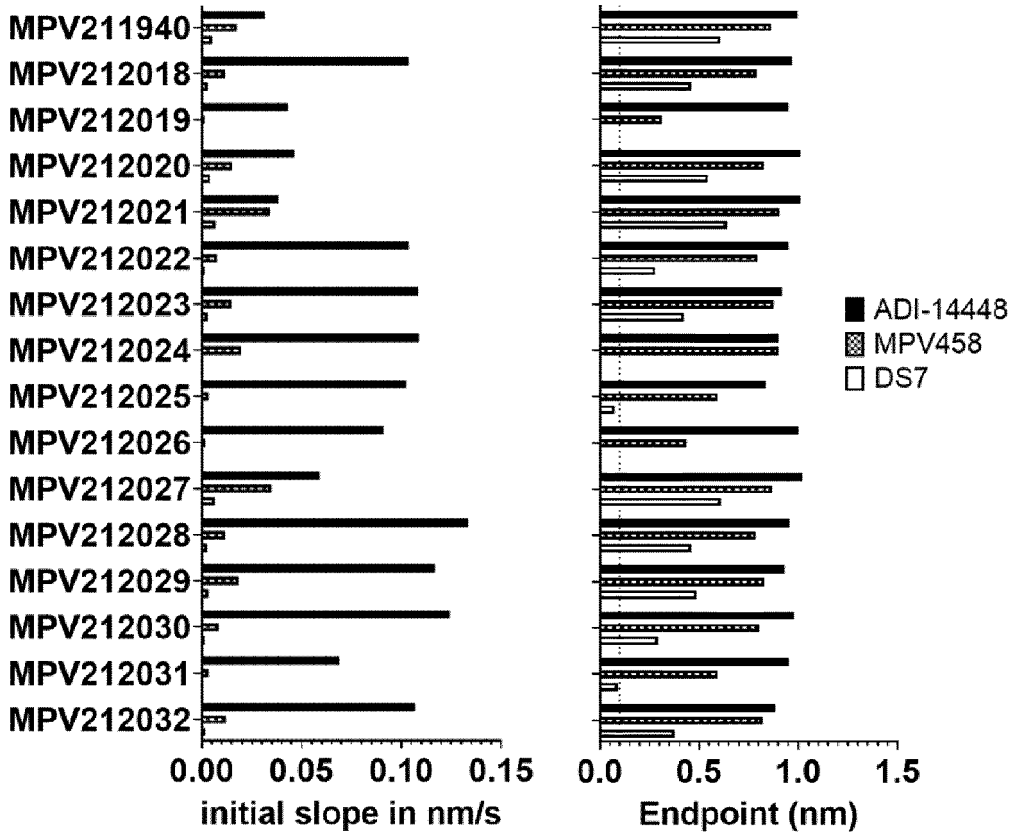
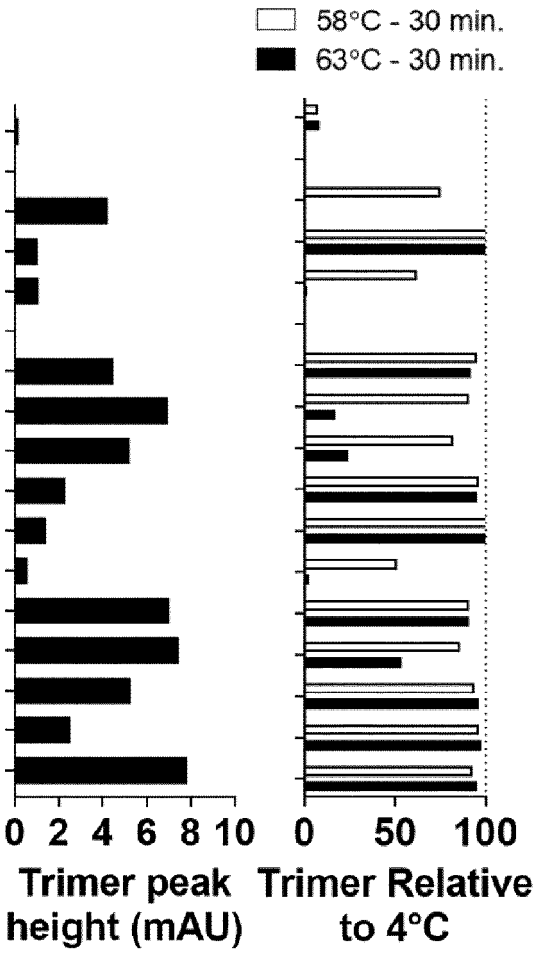


Fig .6

A

	V112	D209	V231	E453
MPV211942	-	-	-	Q
MPV212033	R	-	-	Q
MPV212034	-	E	-	Q
MPV212035	-	-	I	Q
MPV212036	-	-	-	P
MPV212037	R	E	-	Q
MPV212038	R	-	I	Q
MPV212039	R	-	-	P
MPV212040	-	E	I	Q
MPV212041	-	E	-	P
MPV212042	-	-	I	P
MPV212043	R	E	I	Q
MPV212044	R	-	I	P
MPV212045	R	E	-	P
MPV212046	-	E	I	P
MPV212047	R	E	I	P



B

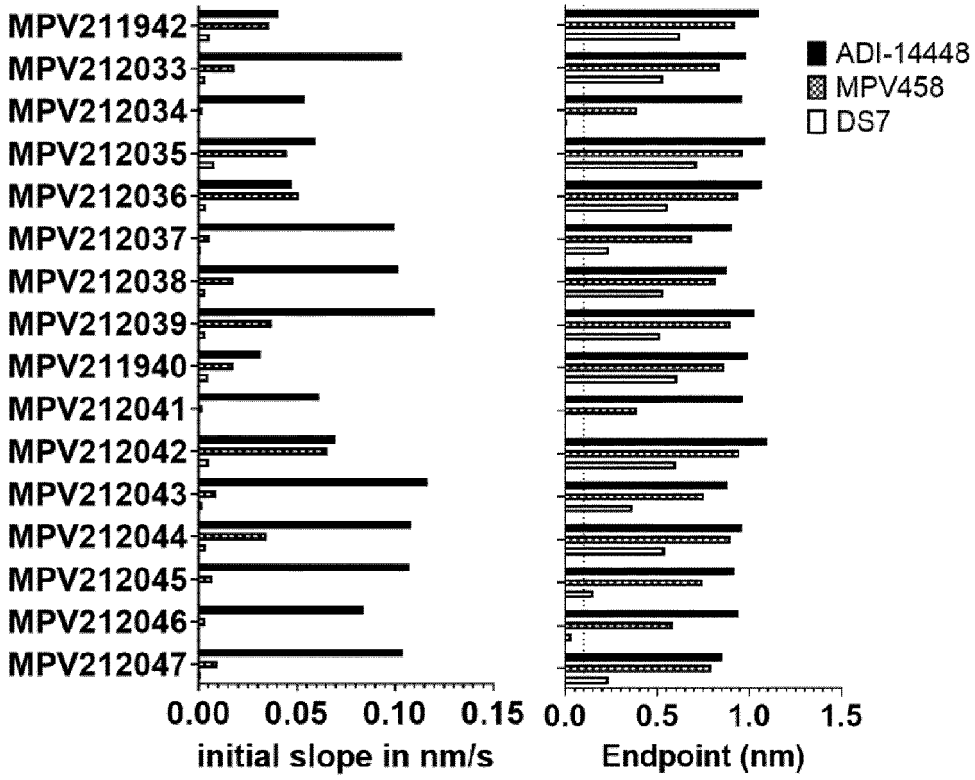


Fig. 7

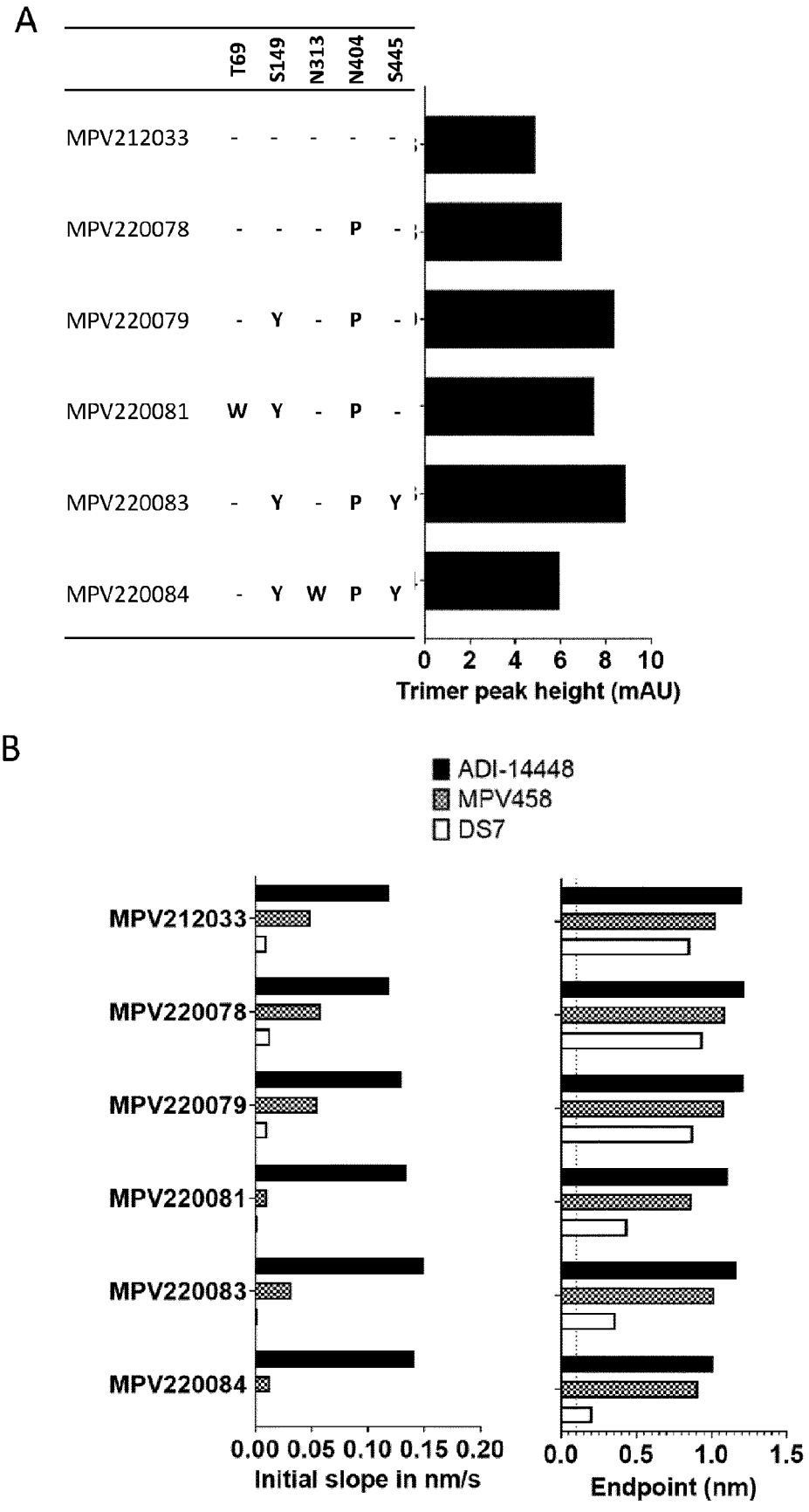

A
T69
S149
N313
N404
S445
MPV212033 - - - - -
MPV220078 - - - P -
MPV220079 - Y - P -
MPV220081 W Y - P -
MPV220083 - Y - P Y
MPV220084 - Y W P Y
0 2 4 6 8 10
Trimer peak height (mAU)
B
ADI-14448
MPV458
DS7
MPV212033
MPV220078
MPV220079
MPV220081
MPV220083
MPV220084
0.00 0.05 0.10 0.15 0.20
Initial slope in nm/s
0.0 0.5 1.0 1.5
Endpoint (nm)

Fig 8

C

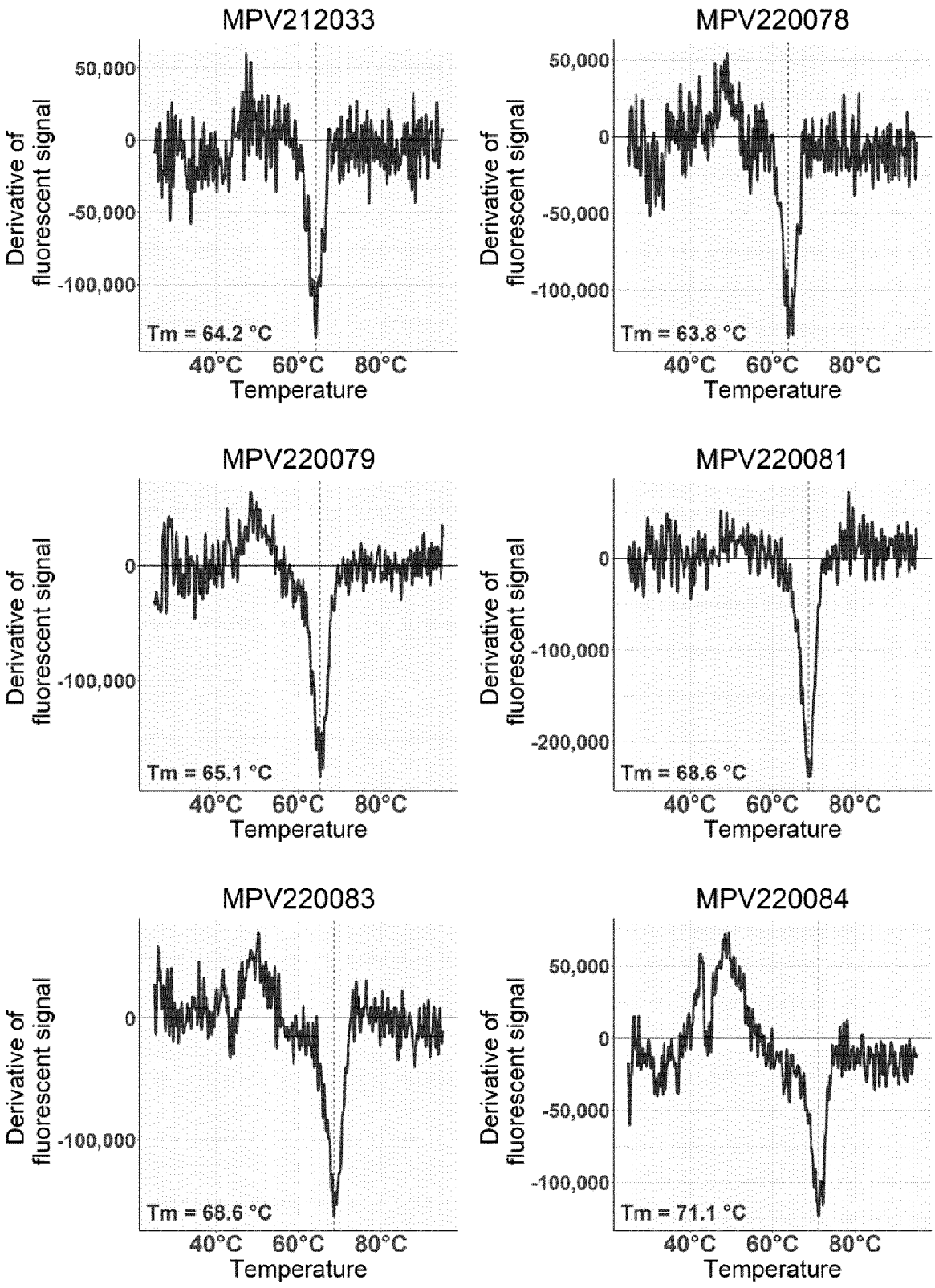
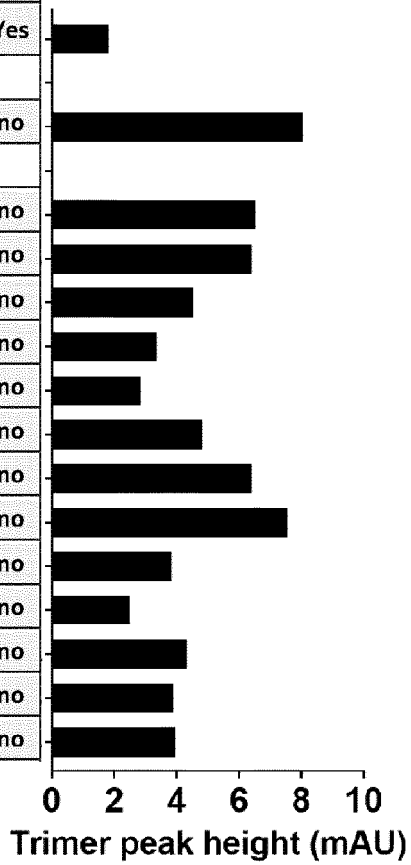


