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(54) VACCINE

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C12N 7/00 (2006.01)

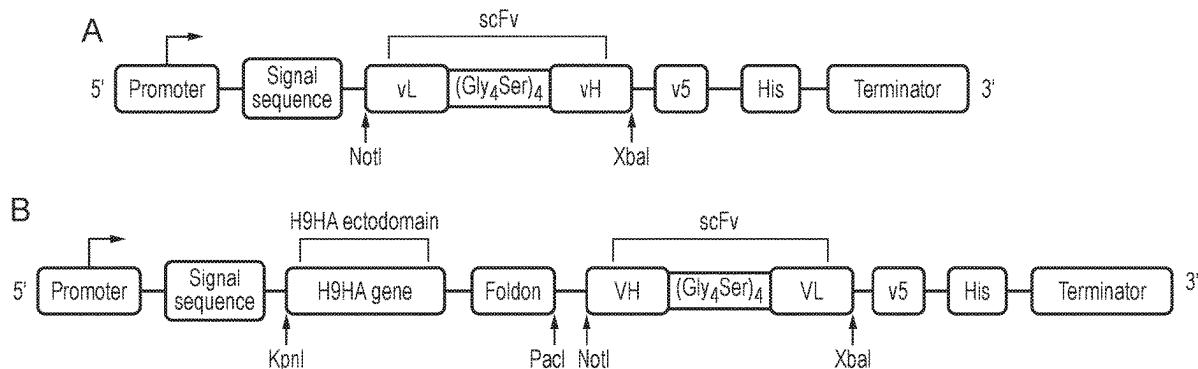
C12N 15/85 (2006.01)

(52) U.S. Cl.
CPC A6IK 39/145 (2013.01); A6IK 39/385 (2013.01); A6IP 31/16 (2018.01); C07K 14/005 (2013.01); C07K 16/2803 (2013.01); C12N 7/00 (2013.01); C12N 15/85 (2013.01); A6IK 2039/552 (2013.01); A6IK 2039/575 (2013.01); A6IK 2039/6056 (2013.01); C07K 2317/622 (2013.01); C12N 2760/16122 (2013.01); C12N 2760/16134 (2013.01)

(57) ABSTRACT

The present invention relates to a genetically engineered protein comprising: at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and a) at least one antigenic polypeptide or b) at least one binding domain which is capable of binding to at least one antigenic polypeptide. The present invention also relates to avian vaccines comprising at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and a) at least one antigenic polypeptide or b) at least one binding domain which is capable of binding to at least one antigenic polypeptide and to the use of such vaccines to treat and/or prevent disease in avian subjects.

Specification includes a Sequence Listing.



