Claims

1. An expression system comprising polynucleotides encoding proteins, wherein the expression system comprises a first polynucleotide encoding at least one protein, peptide or variant thereof, which induces a T cell response, and a second polynucleotide encoding at least one protein peptide or variant thereof, which induces an anti-pathogenic B cell response.

2. The expression system of claim 1 wherein the first polynucleotide and the second polynucleotide are linked such that they are expressed as an artificial polyprotein.

3. The expression system of claim 1, wherein the proteins, peptides or fragments encoded by the first and the second polynucleotide are separated co- or posttranslationally.

4. The expression system of claim 2 or 3, wherein a polynucleotide which encodes a cleavage site is positioned between the first polynucleotide and the second polynucleotide.

5. The expression system of claim 4, wherein the cleavage site is a self-cleaving site or an endopeptidase cleavage site.

~~6.The expression system of claim 5, wherein the self-cleaving site is a 2A cleavage site selected from the group consisting of a viral 2A peptide or 2A-like peptide of Picornavirus, insect viruses, Aphtoviridae, Rotaviruses and Trypanosoma, preferably wherein the 2A cleavage site is the 2 A peptide of foot and mouth disease virus~~

~~7.~~6. The expression-system of any of claims 1 to ~~6~~5, further comprising a third polynucleotide encoding a protein or variant thereof, preferably inducing a T cell response.

~~8.The expression system of claim 7, wherein the third polynucleotide is comprised on a separate or on the same vector as the first polynucleotide and the second polynucleotide.~~

~~9.The expression system of claim 8, wherein the first polynucleotide, the second polynucleotide and the third polynucleotide are linked in such that they are expressed as an artificial polyprotein.~~

~~10.The expression system of any of claims 7 to 9, wherein the protein encoded by the third polynucleotide differs from the protein encoded by first or the second polynucleotide.~~

~~11.The expression system of any of claims 7 to 10, wherein a polynucleotide encoding a linker is positioned between the second polynucleotide and the third polynucleotide.~~

~~12.The expression system of claim 11, wherein the linker is a flexible linker, preferably a flexible linker comprising an amino acid sequence according to SEQ ID NO: 6.~~

~~13.The expression system of any of claims 1 to 12, wherein the protein encoded by the first polynucleotide is located N-terminally with respect to the protein encoded by the second polynucleotide and/or the protein of the optional third polynucleotide is located N-terminally with respect to the protein encoded by the first polynucleotide or is located between the protein encoded by the second polynucleotide and the protein encoded by the first polynucleotide, or~~

~~wherein the protein encoded by the first polynucleotide is located C-terminally with respect to the protein encoded by the second polynucleotide and/or the protein of the optional third polynucleotide is located C-terminally with respect to the protein encoded by the first polynucleotide or is located between the protein encoded by the second polynucleotide and the protein encoded by the first polynucleotide.~~

~~14.~~7. The expression-system of any of the claims 1 to ~~13~~6, wherein the expression system comprises at least one vector ~~or vectors are~~ selected from the group consisting of plasmid vectors, cosmid vectors, phage vectors, preferably lambda phage and filamentous phage vectors, and viral vectors~~, preferably adenovirus vectors, adeno-associated virus (AAV) vectors, alphavirus vectors, herpes virus vectors, arena virus vectors, measles virus vectors, pox virus vectors, vesicular stomatitis virus vectors, retrovirus vectors, lentivirus vectors, viral like particles, and bacterial spores~~.

~~15.~~8. The expression-system of claim ~~14~~7, wherein the viral vector is selected from the group consisting of PanAd1, PanAd2, PanAd3, ChAd55, ChAd 73, ChAd83, ChAd146, ChAd147, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd63 and ChAd82.

~~16.~~9. The expression-system of claim ~~14~~8, wherein the viral vector is an adenoviral vector selected from the group consisting vectors derived from cytomegaloviruses, preferably rhesus cytomegalovirus (RhCMV) vectors and arena virus vectors, preferably lymphocytic choriomeningitis virus (LCMV) vectors.

~~17.~~10. The expression system of any of the claims 1 to ~~16~~9, wherein at least one of the first polynucleotide the second polynucleotide ~~or the optional third polynucleotide~~ is selected from the group consisting of a protein, peptide or variant thereof ~~derived~~ from a pathogen.

~~18.~~11. The expression system of claim ~~17~~10, wherein the pathogen is a virus.