Fig. 8 - continued

A

	473	474	477	484	485	F1 C-term	Foldon
	L	V	S	A	N		
MPV210751						481	Yes
MPV212047			*			489	no
MPV212032	W		I	I		489	no
MPV220115			I			489	no
MPV220116			I	I		489	no
MPV220117		I	I	I		489	no
MPV220118			I	I	I	489	no
MPV220119	I		I	I		489	no
MPV220120	Y		I	I		489	no
MPV220121	I	I		I		489	no
MPV220122	I	I	I	I		489	no
MPV220123	I	I	I	I	I	489	no
MPV220124			L	I		489	no
MPV220125			I	L		489	no
MPV220126			L	L		489	no

*L473W, D475R, Q476K, S477F, N478D, R479E, A484I



B

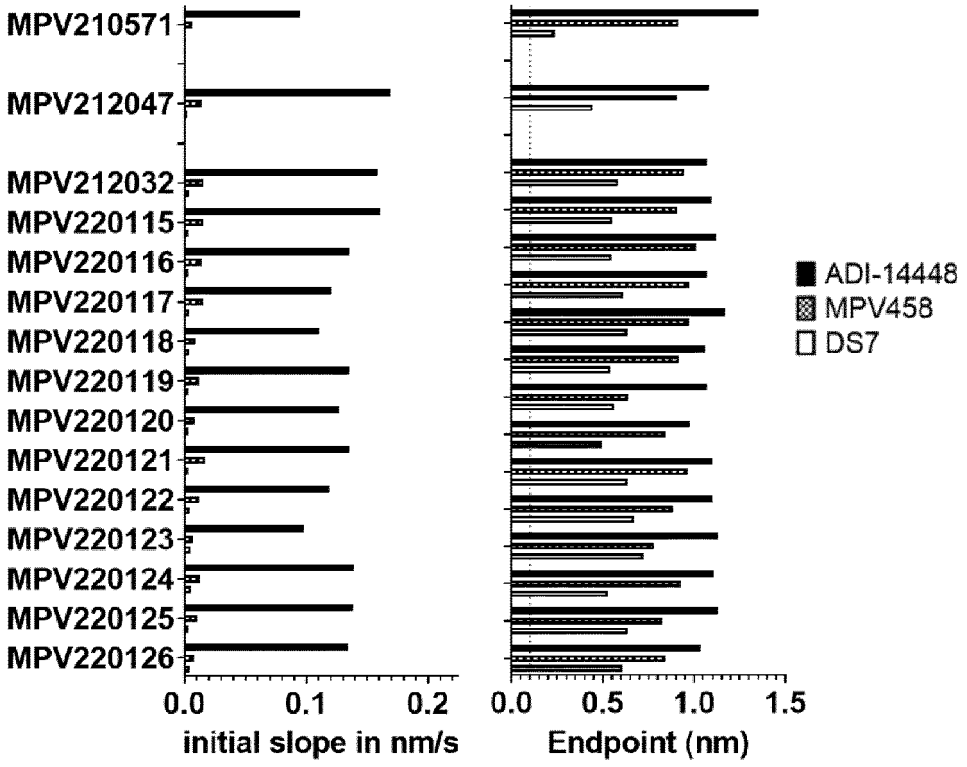


Fig. 9

C

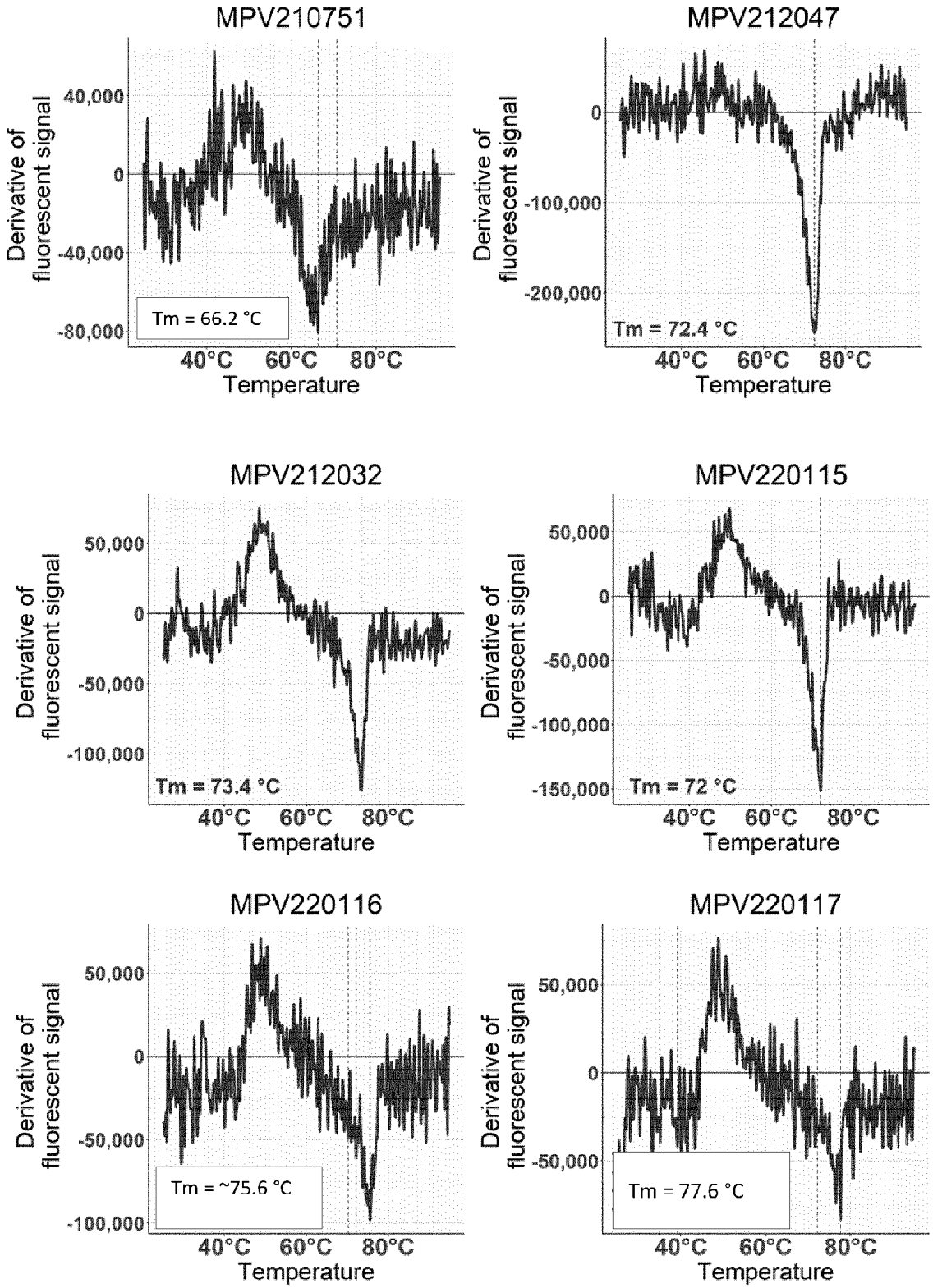


Fig. 9 continued

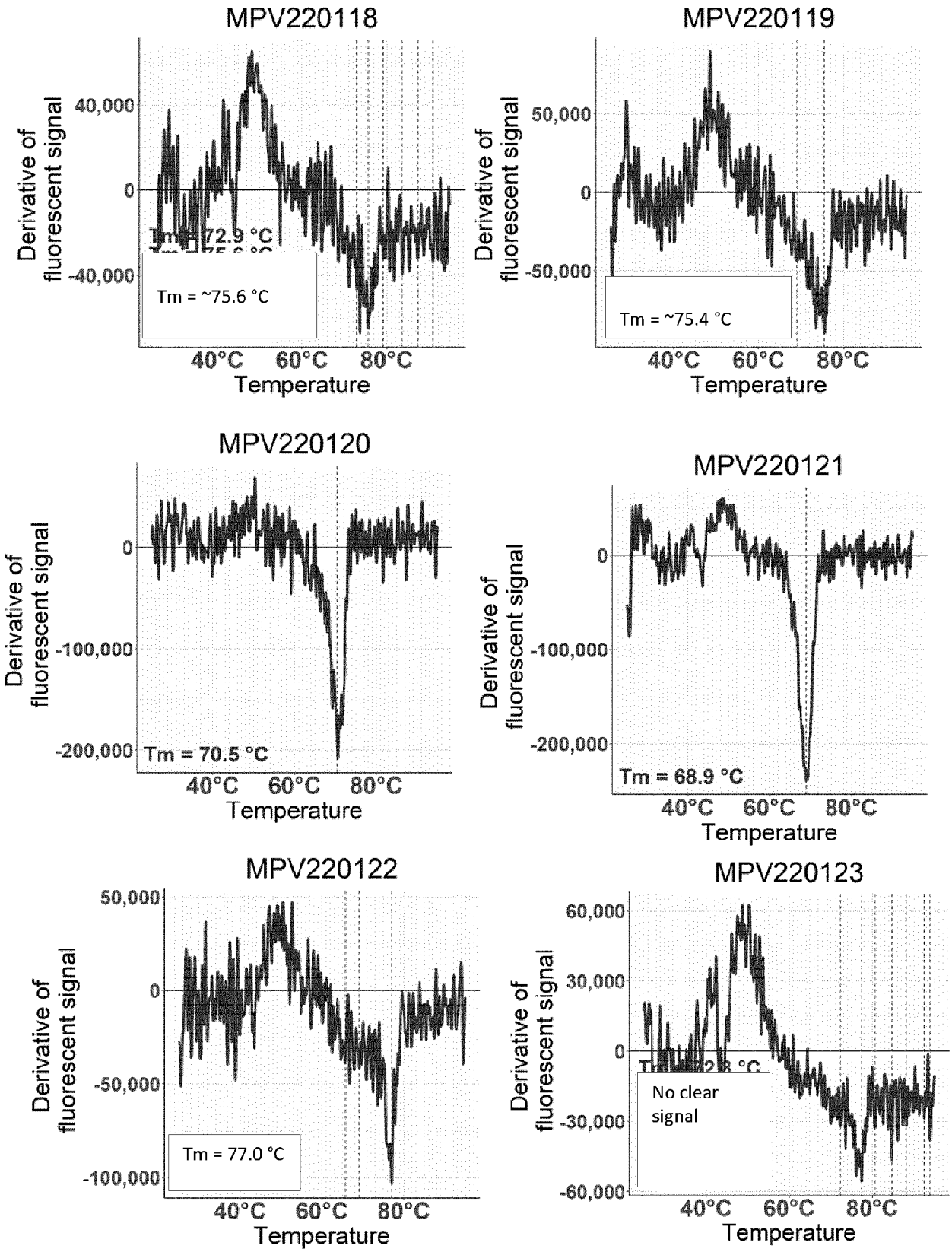


Fig.9 continued

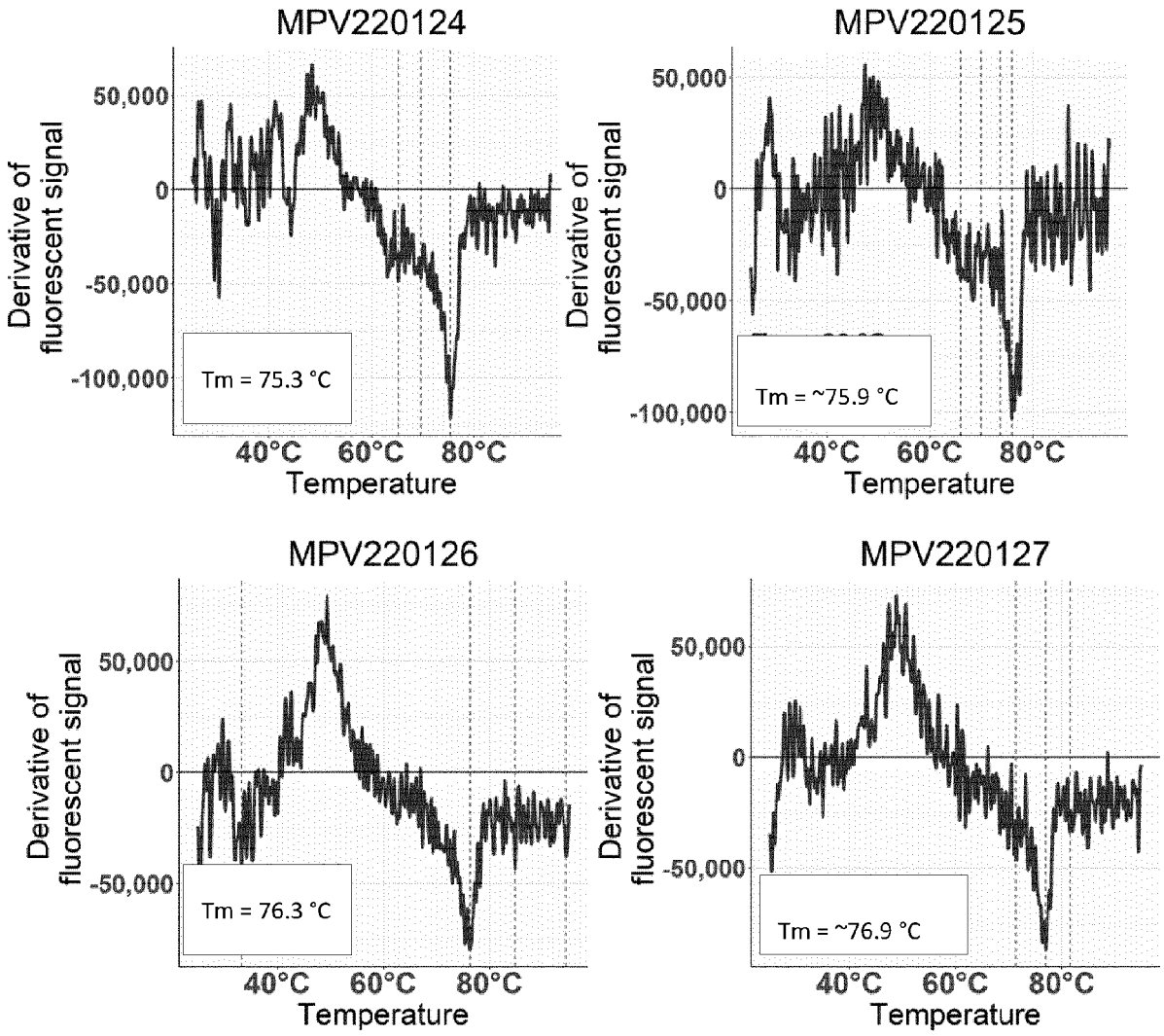
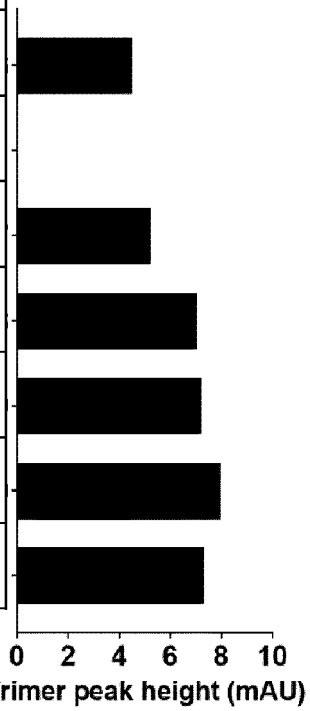