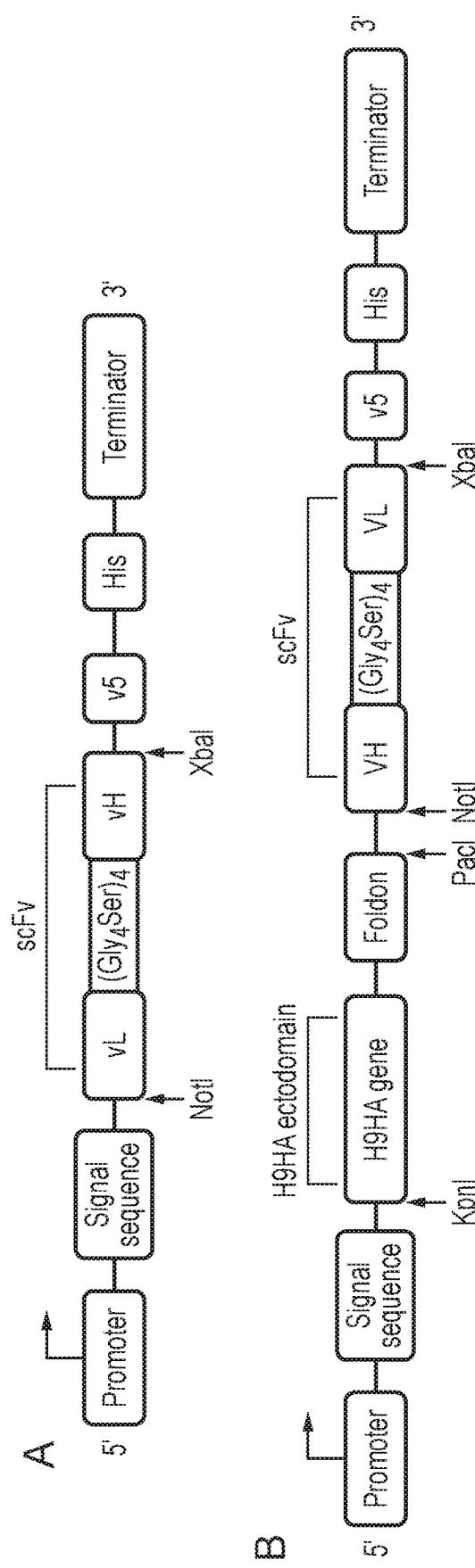


FIG. 1

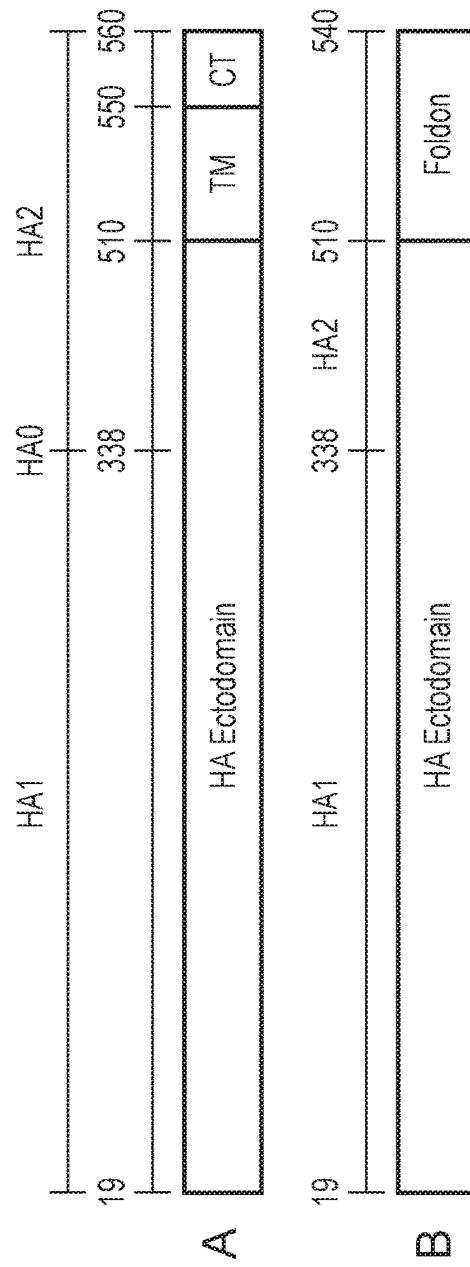


FIG. 2

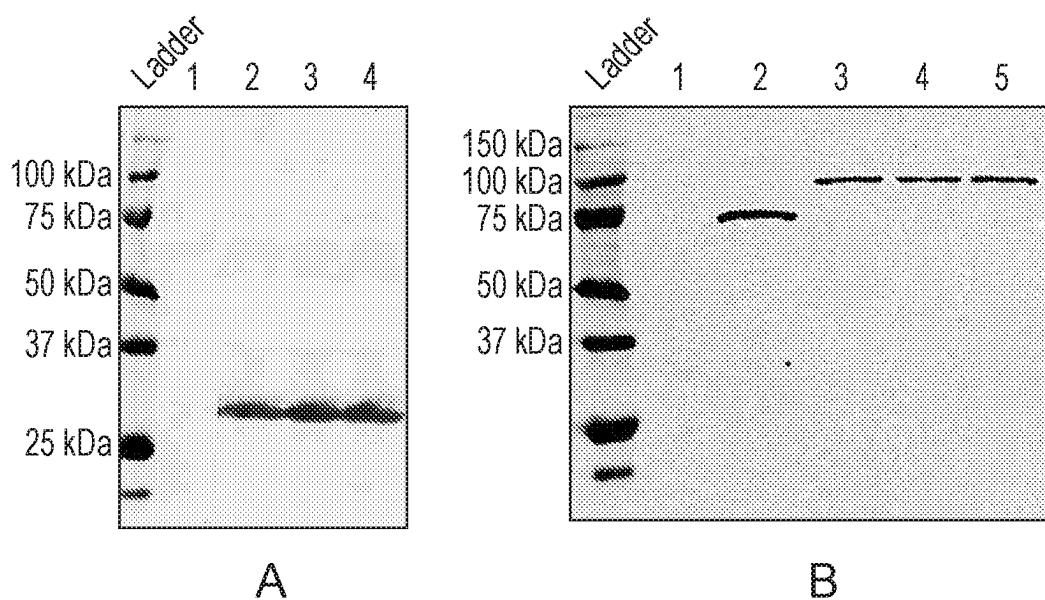


FIG. 3

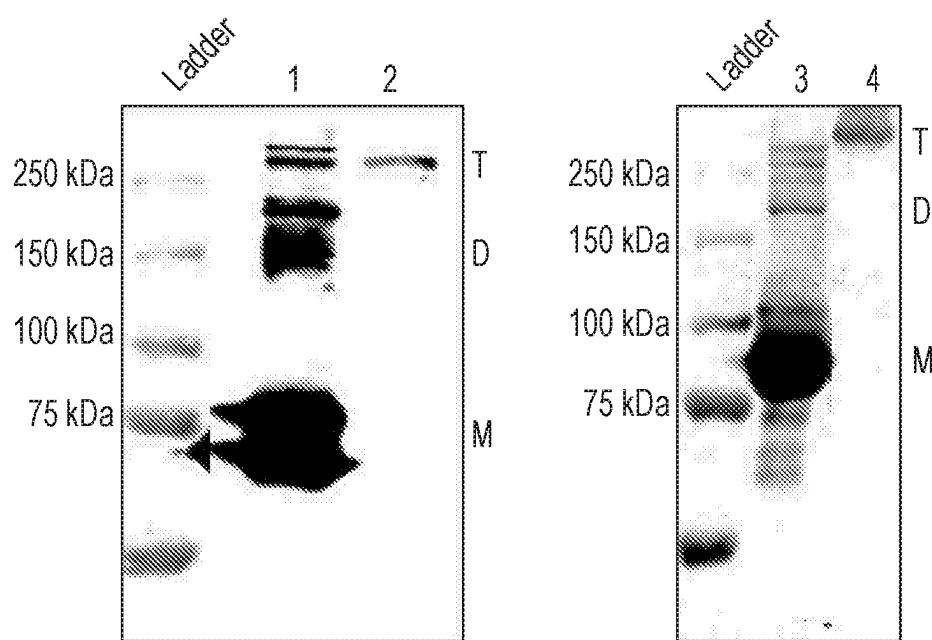


FIG. 4

Table 1

Groups	HA titre (~35 µg of proteins)
PBS control	0
H9HA Foldon	256
H9HA Foldon-Dec205 scFv	512
H9HA Foldon-CD11c scFv	128
H9HA Foldon-CD83 scFv	256