~~19.~~12. The expression system of any of claims 1 to ~~18~~11, wherein the protein, ~~which~~ that induces a T cell response is a non-structural and/or internal protein of a virus, and/or the protein ~~which~~ that induces an anti-pathogenic B cell response is a structural and/or surface protein of a virus.

~~20.The expression system of claim 19, wherein the amino acid sequence of the structural and/or surface and/or non-structural and/or internal protein comprises consecutive segments or a consensus sequence of one or more different virus isolates.~~

~~21.The expression system of claim 19 or 20, wherein the structural and/or surface protein is a protein exposed on the surface of the native virus.~~

~~22.The expression system of claim 21, wherein the membrane attachment domain of the protein exposed on the surface of the native virus is functionally deleted.~~

~~23.The expression system of any of claims 18 to 22, wherein the virus is selected from the group consisting of a DNA virus, a negative sense single stranded RNA virus or an ambisense RNA virus, preferably a negative sense single stranded RNA virus.~~

~~24.~~13. The expression system of ~~any of~~ claims ~~18~~ 11 ~~to~~or ~~23~~12, wherein the virus is selected from the group consisting of paramyxoviruses and orthomyxoviruses.

~~25.The expression system of claim 23 or 24, further comprising a third polynucleotide encoding a non-structural and/or internal protein or variants thereof from a virus as defined in claims 23 or 24.~~

~~26.The expression system of claim 24 or 25, wherein the paramyxovirus is selected from the subfamily of Pneumovirinae, Paramyxovirinae, Fer-de-Lance-Virus, Nariva-Virus, Salem-Virus, Tupaia-Paramyxovirus, Beilong-Virus, J-Virus, Menangle-Virus, Mossmann-Virus, and Murayama-Virus.~~

~~27.~~14. The expression system of claim ~~26~~13, wherein the paramyxoviruses ~~Pneumovirinae~~ is selected from the group consisting of Pneumovirus, ~~preferably human respiratory syncytical virus (RSV), murine pneumonia virus, bovine RSV, ovine RSV, caprine RSV, turkey rinotracheitis and~~ Metapneumovirus, and Respirovirus ~~preferably human metapneumovirus and avian metapneumovirus~~.

~~28.The expression system of claim 26, wherein the Paramyxovirinae is selected from the group consisting of Respirovirus, preferably human parainfluenza virus 1 and 3, and Rubulavirus, preferably human parainfluenza virus 2 and 4.~~

~~29.~~15. The expression system of claim ~~21~~12, wherein the ~~protein exposed on the~~ structural and/or surface protein of the ~~native~~ virus is selected from the group of paramyxovirus proteins consisting of fusion protein (F), and any of the attachment glycoproteins G, H, and HN.

~~30.The expression system of claim 29, wherein F comprises an amino acid sequence of F of one RSV isolate or a consensus amino acid sequence of two or more different RSV isolates, preferably according to SEQ ID NO: 1.~~

~~31.The expression system of claim 29 or 30, wherein F comprises an amino acid sequence according to SEQ ID NO: 2.~~

~~32.~~16. The expression system of any of claims 1~~29~~ to ~~31~~15, wherein the non-structural and/or internal protein is selected from the group of paramyxovirus proteins consisting of nucleoprotein N, Matrix proteins M and M2, Phosphoprotein P, non structural proteins NS1 and NS2, and the catalytic subunit of the polymerase (L).

~~33.The expression system of claim 32, wherein N comprises an amino acid sequence of N, of one RSV isolate or a consensus amino acid sequence of two or more different RSV isolates, preferably according to SEQ ID NO: 3 and wherein M2 comprises an amino acid sequence of M2 of one RSV isolate or a consensus amino acid sequence of two or more different RSV isolates, preferably according to SEQ ID NO: 5.~~

~~34.The expression system of claim 32 or 33, wherein N comprises the amino acid sequence according to SEQ ID NO: 4 and M2 comprises the amino acid sequence according to SEQ ID NO: 5.~~

~~35.The expression system of any of claims 2 to 34, wherein the polyprotein encoded by the first, the second and the optional third polynucleotide has an amino acid according to SEQ ID NO: 7.~~

~~36.~~17. The expression system of claim 13~~24 or 25~~, wherein the orthomyxovirus is selected from Influenza A virus, Influenza B virus, Influenza C virus, Thogotovirus, Isavirus and unclassified Orthomyxoviridae.