Fig. 9 continued

A

	69	112	149	209	231	313	404	445	453
	T	V	S	D	V	N	N	S	E
MPV220116		R		E	I				P
MPV220127		R		E	I		P		P
MPV220128		R	Y	E	I		P		P
MPV220129	W	R	Y	E	I		P		P
MPV220130		R	Y	E	I		P	Y	P
MPV220131		R	Y	E	I	W	P	Y	P



B

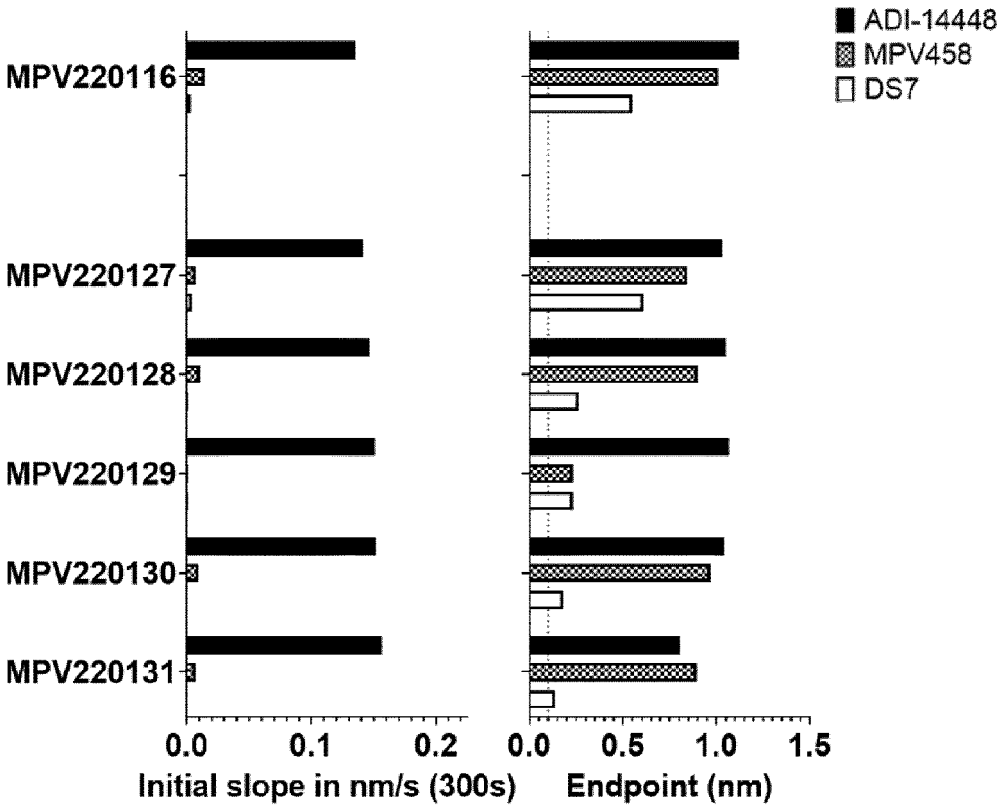


Fig. 10

C

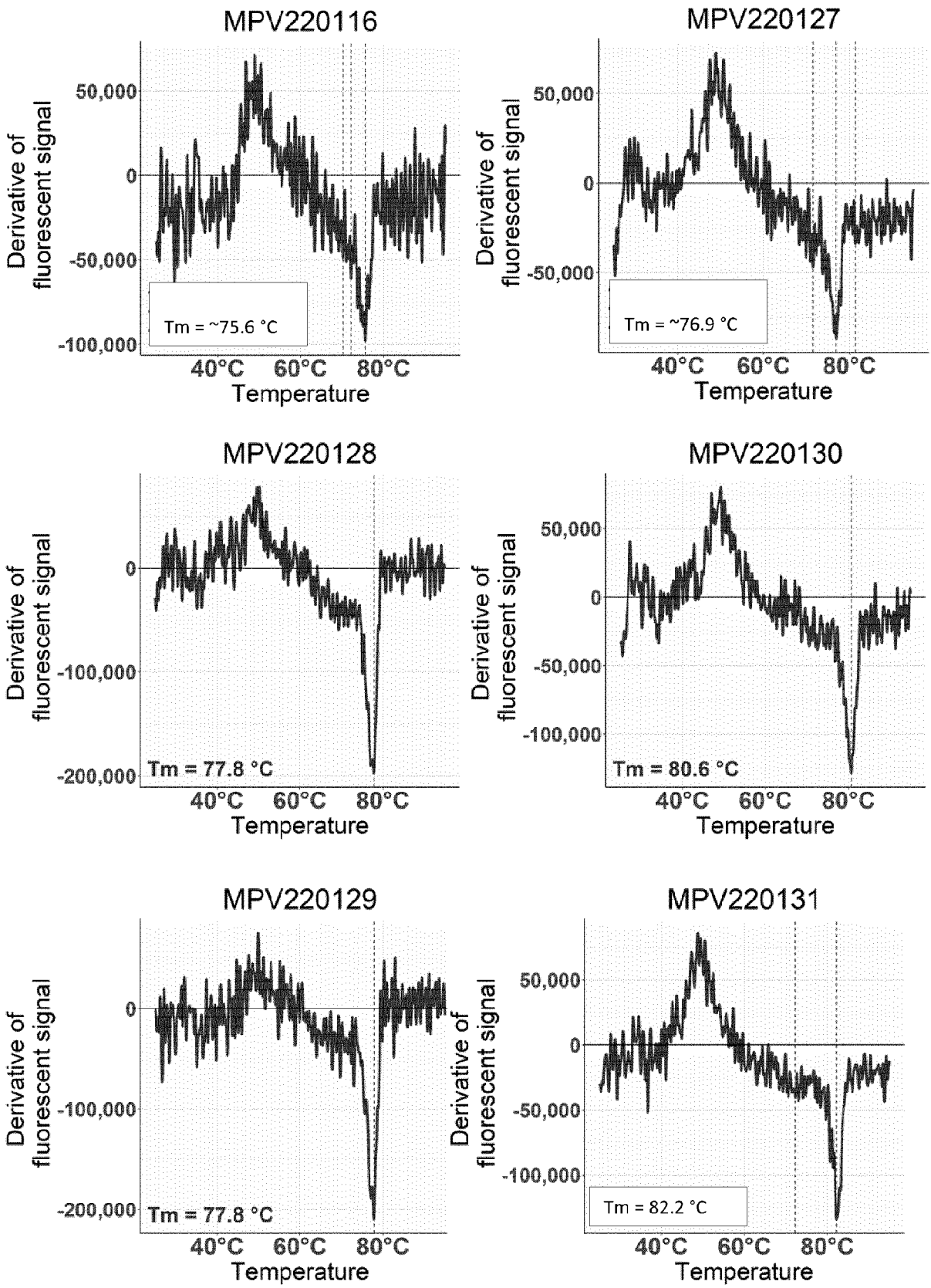
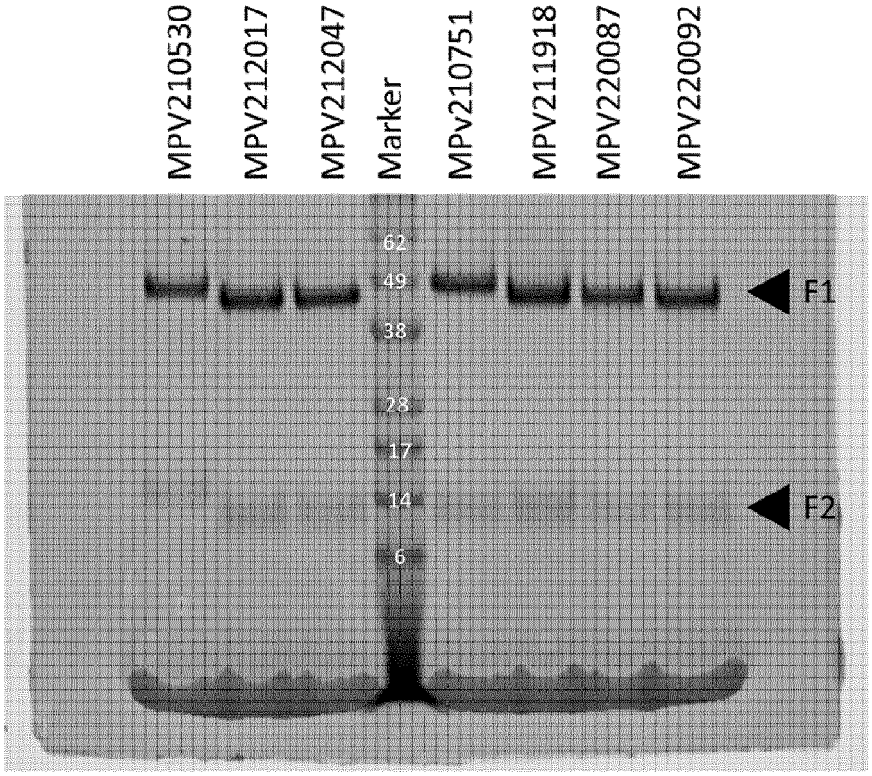


Fig. 10 - continued

A


MPV210530
MPV212017
MPV212047
Marker
MPv210751
MPV211918
MPV220087
MPV220092
62
49
38
28
17
14
6
F1
F2

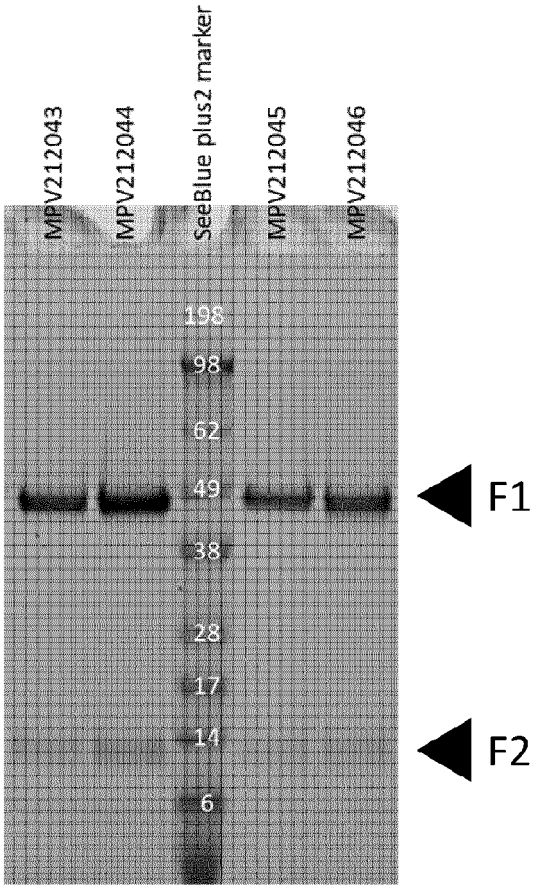

MPV212043
MPV212044
SeeBlue plus2 marker
MPV212045
MPV212046
198
98
62
49
38
28
17
14
6
F1
F2