FIG. 5

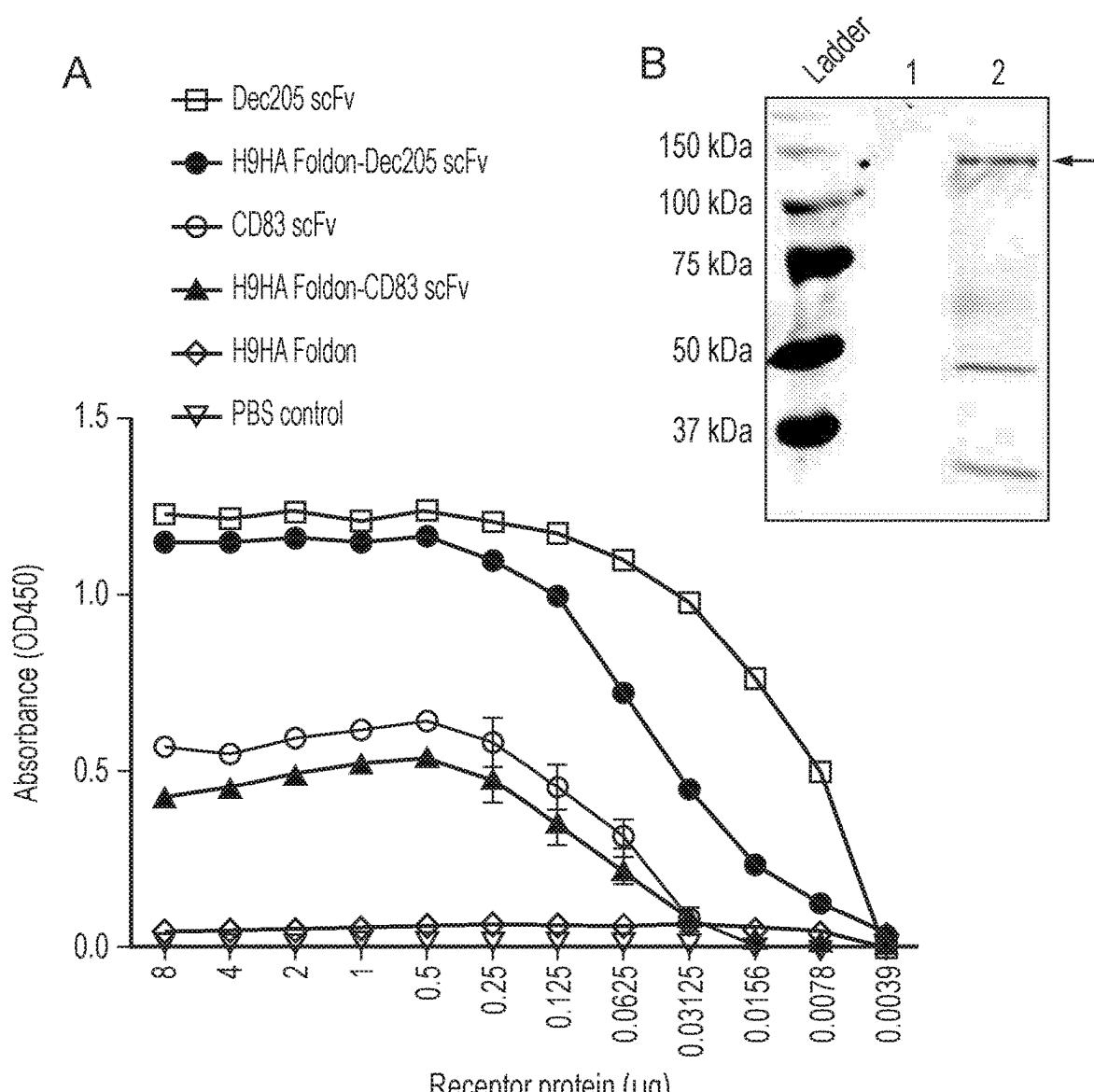


FIG. 6

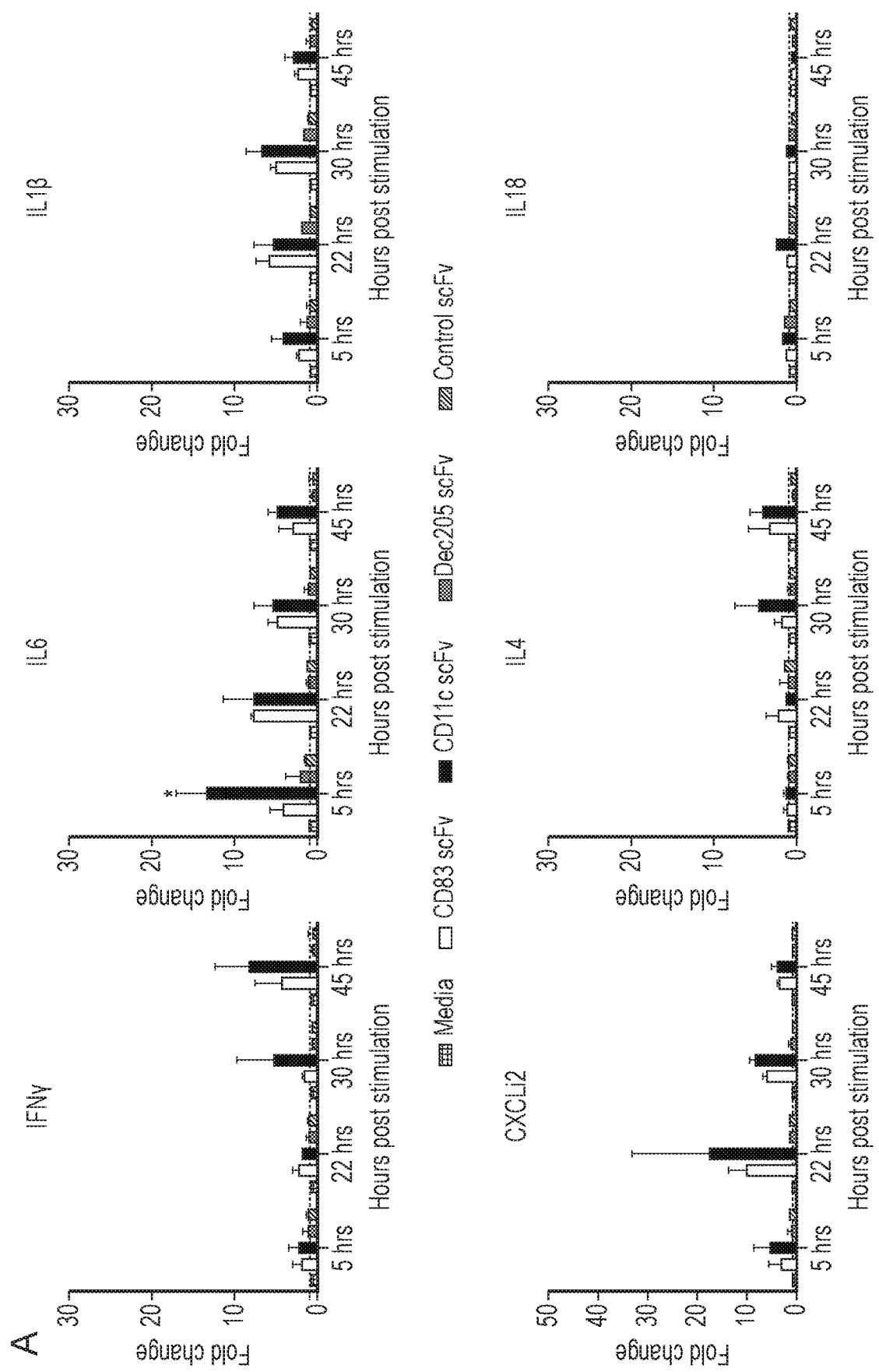


FIG. 7

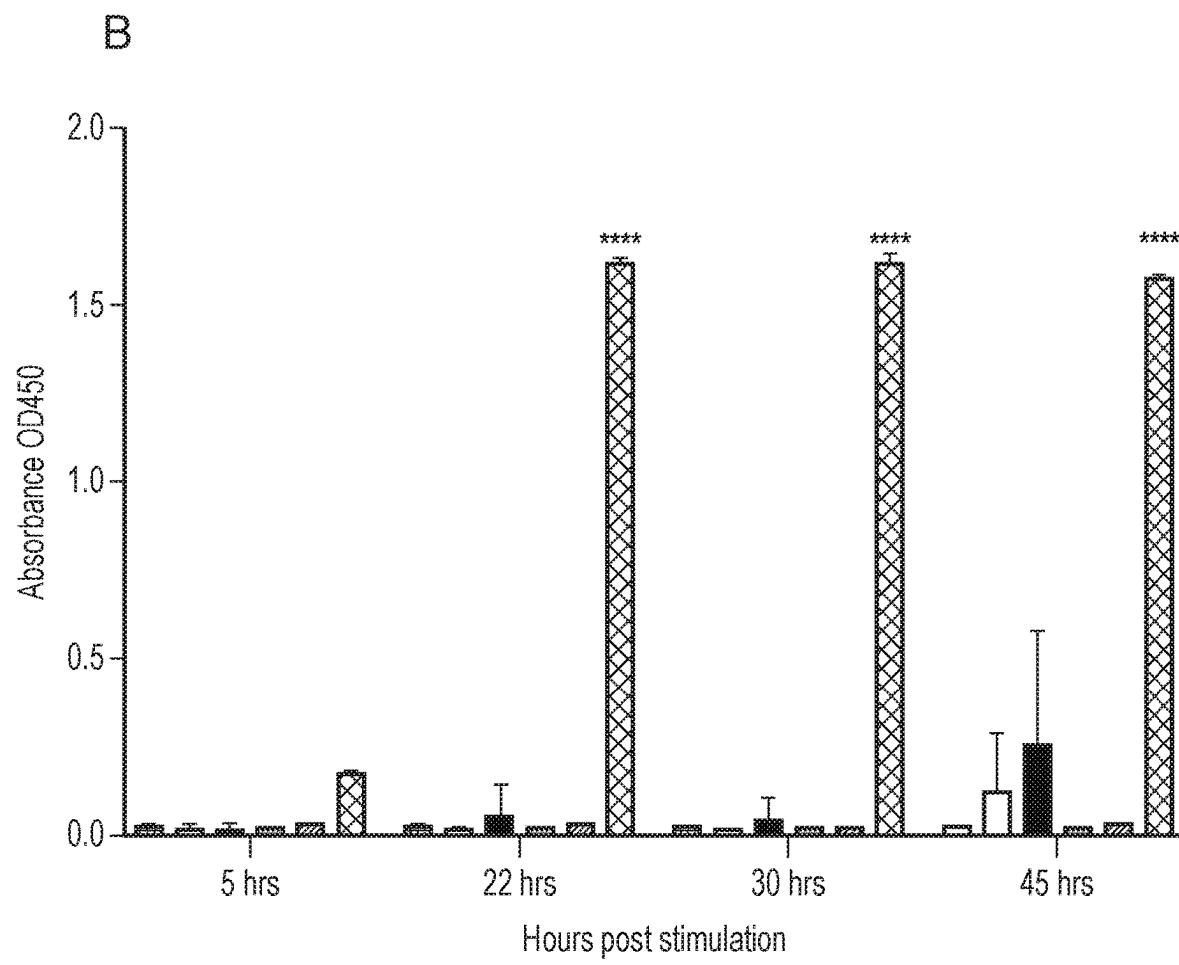


FIG. 7 (Continued)