~~37.The expression system of claim 36, wherein the Influenza A virus is a subtype selected from the group consisting of H1N1, H1N2, H2N2, H3N1, H3N2, H3N8, H5N1, H5N2, H5N3, H5N8, H5N9, H7N1, H7N2, H7N3, H7N4, H7N7, H9N2, H10N7, preferably H1N1.~~

~~38.~~18. The expression system of claim ~~21~~12, wherein the structural and/or ~~protein exposed on the~~ surface protein of the ~~native~~ virus is selected from the group orthomyxovirus proteins consisting of HA and NA, preferably HA

~~39.The expression system of claim 38, wherein HA comprises an amino acid sequence of HA of one Influenza A virus isolate or a consensus amino acid sequence of two or more different Influenza A virus isolates, wherein the amino acid sequence of HA is preferably selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 21 and variants of these sequences.~~

~~40.The expression system of claim 38 or 39, wherein HA comprises an amino acid sequence selected from the group consisting of PQRERRRKKR (SEQ ID NO: 15), PQRESRRKKR (SEQ ID NO: 16), PQGERRRKKR (SEQ ID NO: 17), PLRERRRKR (SEQ ID NO: 18) and PQRETR (SEQ ID NO: 19), preferably PQRERRRKKR (SEQ ID NO: 15).~~

~~41.~~19. The expression system of any of claims ~~19~~ 12, 13~~to 25~~, ~~-~~17 and 18~~and claims 36 to 40~~, wherein the non-structural and/or internal protein(s) is/are selected from the group of orthomyxovirus proteins consisting of NP, M1, M2, NS1, NS2/NEP, PA, PB1, PB2 and PB1-F2 (PB1F2), preferably NP and M1.

~~42.The expression system of claim 41, wherein NP comprises an amino acid sequence of NP of one influenza A virus isolate or subtype or a consensus amino acid sequence of two or more different influenza A virus isolates or subtypes, preferably according to SEQ ID NO: 11, and wherein M1 comprises an amino acid sequence of M1 influenza A virus isolate or subtype or a consensus amino acid sequence of two or more different influenza A virus isolates or subtypes, preferably according to SEQ ID NO: 12.~~

~~43.The expression system of any of claims 2 to 25 and claims 36 to 42, wherein the polyprotein encoded by the first, the second and the optional third polynucleotide has an amino acid sequence according to SEQ ID NO: 13 or a variant thereof and/or is encoded by a polynucleotide having a nucleic acid sequence according to SEQ ID NO: 14 or a variant thereof.~~

~~44.~~20. An isolated protein mixture encoded by the expression system of claims 1 to ~~43~~19.

~~45.~~21. An isolated host cell containing the expression-system according to any one of claims 1 to ~~43~~ 19 and/or the protein mixture of claim ~~44~~20.

~~46.~~22. A composition comprising the expression-system according to any one of claims 1 to ~~43~~ 19 ~~and/or the protein mixture of claim 44~~ and a pharmaceutical acceptable carrier and/or excipient.

~~47.The composition of claim 46, comprising a further active ingredient, preferably selected from the group consisting of an adjuvant and an active ingredient.~~

~~48.The expression system according to any one of claims 1 to 43 and/or the protein mixture of claim 44, an isolated cell according to claim 45, or a composition according to claims 46 or 47, for the use in the treatment or prevention of a disease.~~

~~49.An expression system according to claims the expression-system according to any one of claims 1 to 43 and/or the protein mixture of claim 44, an isolated cell according to claim 41, or a composition according to claims 46 or 47, for the use of claim 48,~~

~~wherein the disease is selected from infectious disease, preferably a viral infectious disease.~~

~~50.The expression system, the isolated protein mixture, the isolated cell or the composition for the use as defined claims 46 or 47, wherein the disease is caused by a pathogen as defined in claims 23, 24, 26 to 28, 36 and 37.~~

~~51.~~23. ~~A method of~~Use of the expression-system according to any one of claims 1 to 19 for the preparation of a medicament for the treatment or prevention of a viral disease comprising administration of an effective amount of the expression-system ~~according to any one of claims 1 to 43 and/or the protein mixture of claim 44, an isolated cell according to claim 45, or a composition according to claims 46 or 47~~19.