Fig 11

B

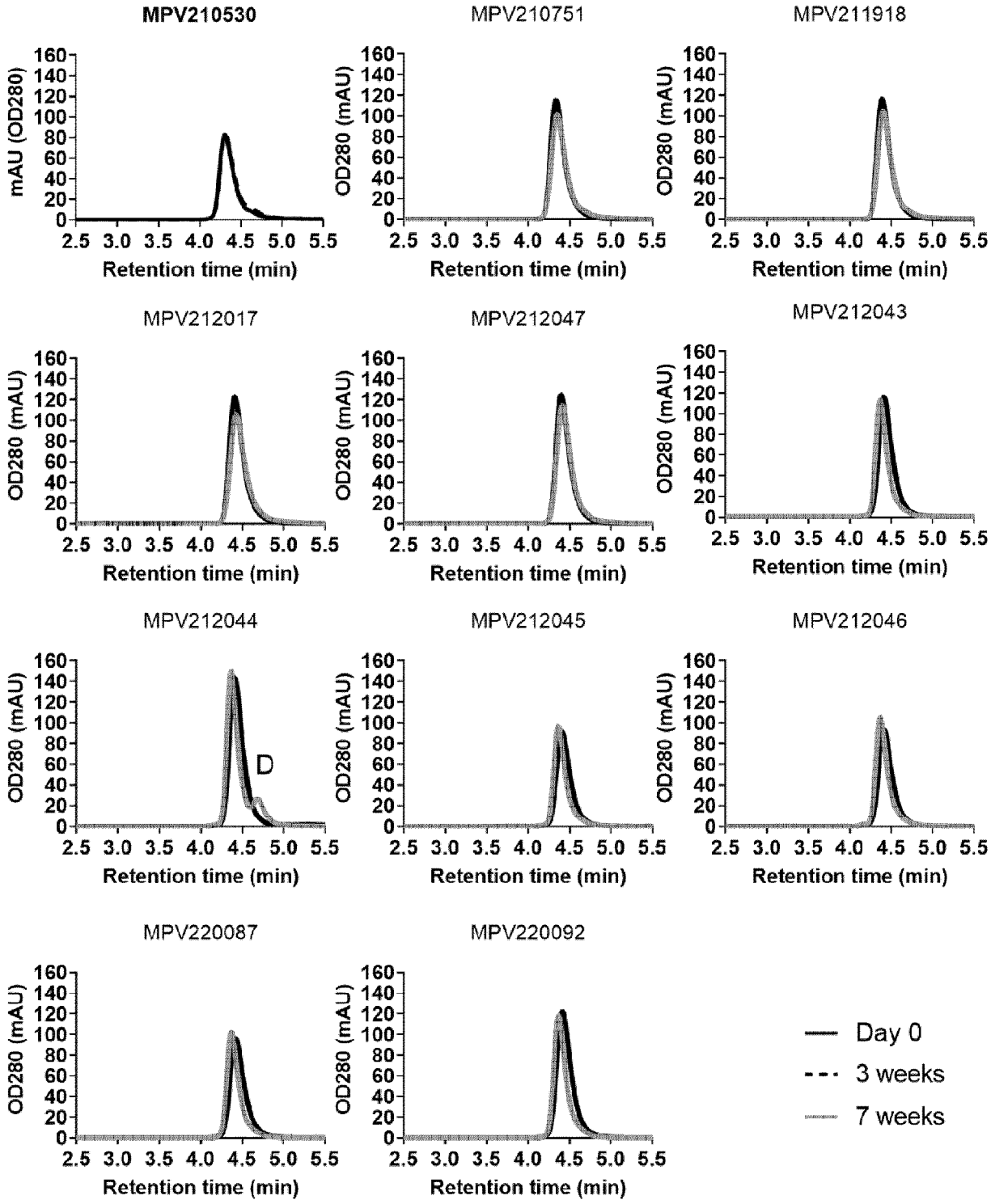


Fig 11 - continued

C

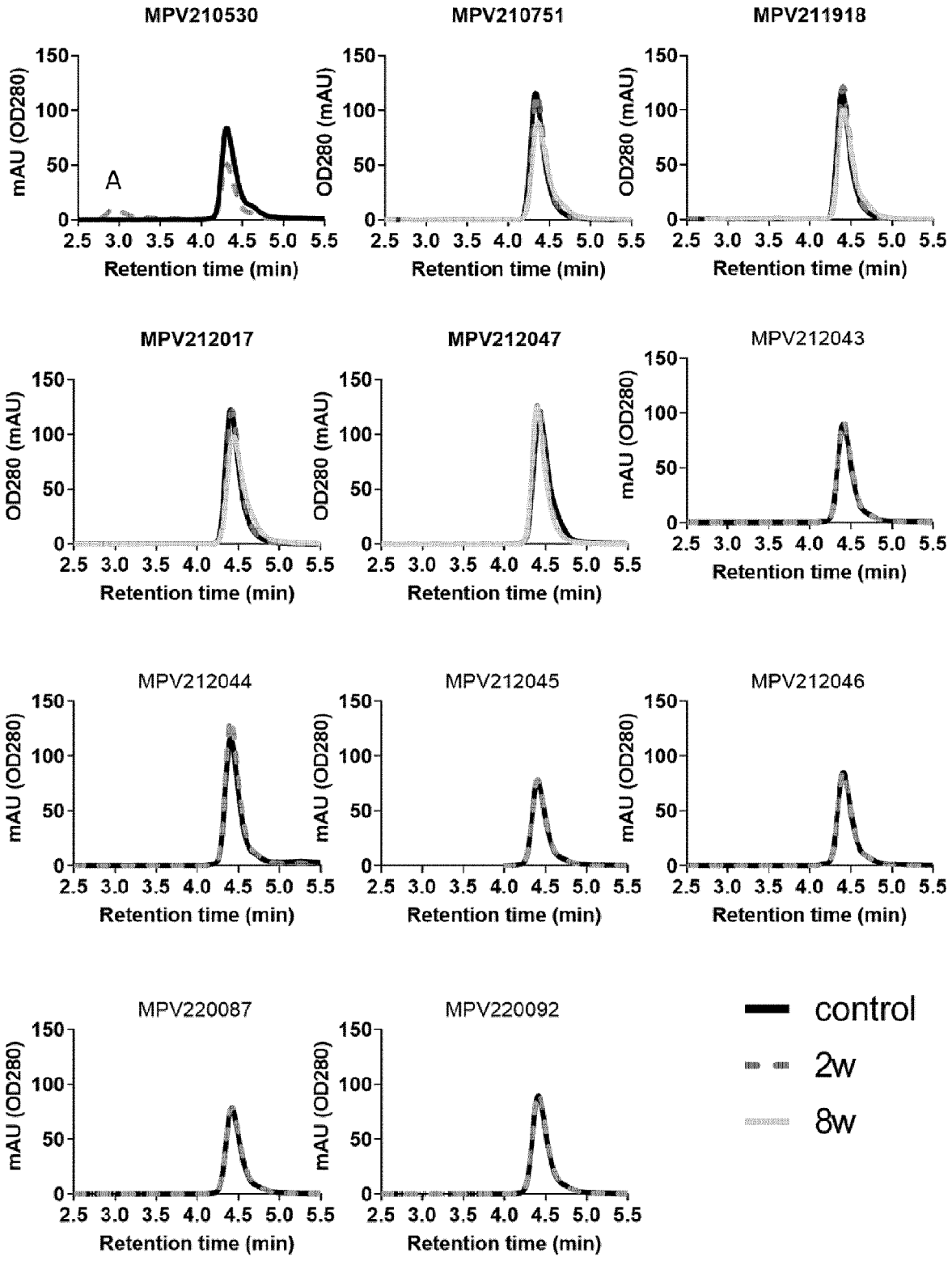


Fig 11 - continued

D

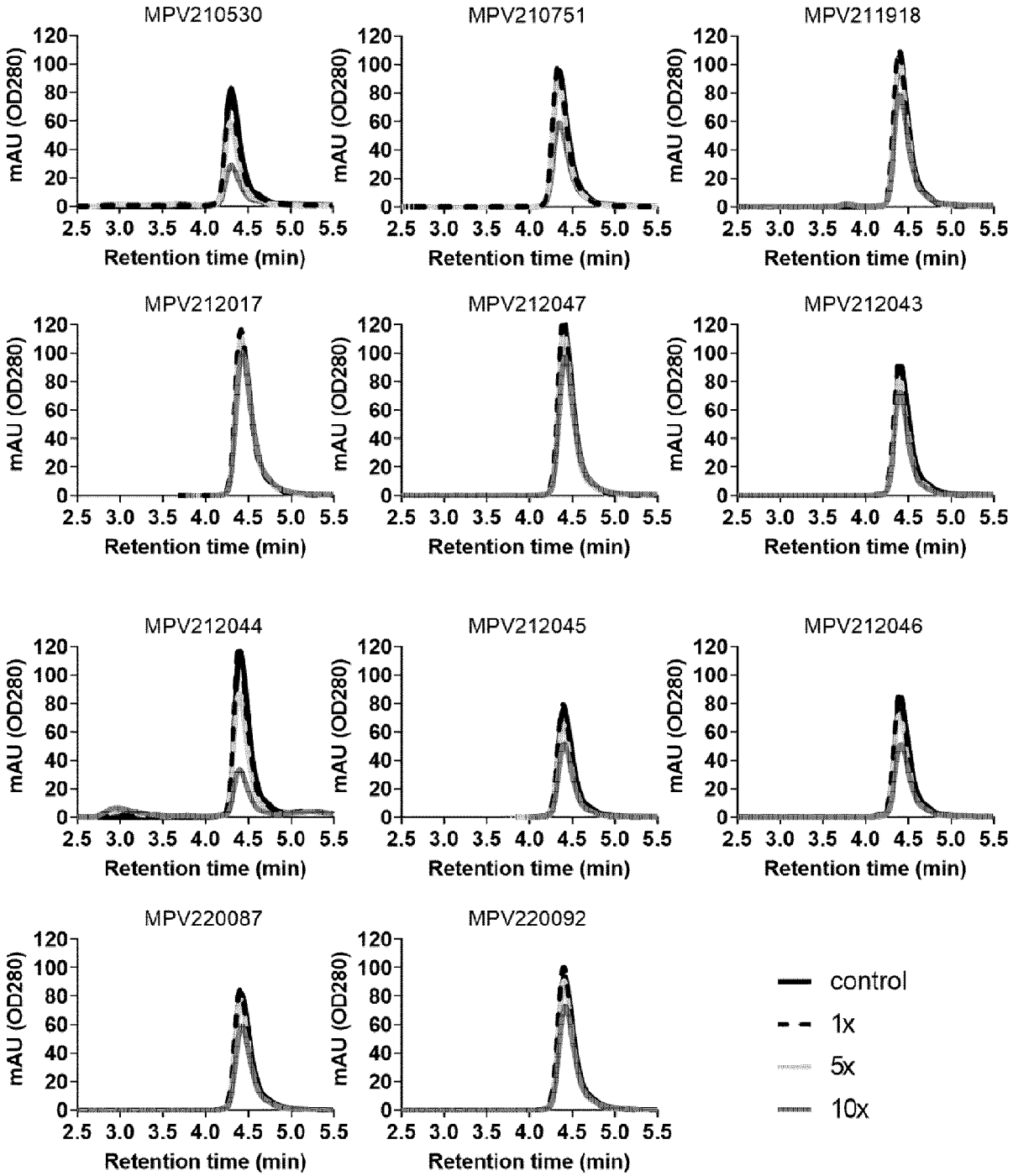


Fig 11 - continued

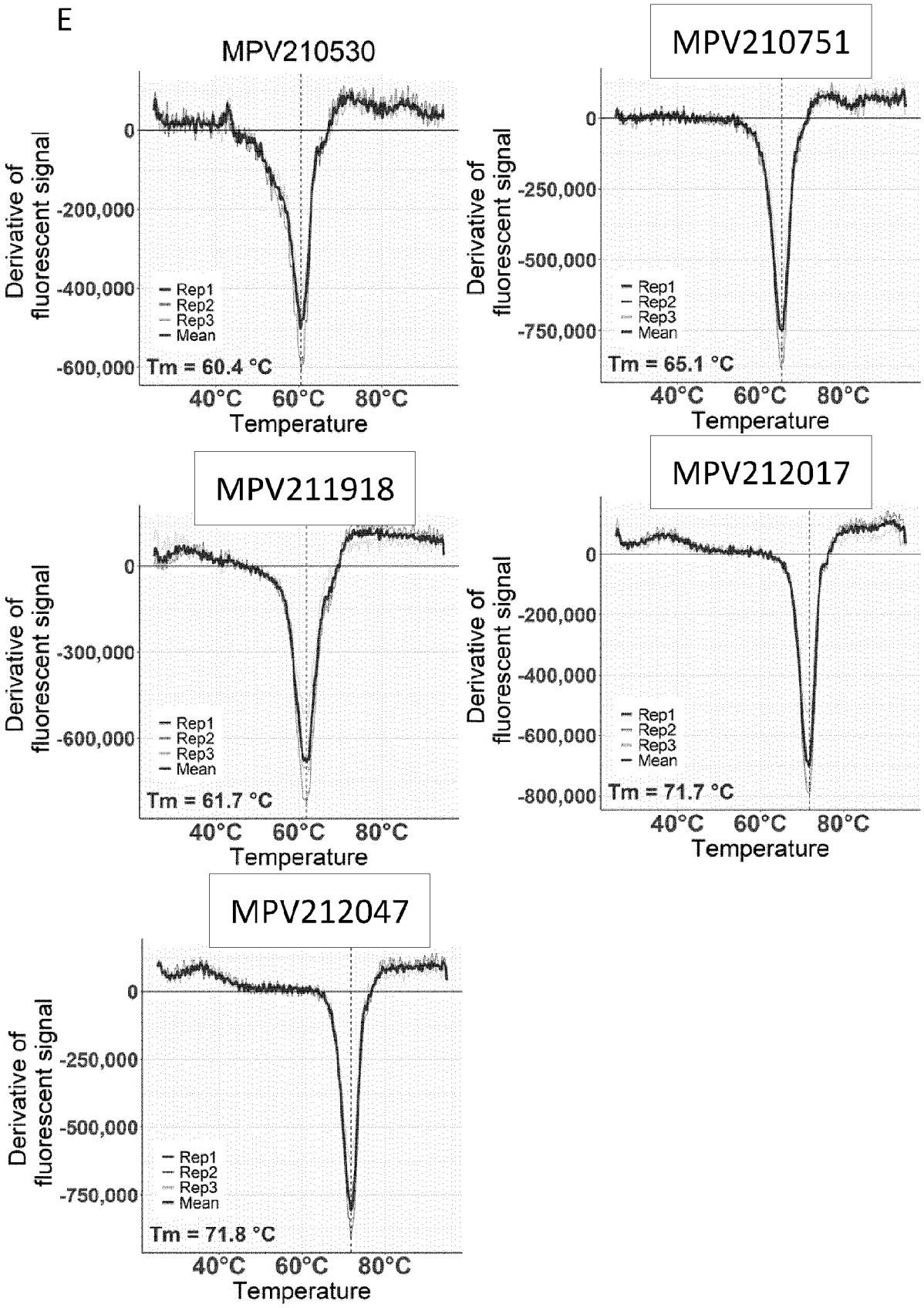


Fig 11 - continued

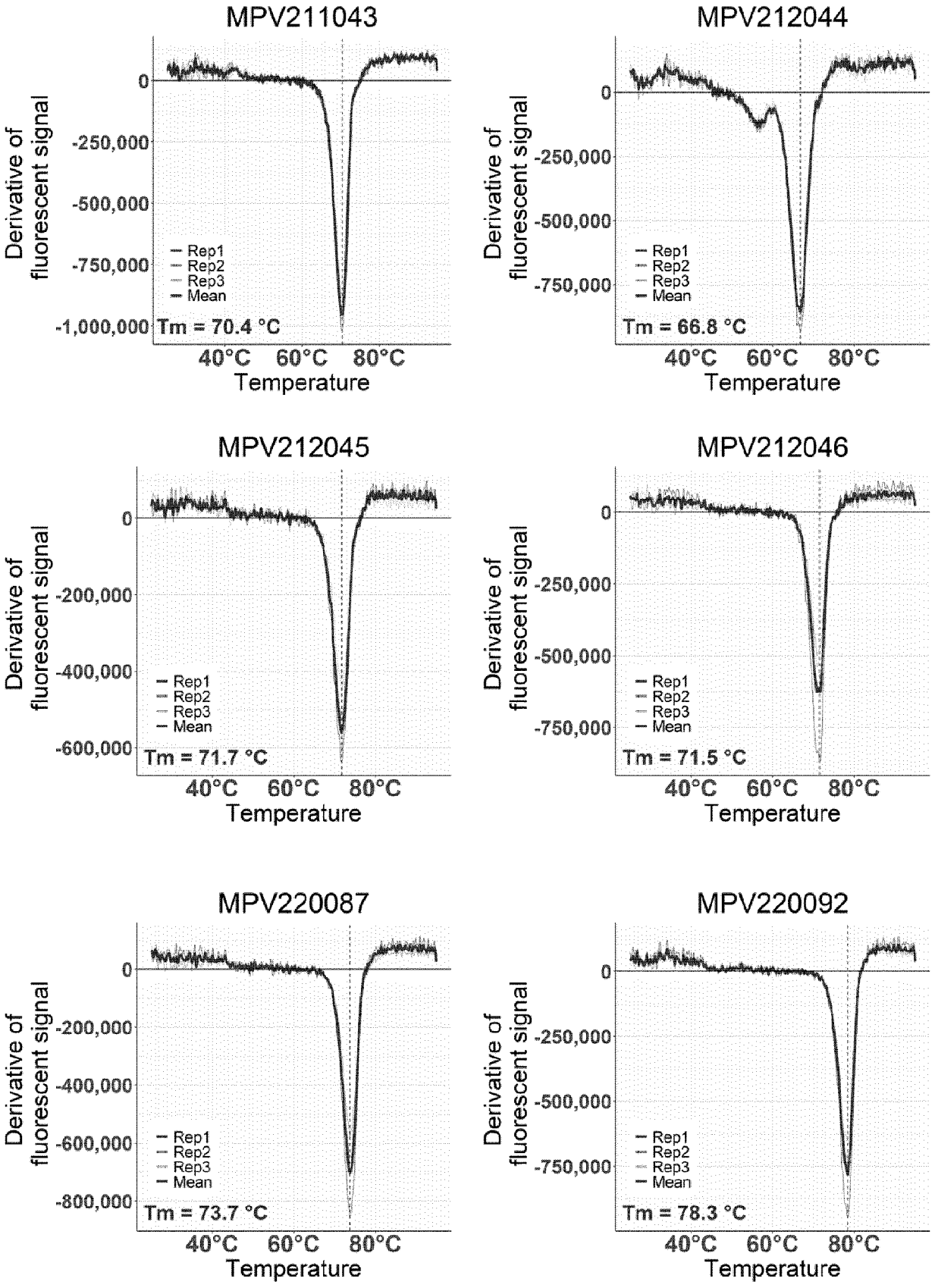


Fig 11 - continued

F

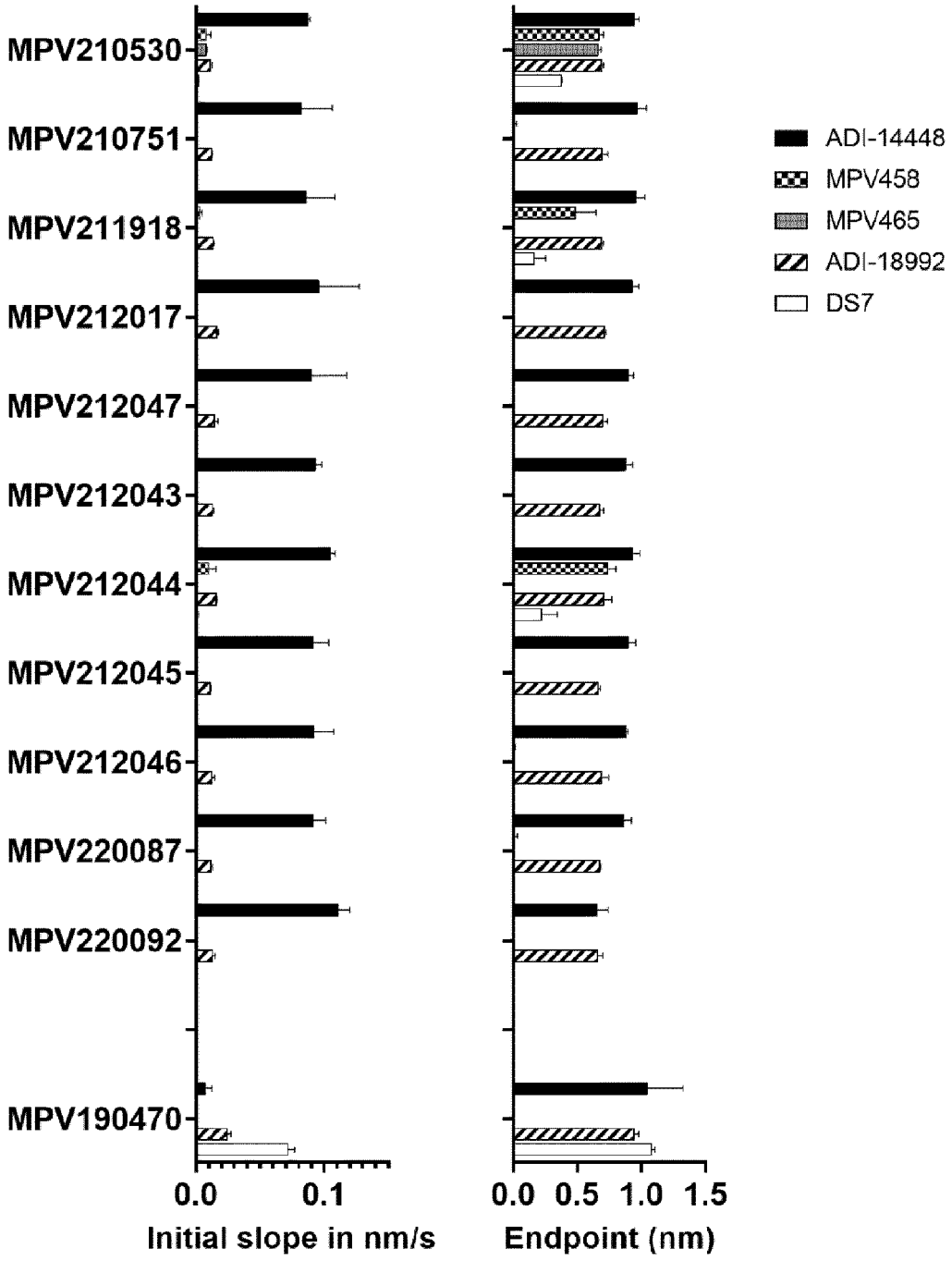


Fig 11 - continued

G

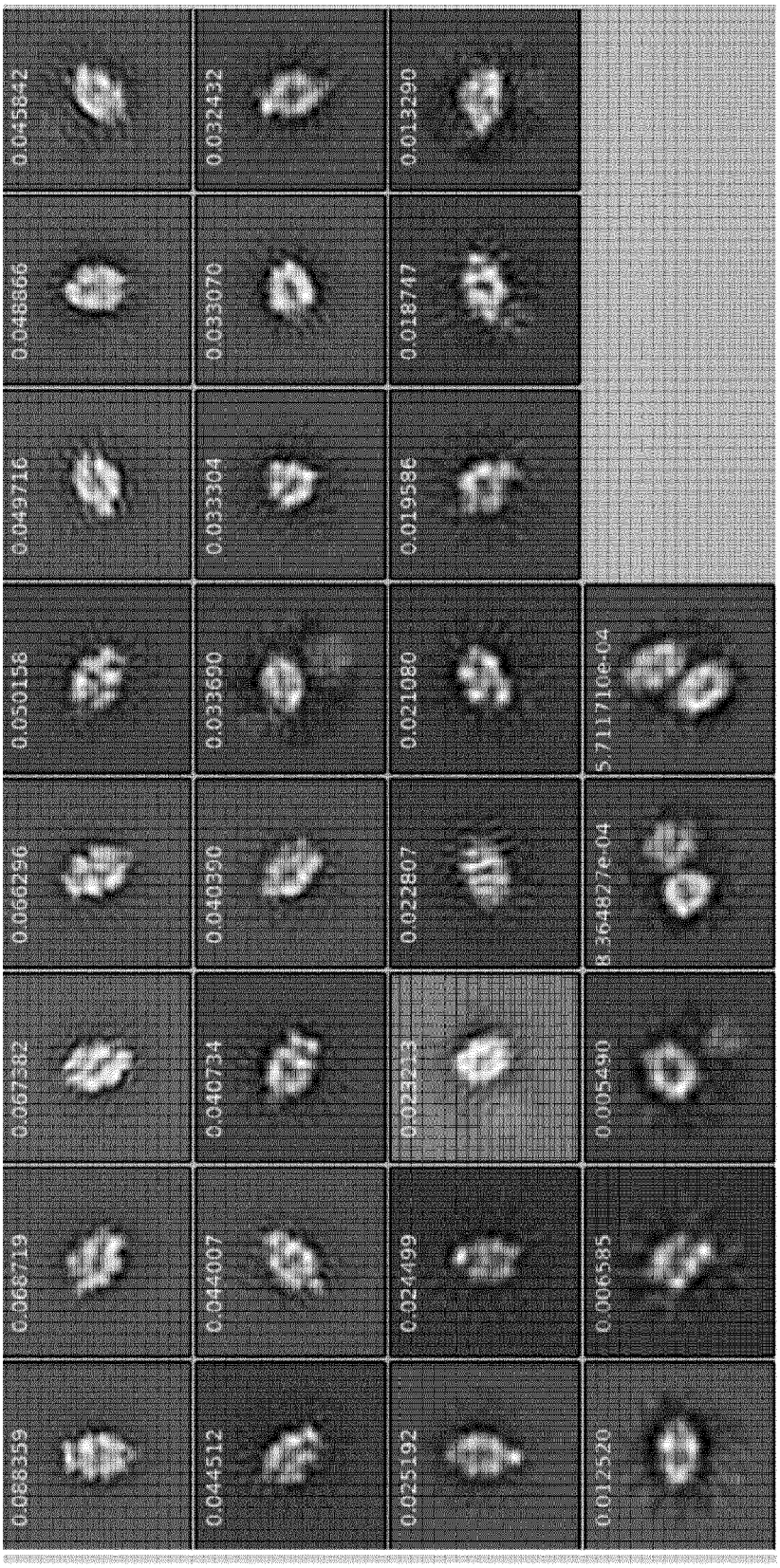


Fig 11 - continued