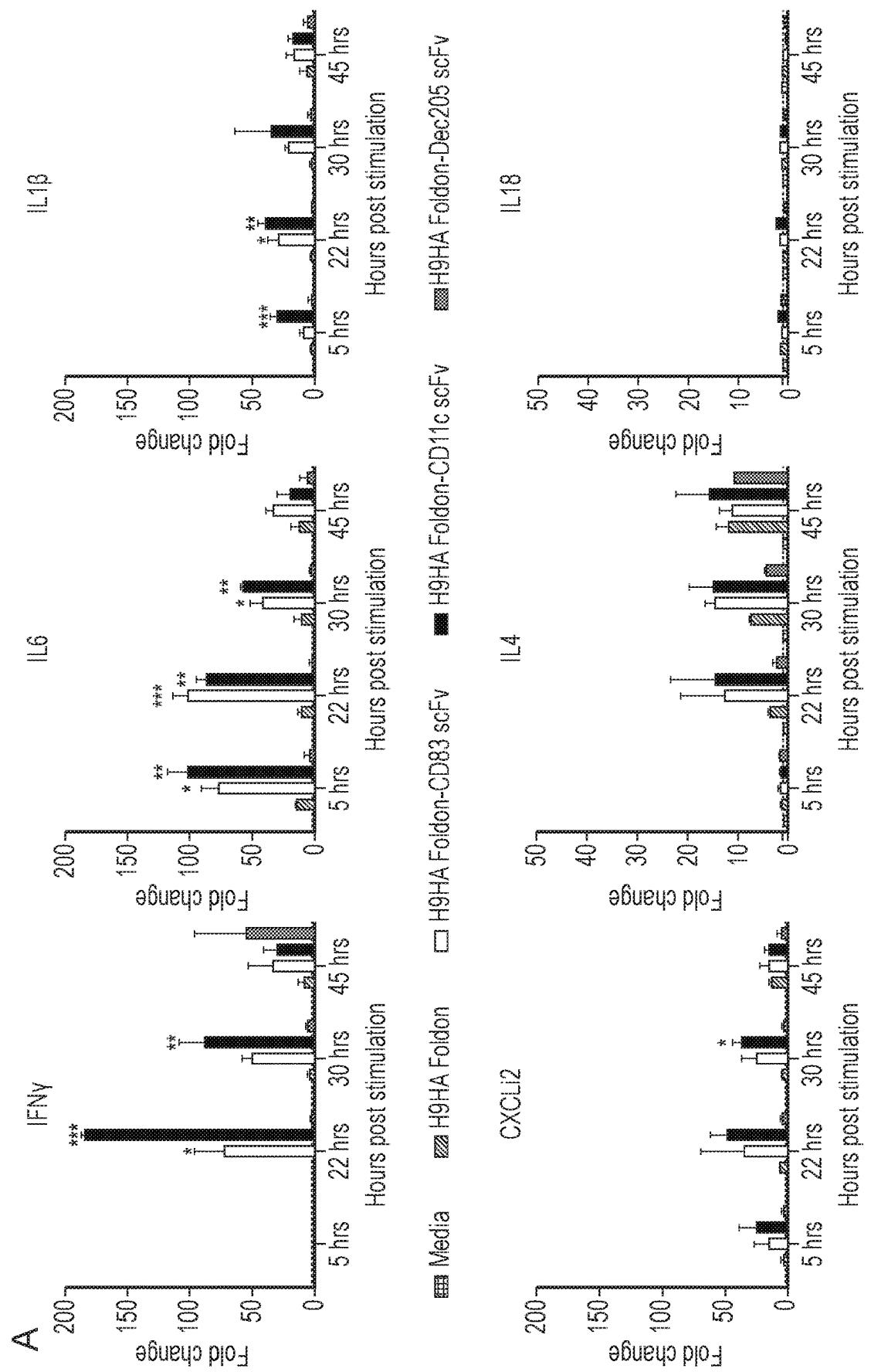


FIG. 8

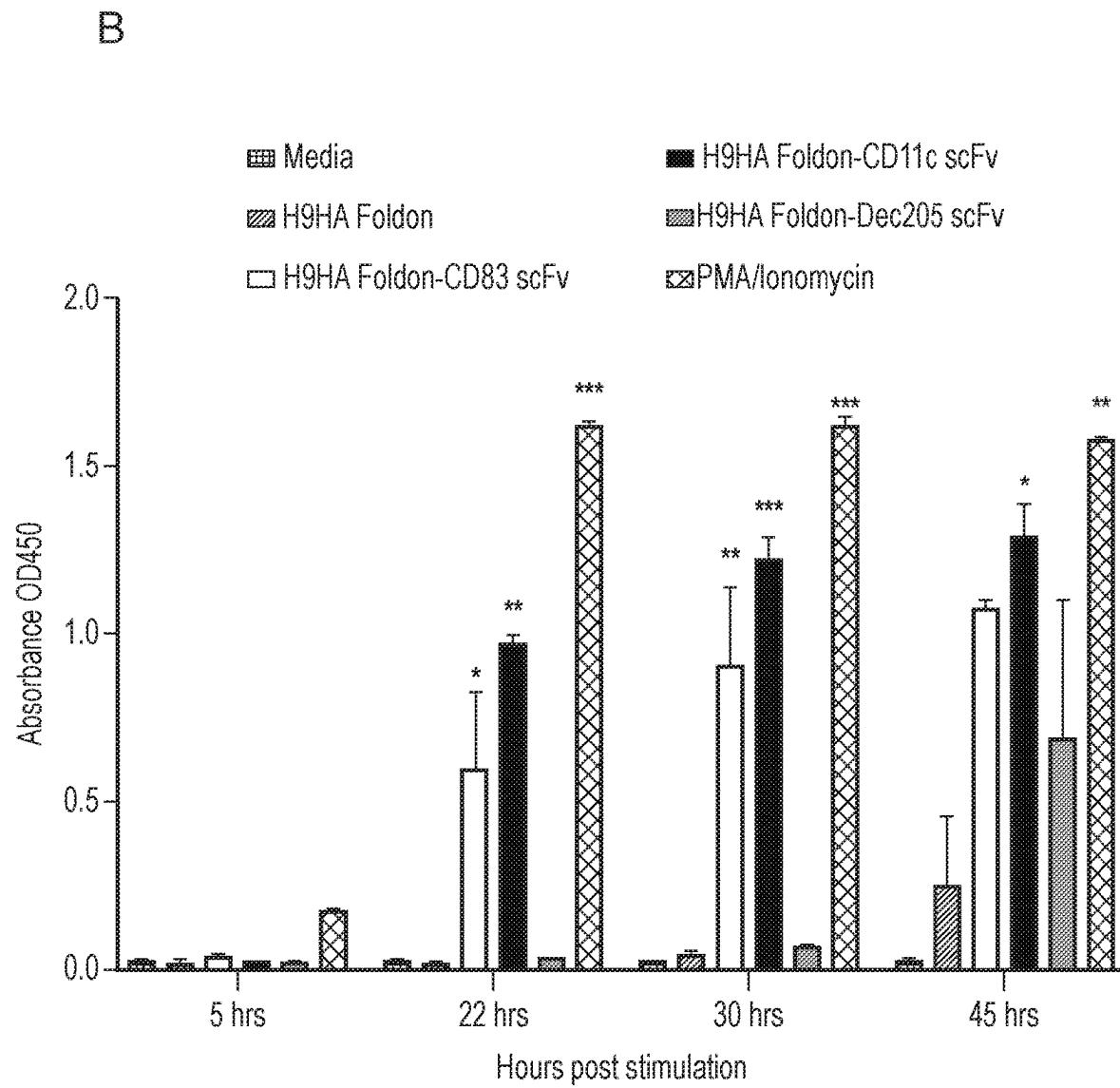


FIG. 8 (Continued)