~~52.~~24. ~~The method~~Use of claim ~~51~~23, wherein the disease is caused by a ~~pathogen as defined in claims 23, 24, 26 to 28, 36 and 37~~virus selected from the group consisting of ~~.~~a DNA virus, a negative sense single stranded RNA virus, an ambisense RNA virus, a negative sense single stranded RNA virus, paramyxoviruses, orthomyxoviruses, Pneumovirinae, Paramyxovirinae, Fer-de-Lance-Virus, Nariva-Virus, Salem-Virus, Tupaia-Paramyxovirus, Beilong-Virus, J-Virus, Menangle-Virus, Mossmann-Virus, Murayama-Virus, human respiratory syncytical virus (RSV), murine pneumonia virus, bovine RSV, ovine RSV, caprine RSV, turkey rinotracheitis, Metapneumovirus, human metapneumovirus and avian metapneumovirus, Respirovirus, preferably human parainfluenza virus 1 and 3, and Rubulavirus, preferably human parainfluenza virus 2 and 4, Influenza A virus, Influenza B virus, Influenza C virus, Thogotovirus, Isavirus , unclassified Orthomyxoviridae, and Influenza A virus is a subtype H1N1, H1N2, H2N2, H3N1, H3N2, H3N8, H5N1, H5N2, H5N3, H5N8, H5N9, H7N1, H7N2, H7N3, H7N4, H7N7, H9N2, or H10N7.

~~53.~~25. ~~Use of T~~the expression-system according to any one of claims 1 to ~~43~~ 19 and/or the protein mixture of claim ~~44~~20, an isolated cell according to claim ~~45~~21, or a composition according to claims 22 ~~46 or 47~~, in the preparation of a medicament

for ~~the use in~~ inducing or enhancing a B-cell response against the protein encoded by the second polynucleotide, which induces an anti-pathogenic B cell response.

~~54.~~26. A nucleic acid construct which comprises~~, essentially consists or consists of~~ a polynucleotide encoding a modified influenza hemagglutinin (HA), wherein the HA0 cleavage site is modified by introducing one or more basic amino acids.

~~55.The nucleic acid construct according to claim 54, wherein the HA is selected from the group of HA subtypes consisting of H1, H2, H3, H4, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16 or a variant thereof or a consensus sequence thereof, or a variant or a consensus sequence of one or more of the HA subtypes selected from the group of HA subtypes consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, preferably the HA subtype H1 or a variant thereof or a consensus sequence thereof.~~

~~56.The nucleic acid construct according to claim 54 or 55, wherein the HA0 cleavage site is modified such that is comprises at least 20% basic amino acids, preferably at least 50% basic amino acids, more preferably at least 70% basic amino acids.~~

~~57.The nucleic acid construct according to any of claims 54 to 56, wherein the HA0 cleavage site has a sequence selected from the group consisting of PQRERRRKKR (SEQ ID NO: 15), PQRESRRKKR (SEQ ID NO: 16), PQGERRRKKR (SEQ ID NO: 17), PLRERRRKR (SEQ ID NO: 18) and PQRETR (SEQ ID NO: 19), preferably the HA0 cleavage site of has the sequence PQRERRRKKR (SEQ ID NO: 15).~~

~~58.~~27. ~~An~~ The expression system of claim 1, comprising at least one polynucleotide encoding a modified ~~n~~ influenza HA protein, wherein the HA0 cleavage site is modified by introducing one or more basic amino acids~~at least one polynucleotide comprises a nucleic acid construct as defined in any of claims 54 to 57, and wherein the expression system is preferably defined according to any of claims 1 to 25~~.

~~59.~~28. An isolated protein mixture encoded by the expression system of claim ~~58~~27.

~~60.~~29. An isolated host cell containing the expression-system according to claim ~~58~~ 27 and/or the protein mixture of claim ~~59~~28.

~~61.~~30. A composition comprising the expression-system according to claim ~~58~~27 ~~and/or the protein mixture of claim 59~~and a pharmaceutical acceptable carrier and/or excipient.

~~62.The composition of claim 61, comprising a further active ingredient, preferably selected from the group consisting of an adjuvant and an active ingredient.~~

~~63.The expression-system according to claim 58 and/or the protein mixture of claim 59, an isolated cell according to claim 60, or a composition according to claims 61 or 62, for use in the treatment or prevention of a disease, preferably an influenza virus A infection.~~

~~64.~~31. A method of treatment or prevention of a viral disease comprising administration of an effective amount of the expression-system according to claim ~~58~~276 ~~and/or the protein mixture of claim 59, an isolated cell according to claim 60, or a composition according to claims 61 or 62~~.

~~65.The expression-system according to claim 58 and/or the protein mixture of claim 59, an isolated cell according to claim 60, or a composition according to claims 61 or 62, for use in inducing or enhancing a B-cell response against HA of influenza A virus.~~