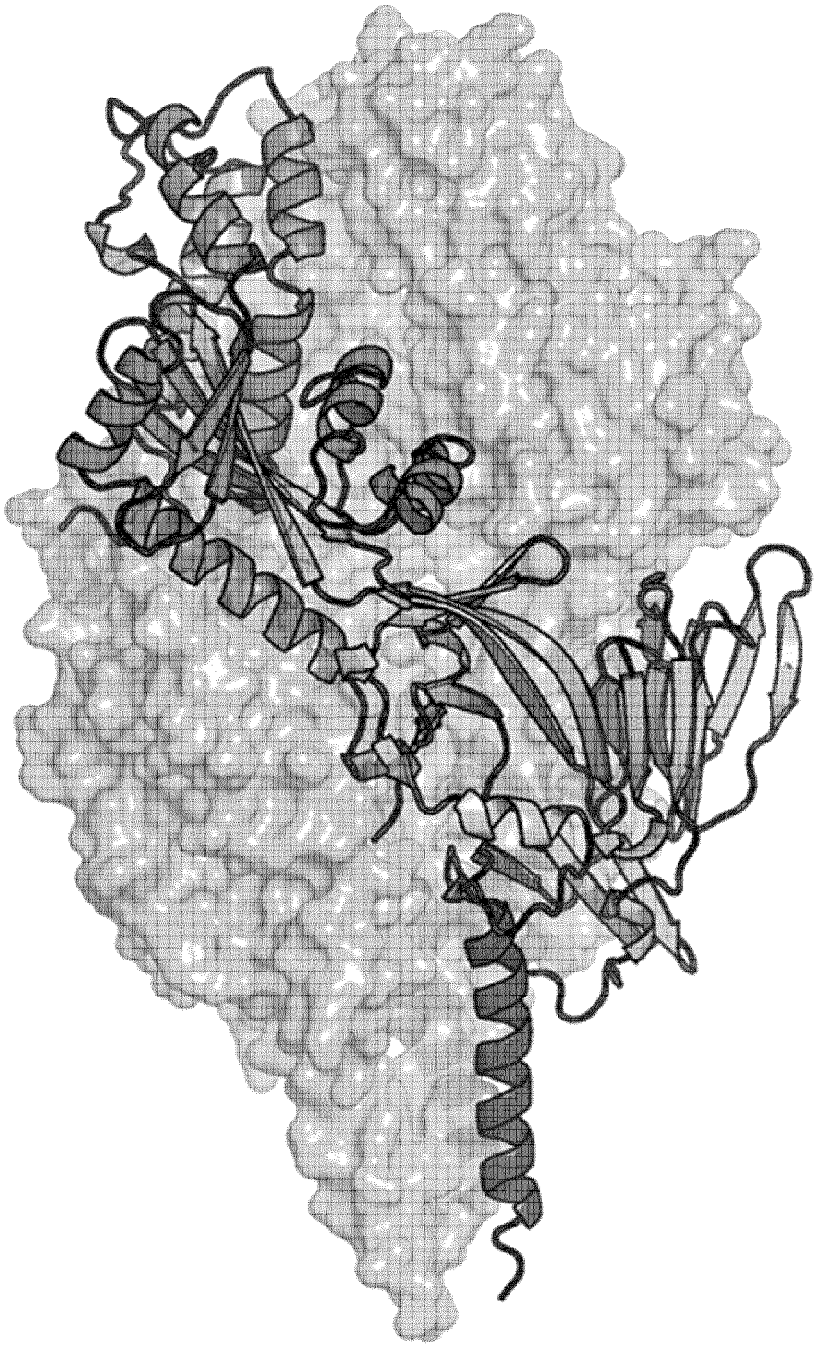
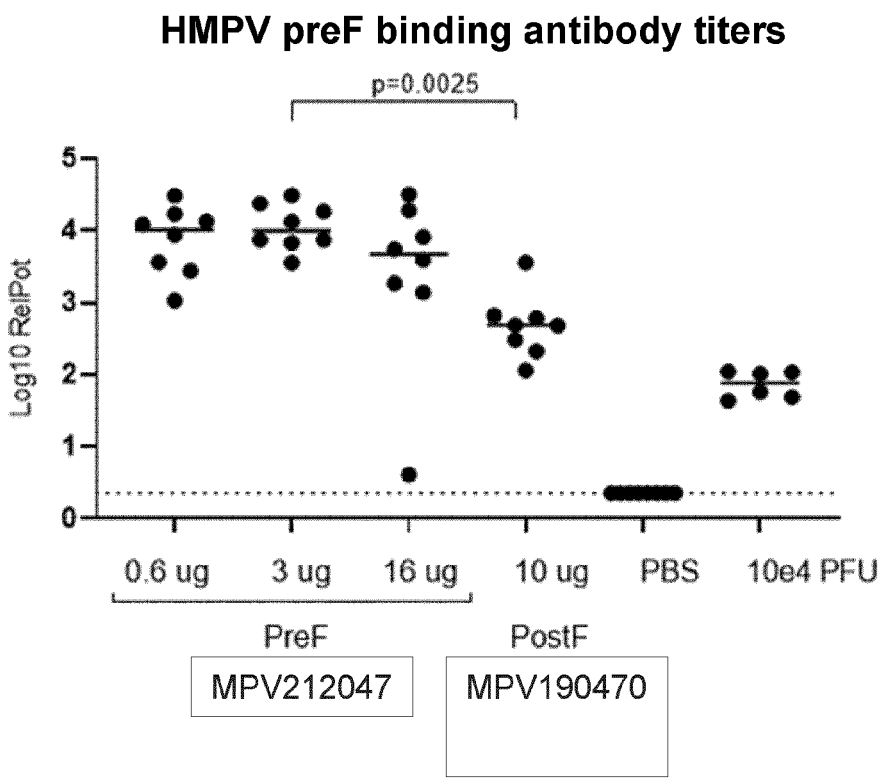


Figure 12

A.


HMPV preF binding antibody titers
p=0.0025
Log10 RelPot
5
4
3
2
1
0
0.6 ug
3 ug
16 ug
10 ug
PBS
10e4 PFU
PreF
PostF
MPV212047
MPV190470

B.

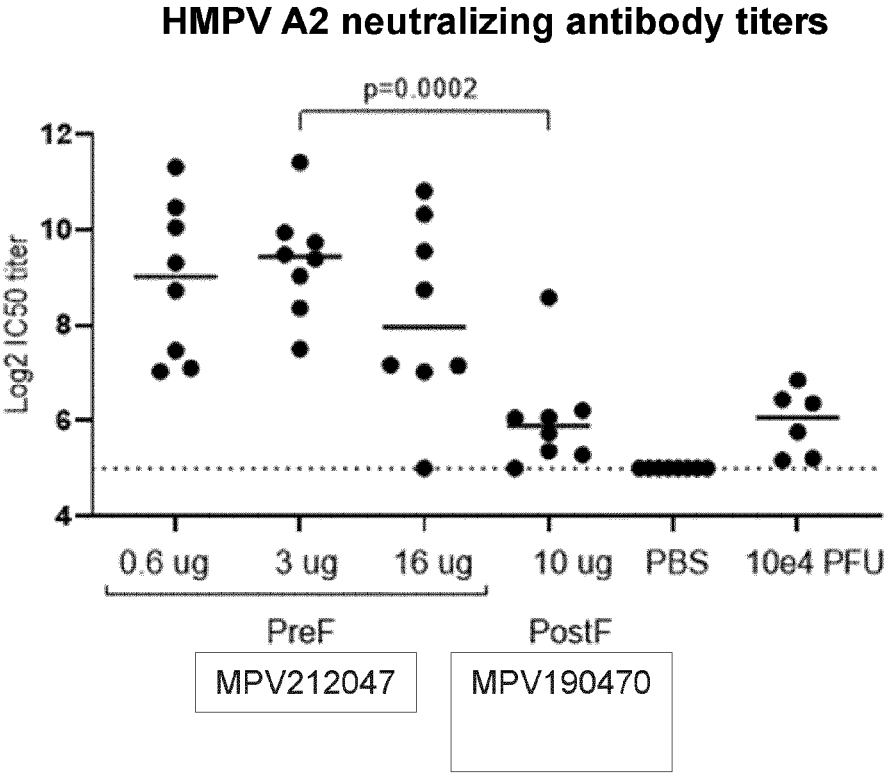

HMPV A2 neutralizing antibody titers
p=0.0002
Log2 IC50 titer
12
10
8
6
4
0.6 ug
3 ug
16 ug
10 ug
PBS
10e4 PFU
PreF
PostF
MPV212047
MPV190470

Figure 13

Figure 13, continued

C.