Table 2

Groups	Average serum HI antibody titre						
	2 µg dose ^P	20 µg dose ^P	35 µg dose ^P /1x10 ⁸ EID ₅₀ per ml ^V	Day 6 ppv	Day 14 ppv	Day 21 ppv	Day 28 ppv
Control PBS	<	<	<	<	<	<	<
Control adjuvant	<	<	<	<	<	<	<
Inactivated H9N2 virus	-	-	-	-	-	<	36
H9HA Foldon	<	160	864	1088	<	185	878
H9HA Foldon-Dec205 scFv	<	100	1312	2240	6	426	2944
H9HA Foldon-CD11c scFv	<	248	3200	2944	9	1078	2432
H9HA Foldon-CD83 scFv	<	99	3384	1264	8	1682	5705

FIG. 9

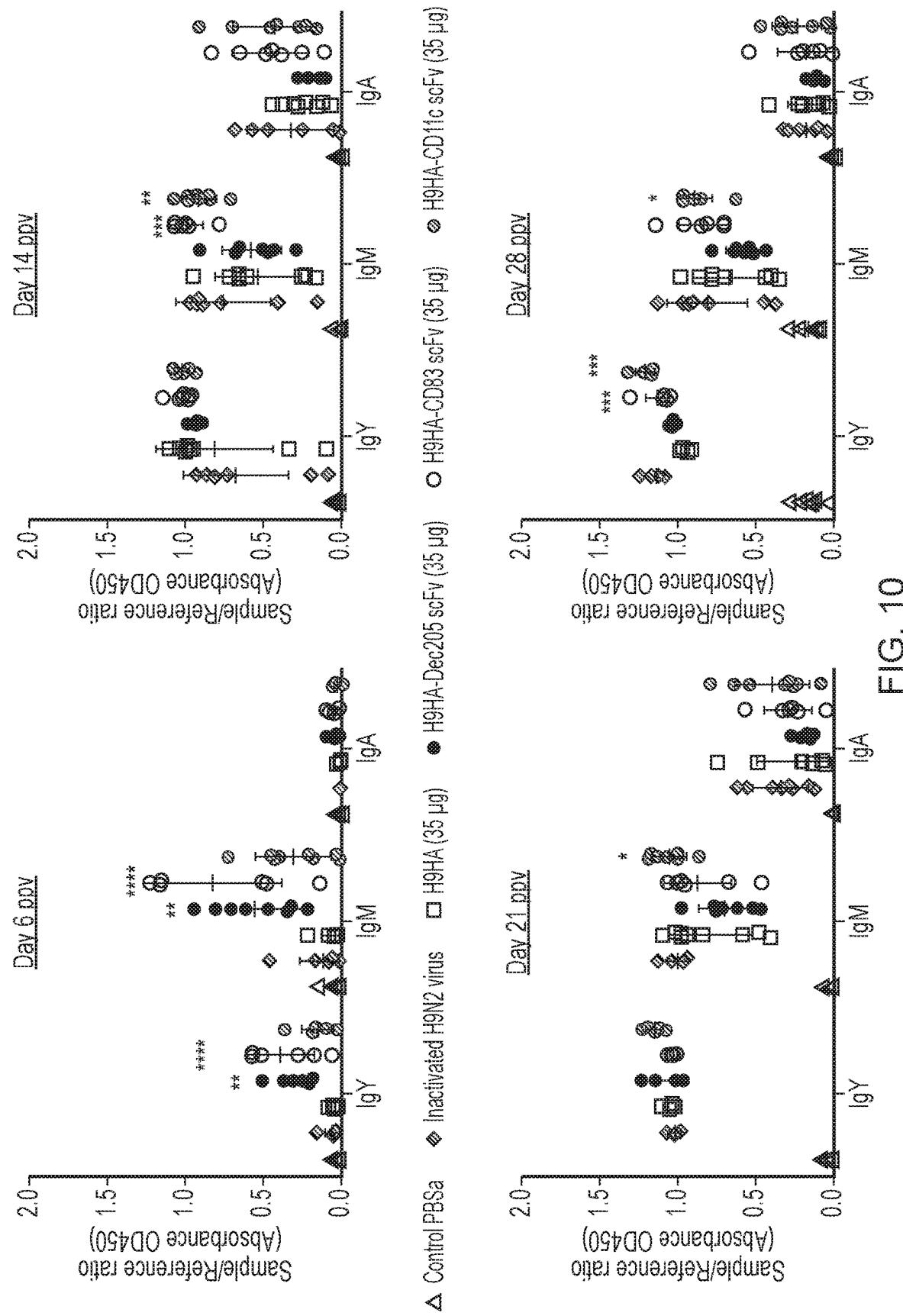


Table 3

Groups	Average MNT titre
PBS control	0
Inactivated H9N2 virus	5800
H9HA Foldon	1450
H9HA Foldon-Dec205 scFv	5400
H9HA Foldon-CD11c scFv	6600
H9HA Foldon-CD83 scFv	24686

FIG. 11

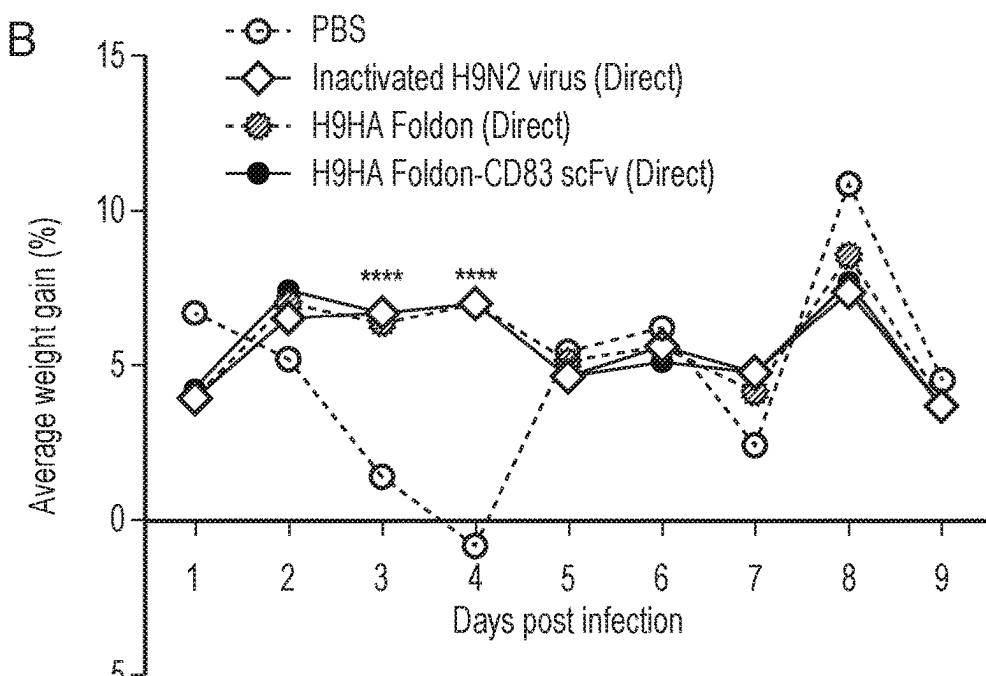
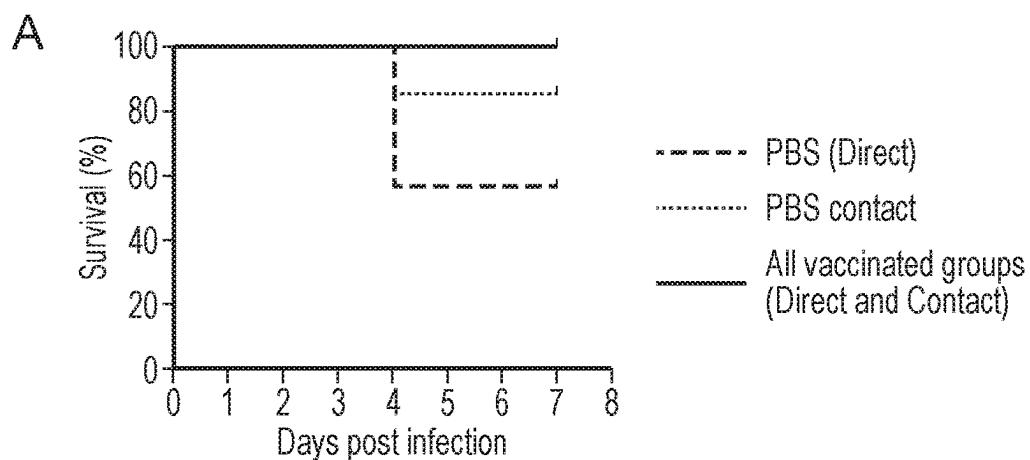
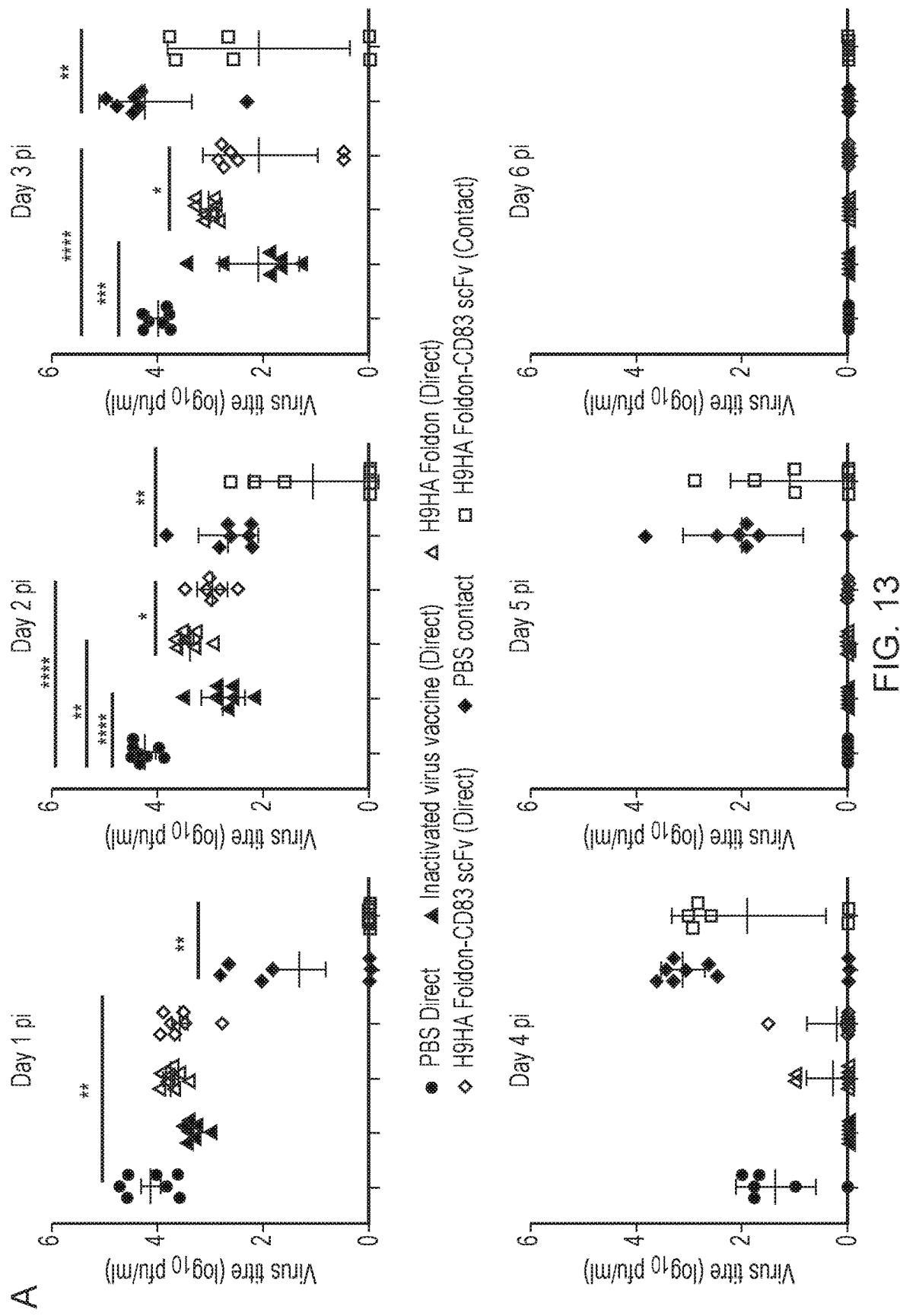