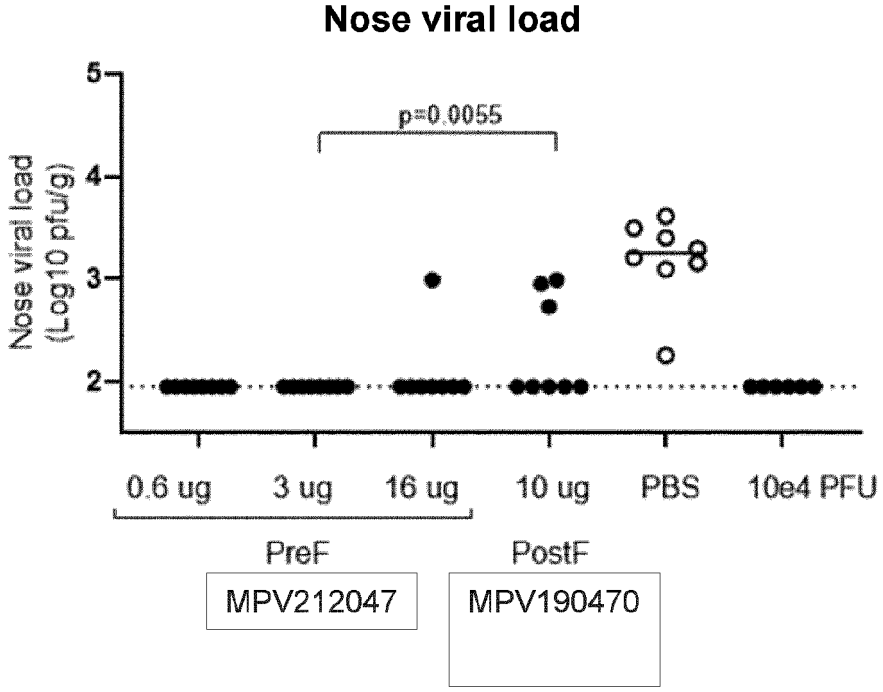
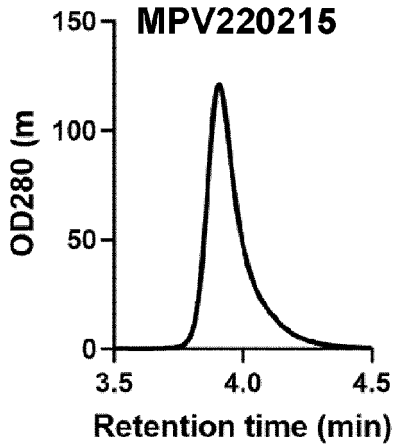


Figure 14

A.



B.

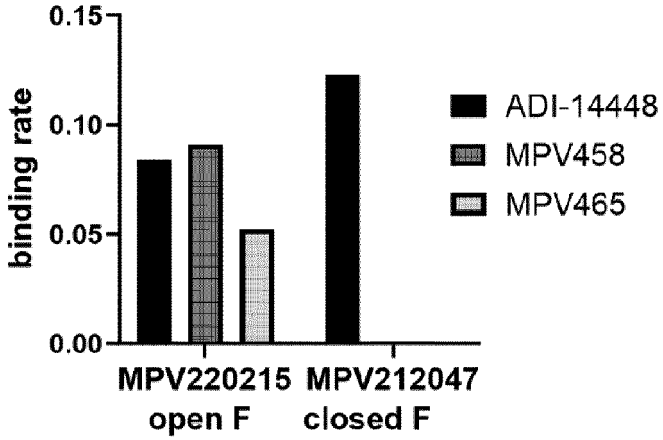
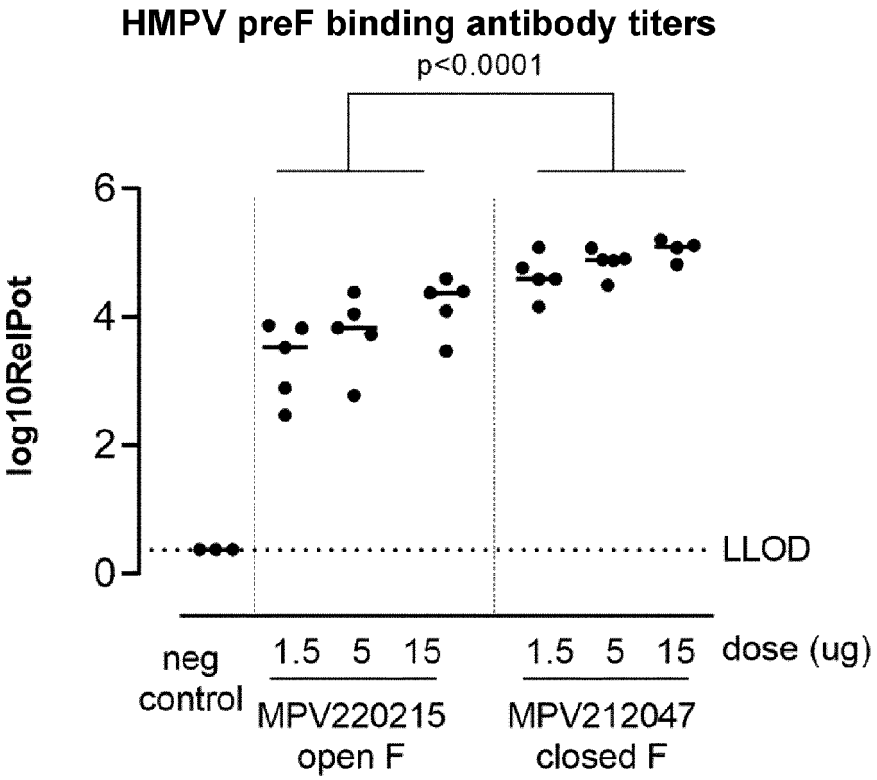
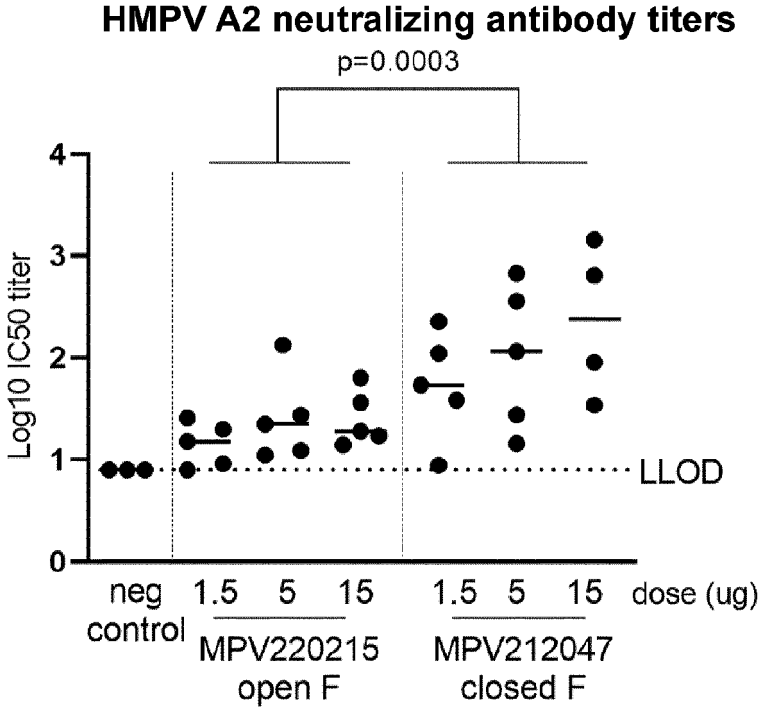


Figure 14, continued

C.



D.



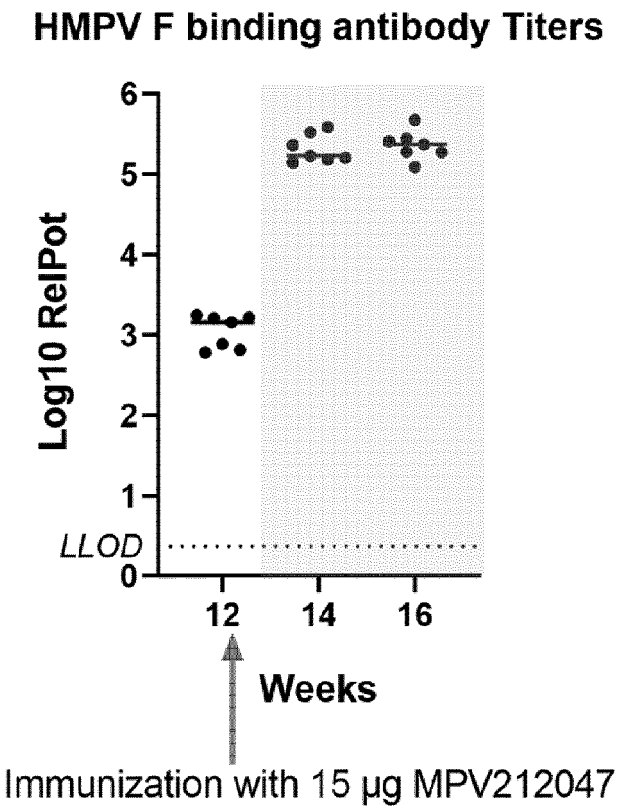
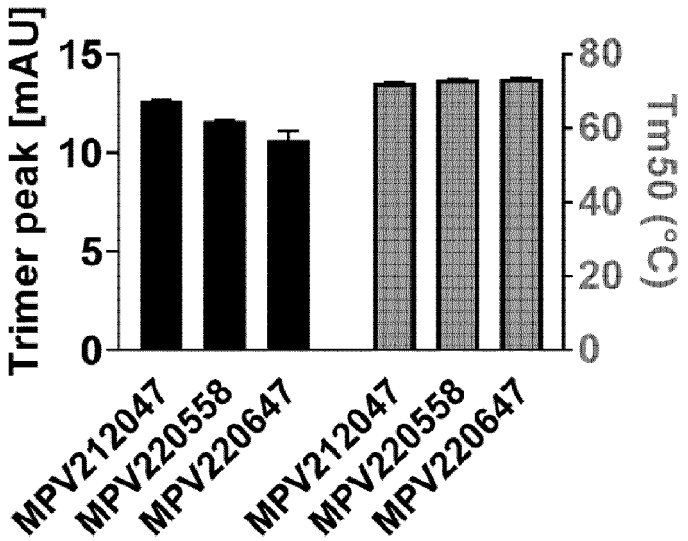

HMPV F binding antibody Titers
Log10 RelPot
6
5
4
3
2
1
LLOD
0
12
14
16
Weeks
Immunization with 15 µg MPV212047

Figure 15

Figure 16

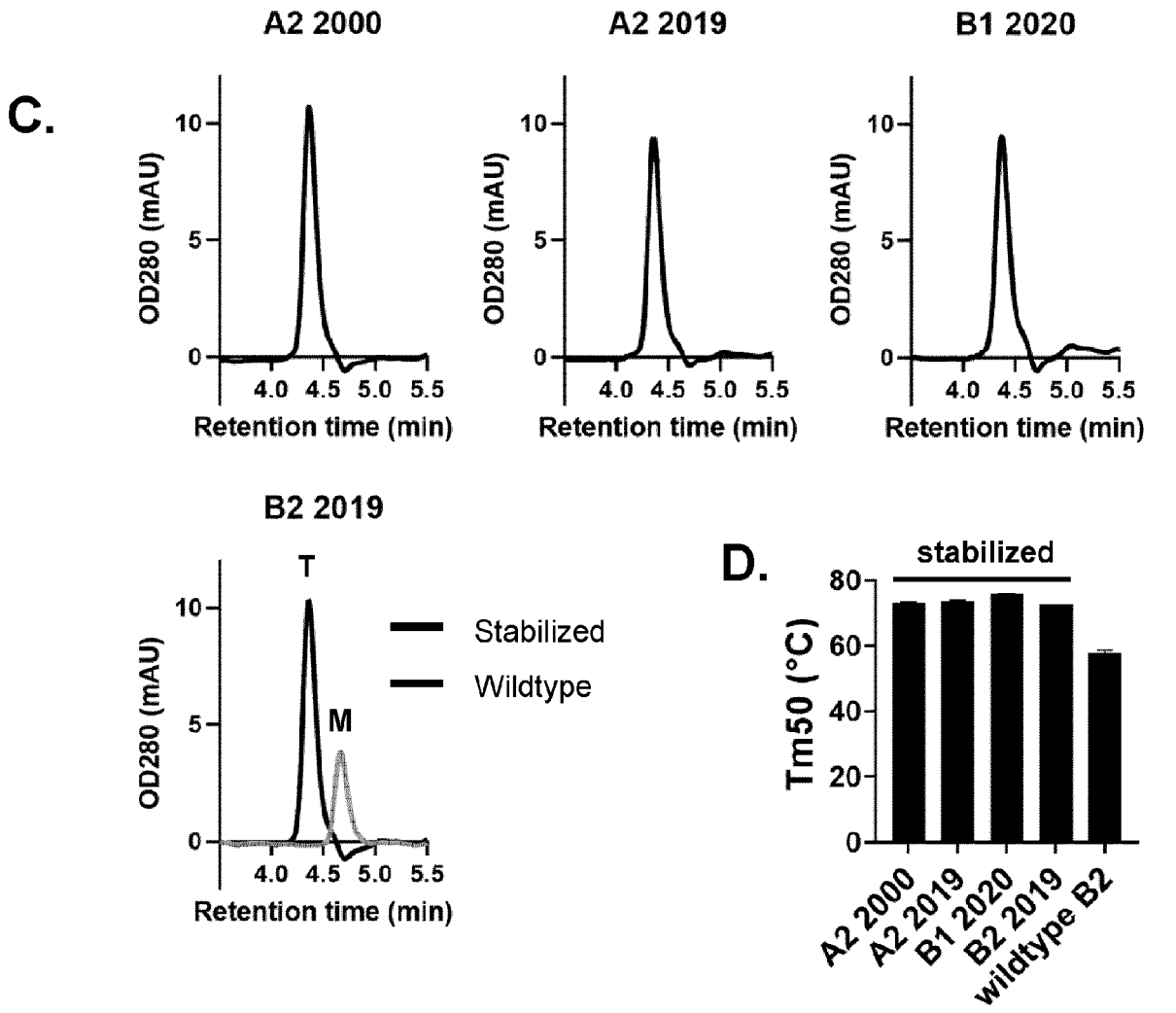
A.



B.