FIG. 12



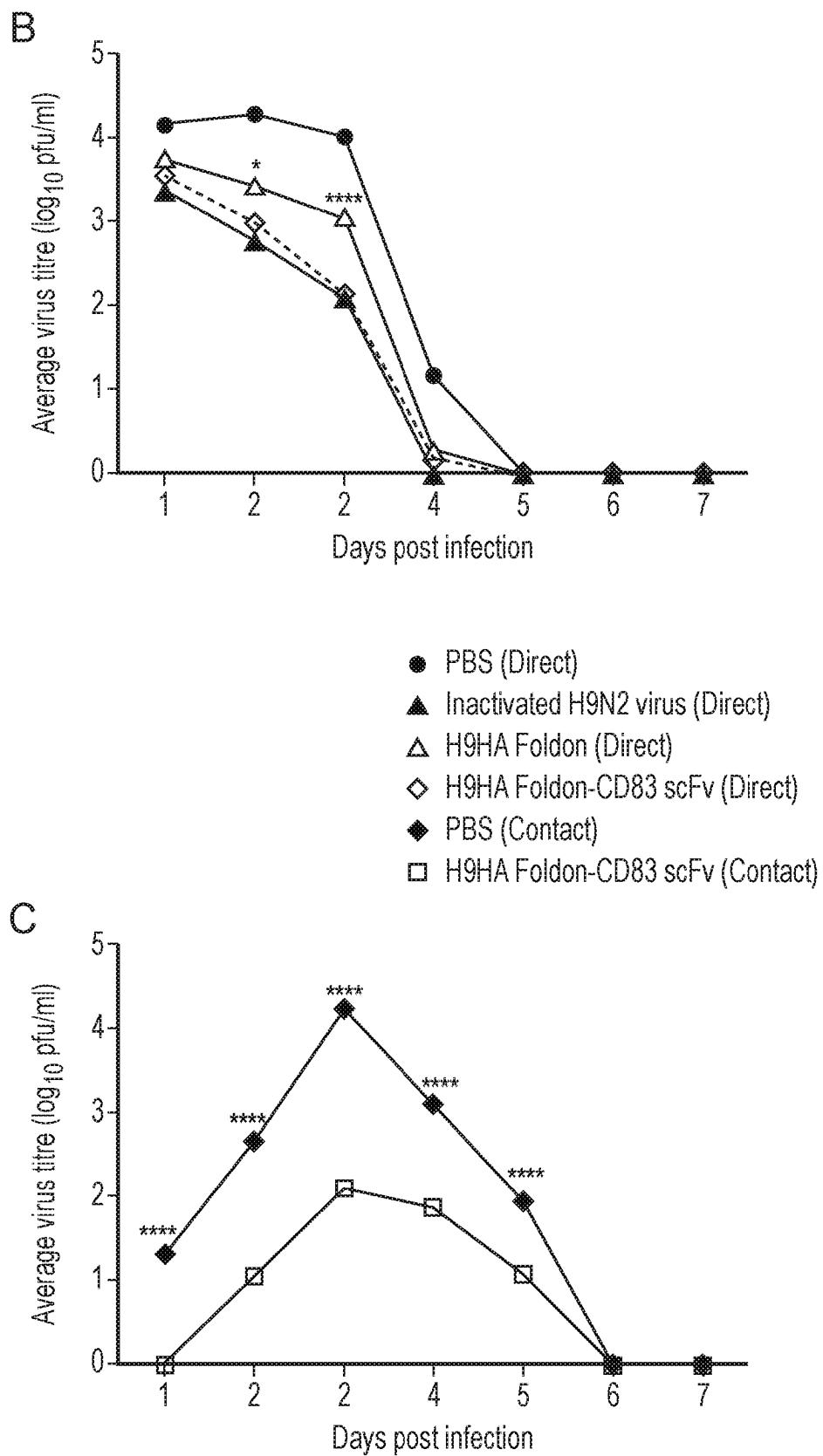


FIG. 13 (Continued)

Table 4

Groups	UDL 01/08 VIRUS					UAE/415 VIRUS				
	Day 6 ppv	Day 14 ppv	Day 21 ppv	Day 28 ppv	Day 35 ppv	Day 6 ppv	Day 14 ppv	Day 21 ppv	Day 28 ppv	Day 35 ppv
Control PBS	^	^	^	^	^	^	^	^	^	^
Commercial vaccine	^	^	26	158	416	630	^	42	79	104
H9HA Foldon (Single)	^	^	5	14	79	69	^	^	^	^
H9HA Foldon (Double)	^	^	18	34	274	416	^	^	8	11
H9HA Foldon-CD83 scFv (Single)	^	^	85	549	1911	1351	^	23	52	69
H9HA Foldon-CD83 scFv (Double)	^	^	91	724	1783	2048	^	39	49	79
Inactivated H9N2 virus	^	^	21	147	208	^	^	^	9	18

FIG. 14

BIP signal-H9HA ectodomain-Foldon-L/NKER-Dec205 scFv-V5-His tag - nucleotide sequence (SEQ ID NO: 65)

ATGAAGTTATGCATATTACTGGCCGTCGTGGCTTGTGGCTCTCGCTCGGATAAGATCTGCATC
GGCCACCAGAGCACCAACAGCACCGAGACCGTGATACCCGACCGAGACAACGGATGCTGTGCCACCAATCTGGCCACCCCTGA
CGCCAAGGAGCTGCTGCACACCGAGACAACGGATGCTGTGCCACCAATCTGGCCACCCCTGA
TCCTGGATACCTGCACCATCGAGGGCTGATCTACGGCAACCCAGCTGCGATCTGCTGCTGGAGGA
CGCGAGTGGTCTACATTGTGGAGCGCCCCAGCGCCGTGAACGGAACCTGCTATCCAGGAACGTGGA
GAACCTGGAGGAGCTGCGCACCTGTTGAGCAGCTGAGCAGCTACCAGCGCATCCAGATCTTCCCCG
ATACCATCTGGAACGTGACCTACACCGGACCAGCAAGAGCTGCGATAGCTTCTACCGAACATG
CGCTGGCTGACCCAGAAGTCCGGCTGTACCCAGTGCAGGATGCCAGTACACCAACAATCGCGCAA
GGACATCCTGTTGTCGAGGGCATCCACCACCCCCAACCGATACGCCAGACCAATCTGTACACCC
GCACCGATAACCACCAGCGTGACCACCGAGAAATCTGGATCGCACCTCAAGCCGTGATCGGCCA
CGCCCACCTCGTAATGGACTGATCGGCCGATCAACTACTATTGGAGCGTGTGAAGCCGGCAGAC
CCTGCGCGTGCAGCAATGAAATCTGATCGCCCGTGGTACGCCACGTGCTGAGCGGAGAGAGCC
ACGGCCGCATTCTGAAGACCGATCTGAACAGCGCAACTGCGTGGTGCAGTGCAGACCCAGAAGGGC
GGCCTGAATAGCACCTGCCCTTCCACAACATCTCGAAGTACGCCCTCGGAAACTGCCCAAGTACAT
CGGCGTGAAGTCCCTGAAGCTGGCATTGAGGGCGCTGGCCAGGACTGGTGGCCGGATGGTACGGATTCCAG
CACAGCAACGATCAGGGCGTGGGAATGGCCGCCATCGCGATAGTACCCAGAAGGCCGTGGATAAGAT
CACCTCCAAAGTGAACAACATCGTGGACAAGATGAACAAGCAGTACGAGATCATGACCACGAGTTCA
GCGAGGTGGAGACCCGCTGAACATGATCAACACAAGATCGACGACCAGATCCAGGATGTGTGGCC
TACAACGCCGAGCTGCTGGTGTCTGGAGAACCGAGAACGCCCTGGACGAGCACGATGCCAACGTGAA
CAATCTGTATAACAAAGTGAAGCGCCCTGGCAGCAACGCCATGGAGGATGAAAGGGATGCTTCG
AGCTGTACCAAGTGCACGATCAGTGCATGGAGACCATCCGCAACGGCACCTACAACCGCCGCAAG
TACAAGGAGGAGAGCCGCTGGAGGCCAGGGCAGCGCTACATCCAGAGGCCACCGACGGACA
GGCCTATGTGCGCAAGGATGGCGAGTGGGTGCTGCTGAGCACCTCCTG **GGTCTGGCTCTGGT**GAGA
TCGTGCTGACACAGAGCCCAGCCTGATGGCTGCTAGCCCAGGCAGAAAGTGACCATTACCTGCAGC
GTGTCCAGCAGCATCAGCAGCGCAACTCCACTGGTATCAGCAGAAGTCCGGCACCTGCCGAAGCT
GTGGATCTACGGAACAAGCAATCTGGCAGCGGAGTGCCTAGTGGAAAGTGGAAAGCGGCA
CCAGCTACGCCCTGACCATCAGTAGCATGGAAGCCGAGGATGCCGACCTACTATTGCCAGCAGTGG
TCGAGCTACCCCTTCACCTCGGAGTGGCACCAAGCTGAAAGTCCGGCACCTGCCGAAGCTGGTGG
CGGAGGTTAGGTGGTGGATCAGGCCAGGGTAGTGAAGTGCAGCTGGTGAAGCGGAGGCGGAAGTGGTGG
ACCTGGTTAAGCCAGCGGAAGCCTGAAGCTGAGTTGCGCTGCCAGCGGATTACCTCAGCTCCTAC
GGCATGAGCTGGTCCGACAGACACCCGATAAGCGCTGGAGTGGTTGCCACCATAGCAGCGGAGG
CAGCTACACGTACTACCCGATAGTGTGAAGGGACGCTTACCATCAGCCGCAACGCCAACAAACA
TCCGTACCTGCAGATGAGCAGCCTGAAGTCCGAGGACCCGATGTATTACTGCGCCCGTCTGAGC
ACCTGGGATTGGTACTTCGATGTGTGGGGACCGGAACCACCGTGACAGTTAGTAGTGGTTCTGGCTC
TGGT**GGTAGCCTATCCCTAACCCCTCTCGGCTCTGATTCTACGCATCATCACCATCACCAT**