Construct ID	Subtype	Collection year	HMPV strain identifier	Genbank ID
MPV220647	A2	2000	TN/00/3-14	EU857553.1
MPV220639	A2	2019	HMPV/Yokohama.JPN/P9767/2019	BBI90013.1
MPV220640	B1	2020	B/NSW/WM2170539/17	QTW05440.1
MPV220641	B2	2019	2019_1360	QQY99338.1

Figure 16, continued



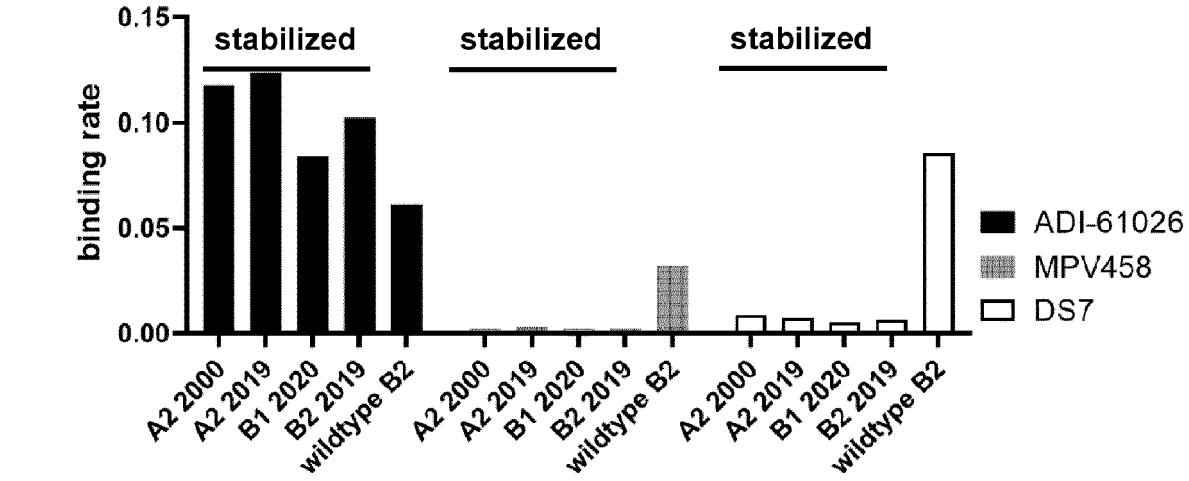
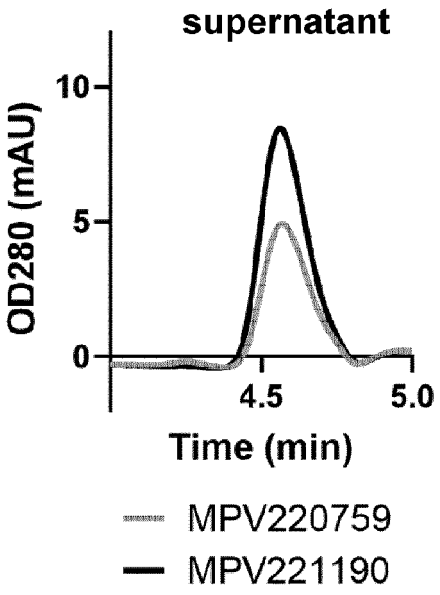
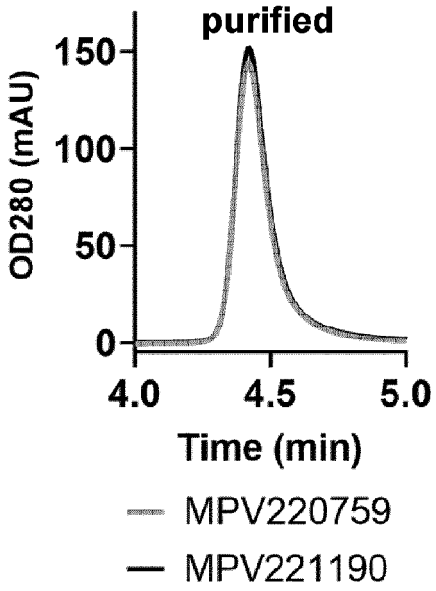


Figure 17

A.



B.



C.

	MPV220759	MPV221190
Determined weight (kDa)	157.0 (±0.668%)	157.4 (±0.595%)
Theoretical weight (kDa)	151.6	151.8
Likely conformation	Trimer	Trimer

D.

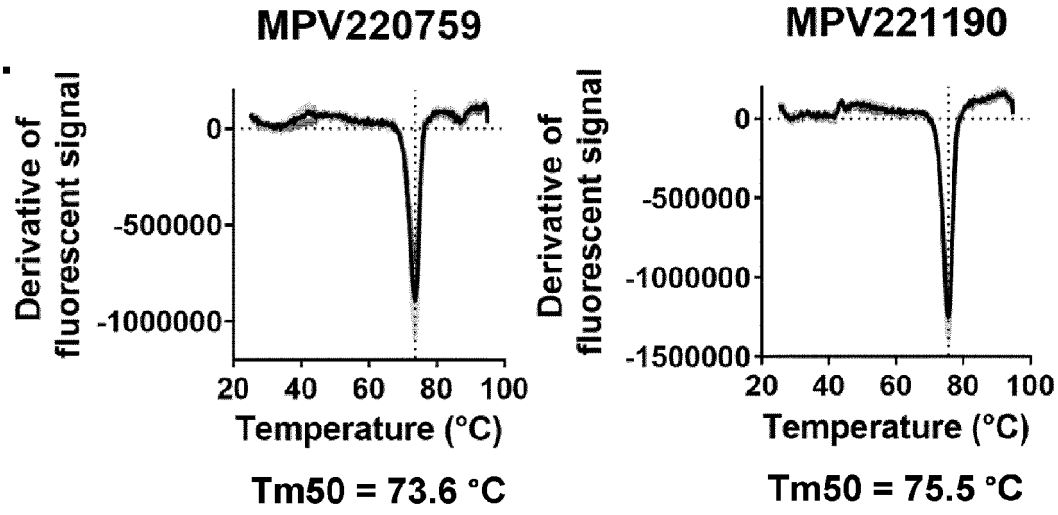


Figure 17, continued

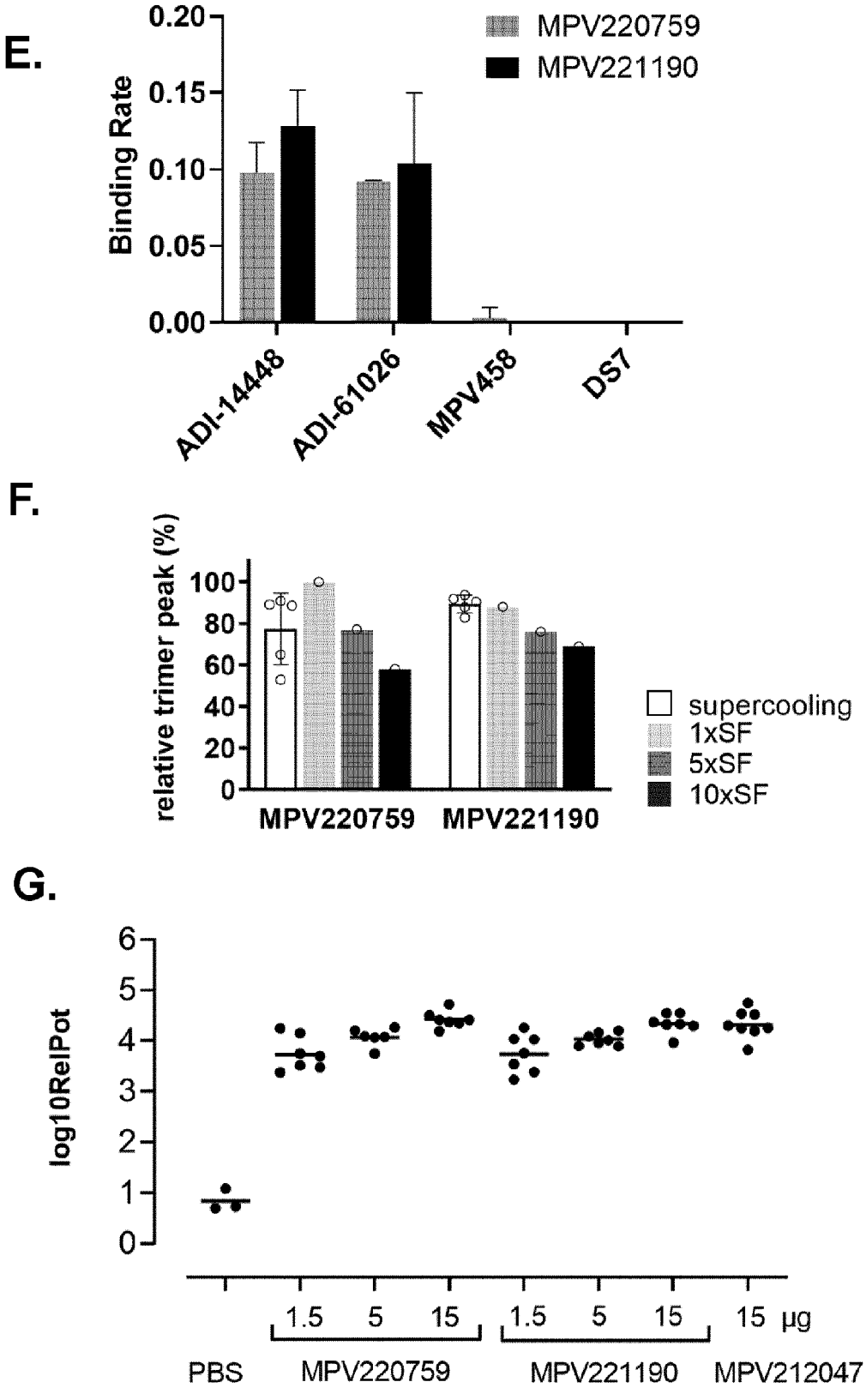
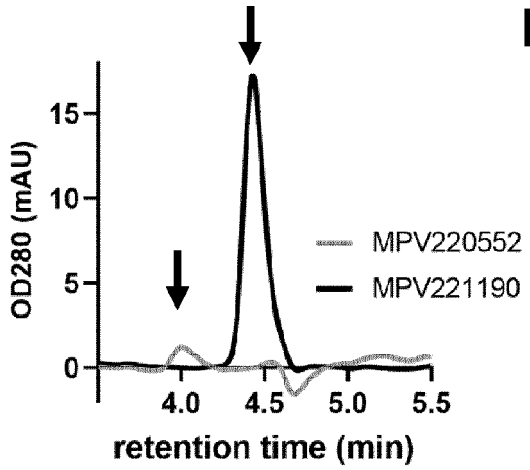


Figure 18

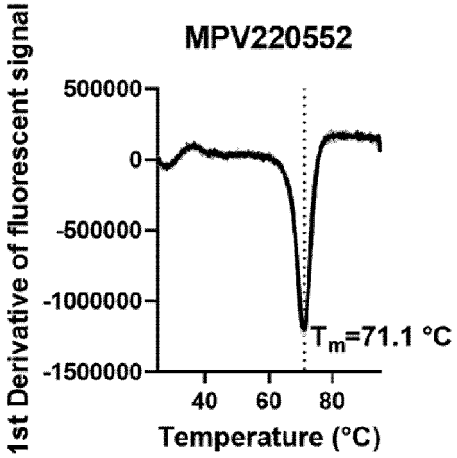
A.



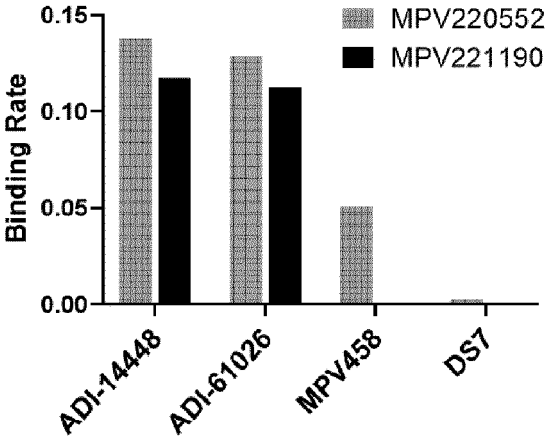
B.

MPV220552	
Determined weight (kDa)	177.9 (±0.221%)
Theoretical weight (kDa)	175.0
Likely conformation	Trimer

C.



D.



E.

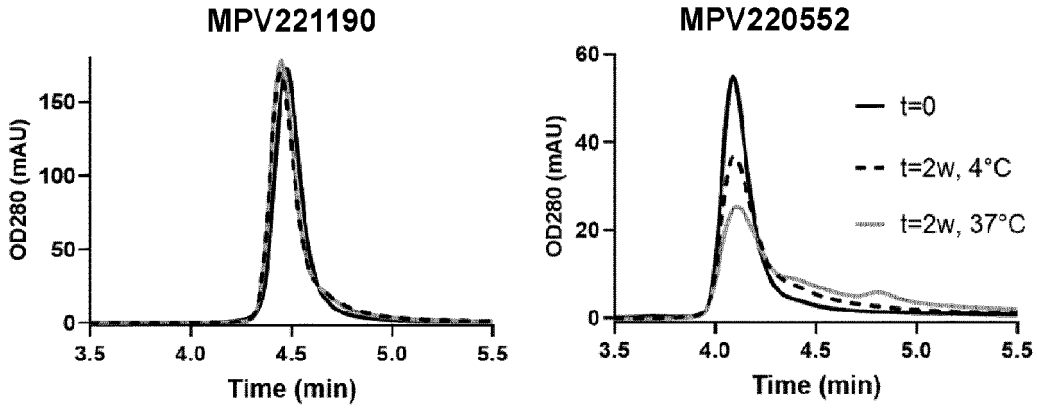


Figure 19

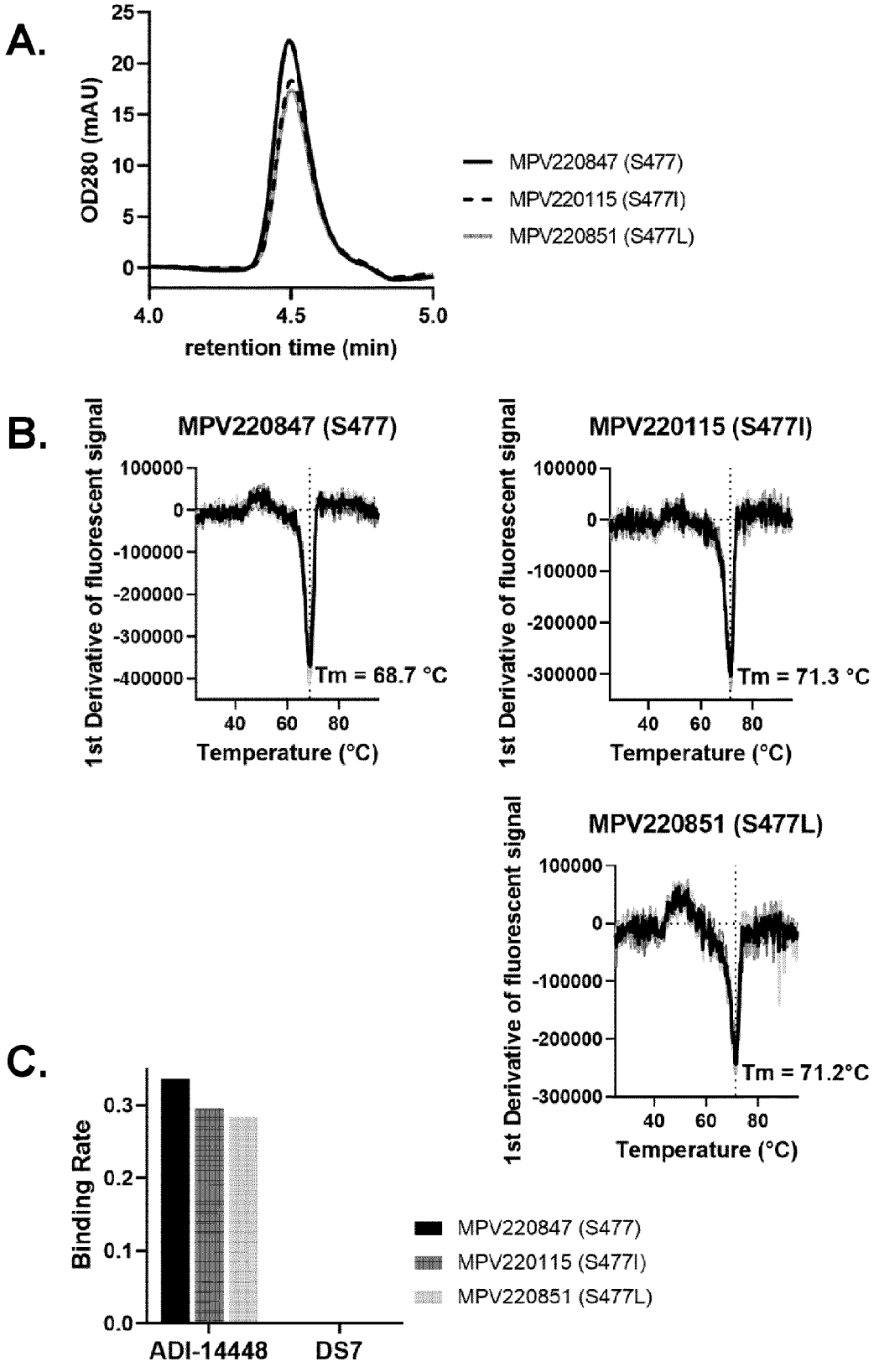

A.
OD280 (mAU)
retention time (min)
MPV220847 (S477)
MPV220115 (S477I)
MPV220851 (S477L)
B.
MPV220847 (S477)
MPV220115 (S477I)
MPV220851 (S477L)
1st Derivative of fluorescent signal
Temperature (°C)
Tm = 68.7 °C
Tm = 71.3 °C
Tm = 71.2°C
C.
Binding Rate
ADI-14448
DS7
MPV220847 (S477)
MPV220115 (S477I)
MPV220851 (S477L)

Figure 20

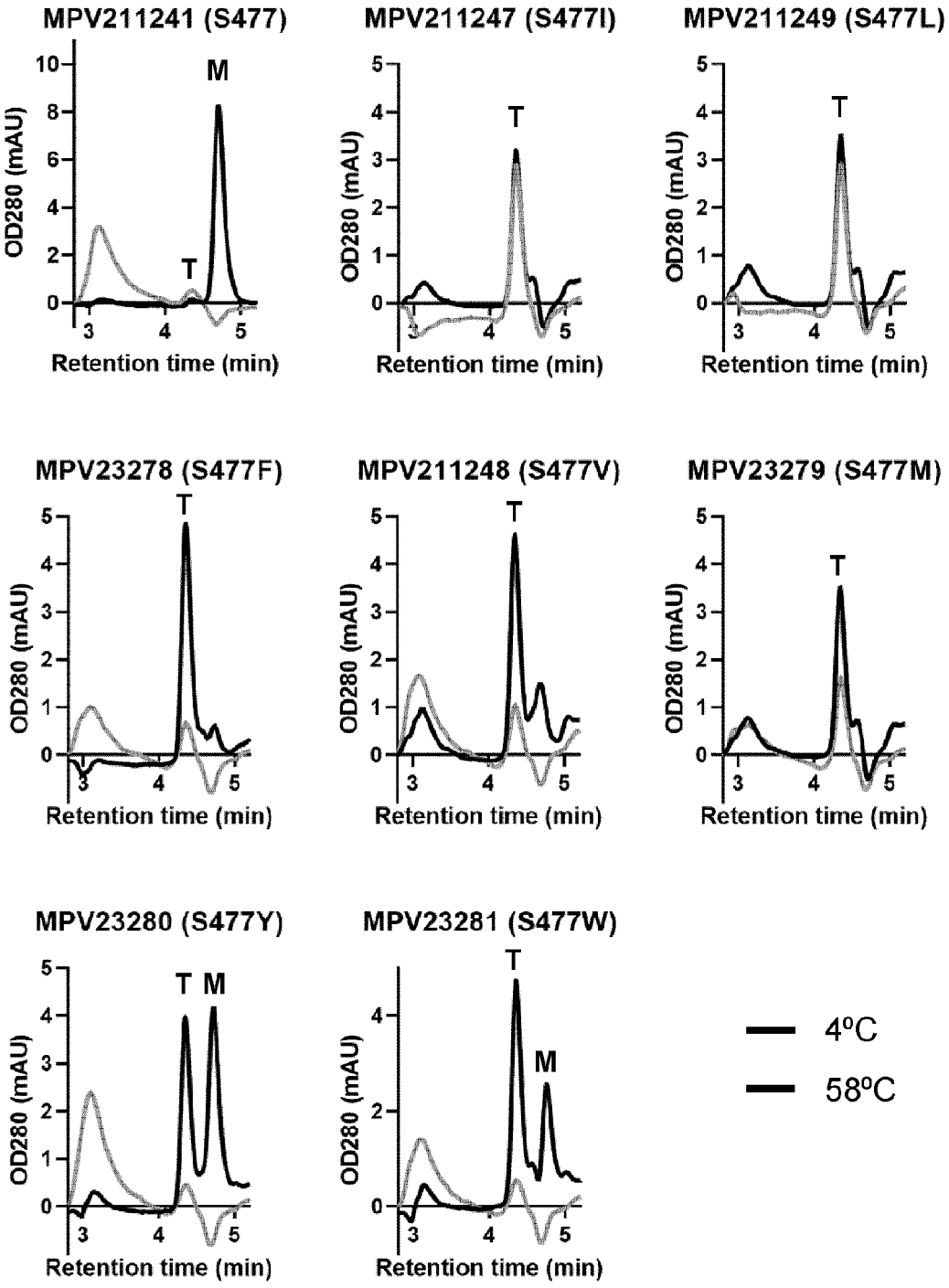

MPV211241 (S477)
MPV211247 (S477I)
MPV211249 (S477L)
MPV23278 (S477F)
MPV211248 (S477V)
MPV23279 (S477M)
MPV23280 (S477Y)
MPV23281 (S477W)
OD280 (mAU)
Retention time (min)
T
M
4°C
58°C

Figure 21

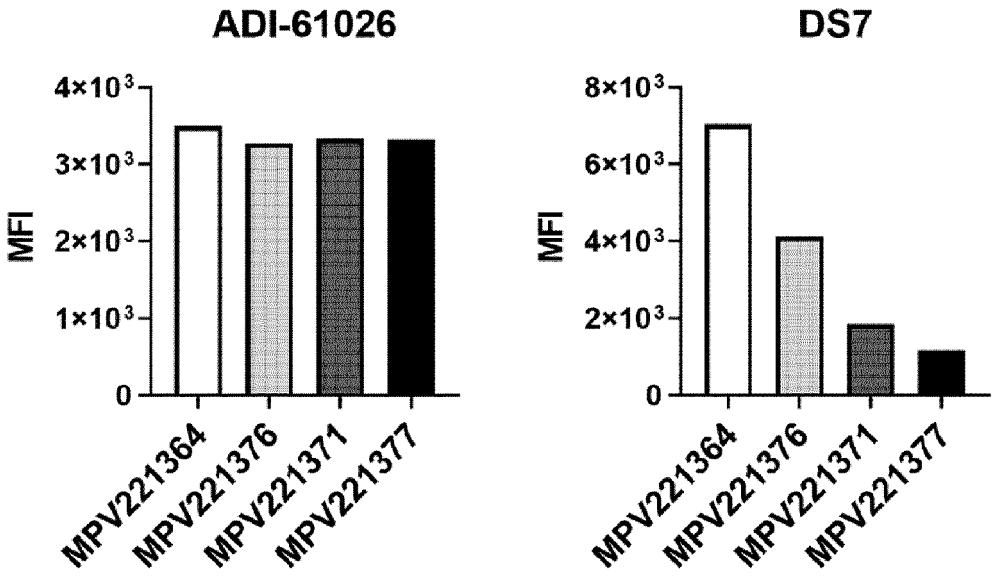

ADI-61026
MFI
4×10³
3×10³
2×10³
1×10³
0
MPV221364
MPV221376
MPV221371
MPV221377
DS7
MFI
8×10³
6×10³
4×10³
2×10³
0
MPV221364
MPV221376
MPV221371
MPV221377

Figure 22

A.

Construct	Description	P27 sequence
MPV23260	p27-B	RRRREAPQYMNYTINTTKNLNVSISKKRKRR
MPV23259	p27-A	RRRRELPRFMNYTLNNTKNTNVTLSKKRKRR
MPV23261	p27-A_ΔGlycan1	RRRRELPRFMQYTLNNTKNTNVTLSKKRKRR
MPV23262	p27-A_ΔGlycan2	RRRRELPRFMNYTLQNTKNTNVTLSKKRKRR
MPV23263	p27-A_ΔGlycan3	RRRRELPRFMNYTLNNTKNTQVTLSKKRKRR
MPV23264	p27-A_ΔGlycan1+2	RRRRELPRFMQYTLQNTKNTNVTLSKKRKRR
MPV23265	p27-A_ΔGlycan2+3	RRRRELPRFMNYTLQNTKNTQVTLSKKRKRR
MPV23266	p27-A_ΔGlycan1+3	RRRRELPRFMQYTLNNTKNTQVTLSKKRKRR
MPV23267	p27-A_ΔGlycan1+2+3	RRRRELPRFMQYTLQNTKNTQVTLSKKRKRR
MPV23268	p27-A_deletion-1	RRRRE---------NNTKNTNVTLSKKRKRR
MPV23269	p27-A_deletion-2	RRRRELPRFMNYTL----------KKRKRR

B.

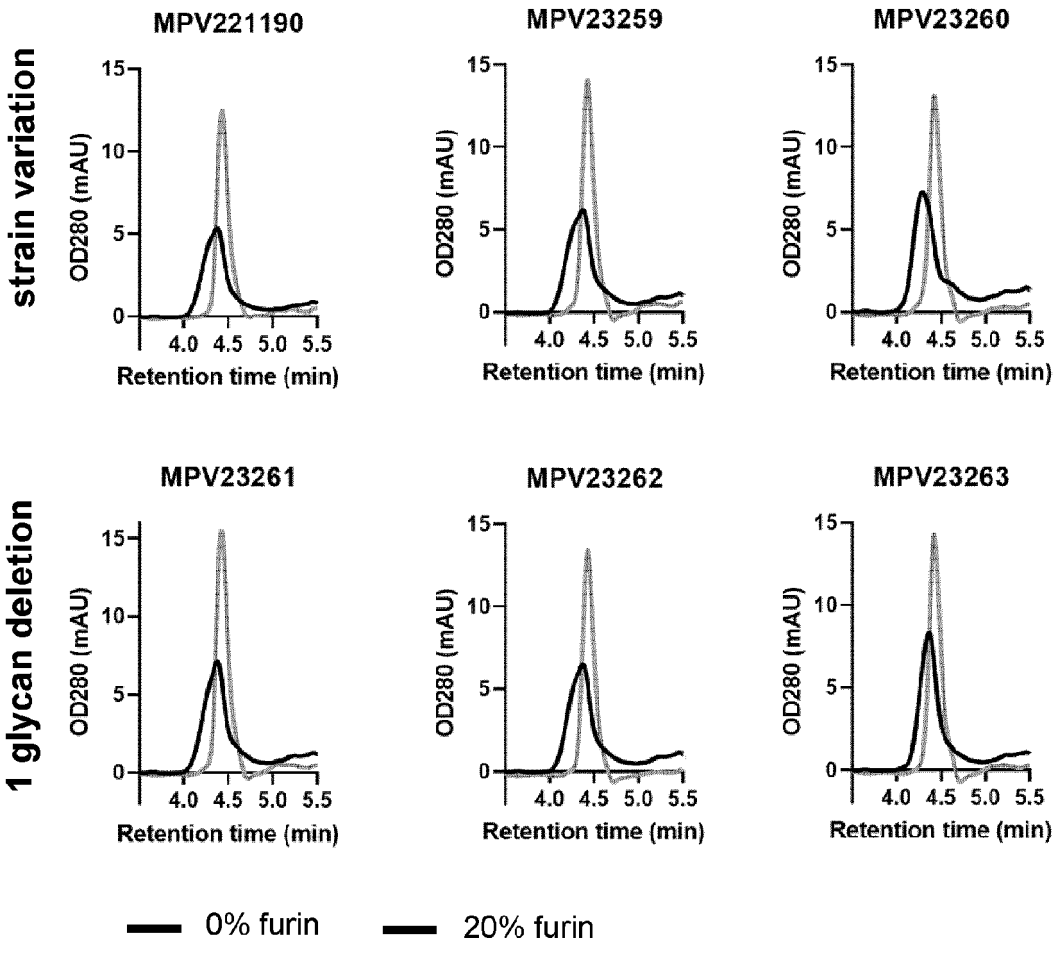
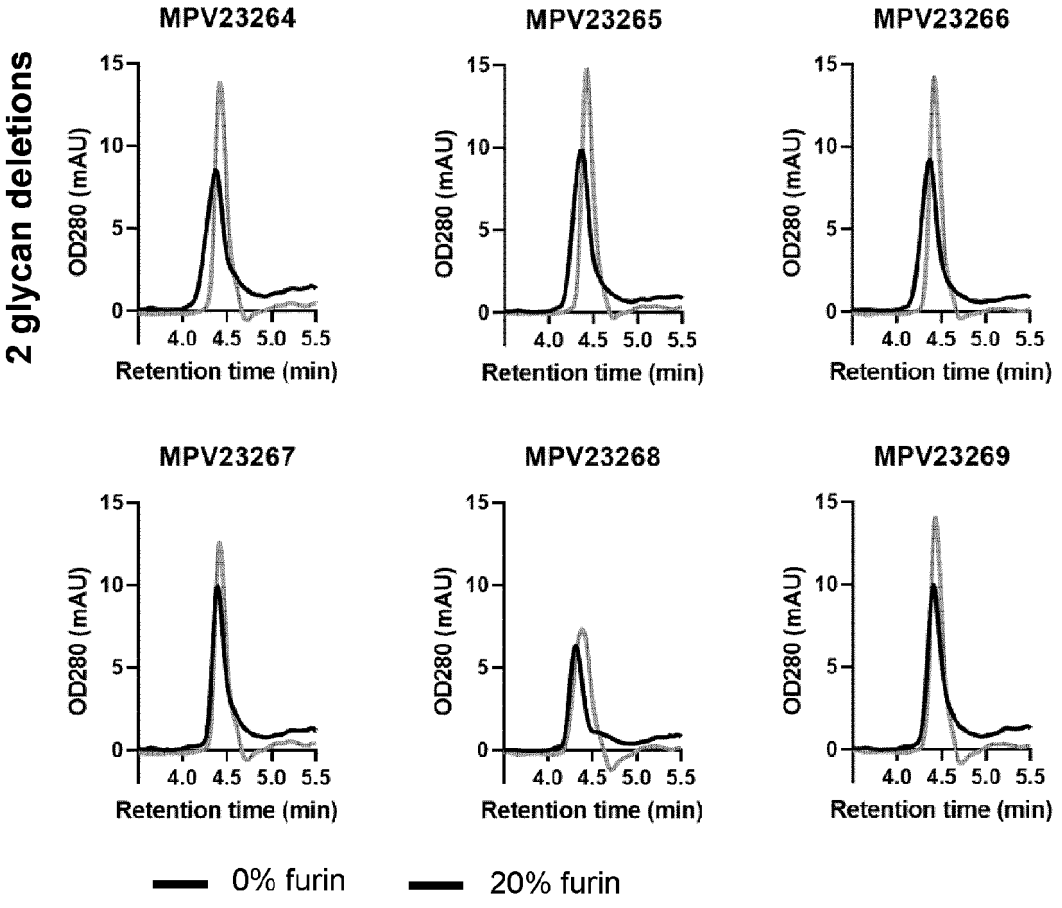


Figure 22, continued



C.