FIG. 15

**BIP signal-H9HA ectodomain-Foldon-LINKER-CD83 scFv-V5-His tag - nucleotide sequence
(SEQ ID NO: 66)**

ATGAAGTTATGCATATTACTGGCCGTCGTGGCCTTGTGGCTCTCGCTCGGATAAGATCTGCATC
GGCCACCAGAGCACCAACAGCACCGAGACCGTGGATAACCTGACCGAGACCAACGTGCCAGTGACCCA
CGCCAAGGAGCTGTCACACCGAGCACACGGAAATGCTGCGCCACCAATCTGGCCACCCCTGA
TCCTGGATACCTGCACCATCGAGGGCTGATCTACGGCAACCCCAGCTGCGATCTGCTGGAGGA
CGCGAGTGGTCCACATTGGAGCGCCCCAGCGCCGTGAACGGAACCTGCTATCCAGGCAACGTGGA
GAACCTGGAGGAGCTGCGCACCCCTGTTGAGCTCGAGCAGCTACAGCGCATCCAGATCTTCCCCG
ATACCATCTGGAACGTGACCTACACCGCACAGCAAGAGCTGAGCGATAGCTTCTACCGAACATG
CGCTGGCTGACCCAGAAGTCCGGCTGTACCCAGTGCAGGATGCCAGTACACCAACAATCGCGGCAA
GGACATCCTGTTGTCGGCATCCACCACCCCCAACCGATACGCCAGACCAATCTGTACACCC
GCACCGATAACCACCACCGGTGACCACCGAGAAATCTGGATCGCACCTCAAGCCGTGATCGGCCA
CGCCCACTCGTAATGGACTGATCGGCCGATCAACTACTATTGGAGCGTGTGAAGCCGGCCAGAC
CCTGCGCGTGCAGCAATGAAATCTGATCGCCCGTGGTACGCCACGTGCTGAGCGGAGAGAGCC
ACGGCCGCATTCTGAAGACCGATCTGAACAGCGCAACTCGCTGGTGCAGTGCAGACCCGAGAAGGGC
GGCCTGAATAGCACCCCTGCCCTTCCACAACATCTGAAGTACGCCCTCGGAAACTGCCCAAGTACAT
CGGCGTGAAGTCCCTGAAGCTGCCATCGCCCTGCGCAATGTGCCAGCCCGCAGTAGTCGCGGACTGT
TCGGAGCCATTGCCGGCTTCAATTGAGGGCGCTGCCAGGACTGGTGGCCGGATGGTACGGATTCCAG
CACAGCAACGATCAGGCGTGGGAATGCCGCCATCGCAGTACGAGATACCCAGAAGCCGTGGATAAGAT
CACCTCCAAAGTGAACAACATCGTGGACAAGATGAACAAGCAGTACGAGATCATGACCACGAGTTCA
GCGAGGTGGAGACCCGCTGAACATGATCAACAAACAAGATCGACGACCAGATCCAGGATGTGTGGCC
TACAACGCCGAGCTGCTGGTGTGGAGAACAGAACCCCTGGACGAGCACGATGCCAACGTGAA
CAATCTGTATAACAAAGTGAAGCGCCCTGGCAGCAACCCATGGAGGATGAAAGGGATGCTTCG
AGCTGTACCAAGTGCAGCATCGTGGAGACCATCCGCAACGGCACCTACAACCGCCGCAAG
TACAAGGAGGAGAGCGCGCTGGAGCGCCAGGGCAGCGCTACATCCAGAGGCCAACGCGACGGACA
GGCCTATGTGCGCAAGGATGGCGAGTGGTGCTGCTGAGCACCTCCTG **GGTCTGGCTCTGGT**GATA
TCGTGATGACCCAGTCGCAAGCAGTCTGGCTGTCCGTGGACAGAAAGTGACCATGAGCTGCACC
AGCAGCCAGGTGCTGCTGCACAGCCCCAACAGAAAGAATTACCTGCCCTGGTACGCCAGAAGCCCG
CCAAAGTCCGAAGCTGCTGGCTACTTGCACACGCCAGCGAGAGCGGAGTGCAGATGTTTACCG
GAAGCGGCAGCGGCCACCGATTCAACCTGACAATTAGTAGCGTGCAGGCCAGGATCTGCCGTGTAT
TACTGCCAGCAGCACTACAGCACCCGCTGACATTGGCGCCGGAACGAAGCTGGAAGTGAAGTCAACTGC
AGGTGGTAGTGGTGGCGGGAGGATCAGGTGGTGGTCTGGCGGTGGTGGAAAGTGAAGTCAACTGC
AGCAGAGCGGCCAGAGCTGGTCAAACCCAGGTGCCAGCGTGAAGATCAGCTGCAAGGCCAGCGGATAC
ACCTTCACCGATTACTACATCAACTGGGTCAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGCGA
TATCAACCCACCAACGGCGATAGCACCTACAGCCAGAAGTTCAAGGGCAAAGCCACGCTGACCGTGG
ATAAGAGTAGCAGCACCGCCTACATGGAACGTGCGCAGCCTGACAAGCGAAGTGTCCGCCGTACTAT
TGCGCCGTGATTACGCCATGGATTACTGGGGACAGGGCACCAGTGTGACCGTTAGTAGT **GGTAAGCC**
TATCCCTAACCCCTCTCGGTCTCGATTCTACGCATCATCACCATCACCAT

BIP signal-H9HA ectodomain-Foldon-L/NKER-CD11c scFv-V5-His tag - nucleotide sequence (SEQ ID NO: 67)

ATGAAGTTATGCATATTACTGGCCGTCGTGGCCTTGTGGCTCTCGCTCGGATAAGATCTGCATC
GGCCACCAGAGCACCAACAGCACCGAGACCGTGATAACCTGACCGAGACCAACGTGCCAGTGACCCA
CGCCAAGGAGCTGCTGCACACCGAGACAACGGAATGCTGTGCGCACCAATCTGGCCACCCCTGA
TCCTGGATACTGCACCATCGAGGGCCTGATCTACGGCAACCCCAGCTGCGATCTGCTGCTGGAGGA
CGCGAGTGGTCCCTACATTGTGGAGCGCCCCAGCGCCGTGAACGGAACCTGCTATCCAGGAAACGTGGA
GAACCTGGAGGAGCTGCGCACCCCTGTTCACTGAGCAGCTGAGCAGCTACAGCGCATCCAGATCTTCCCCG
ATACCATCTGGAACGTGACCTACACCGGACCAGCAAGAGCTGAGCGATAGCTTCTACCGAACATG
CGCTGGCTGACCCAGAAGTCCGGCTGTACCCAGTGCAGGATGCCAGTACACCAACAAATCGCGGCAA
GGACATCCTGTTGTCGTGGGCATCCACCACCCCCAACCGATAACGCCAGACCAATCTGTACACCC
GCACCGATAACCACCAGCGTGACCACCGAGAATCTGGATCGCACCTCAAGCCGTGATCGGCCA
CGCCCACCTCGTAATGGACTGATCGGCCGATCAACTACTATTGGAGCGTGCTGAAGCCGGCCAGAC
CCTGCGCGTGCAGCAATGAAATCTGATCGCCCCGTGGTACGCCACGTGCTGAGCGGAGAGAGCC
ACGGCCGCATTCTGAAGACCGATCTGAACAGCGCAACTGCGTGGTGCAGTGCAGACCCAGAAGGGC
GGCCTGAATAGCACCCCTGCCCTTCCACAACATCTCGAAGTACGCCCTCGGAAACTGCCCAAGTACAT
CGGCGTGAAGTCCCTGAAGCTGCCATCGGCCCTGCGCAATGTGCCAGCCCGCAGTAGTCGCGGACTGT
TCGGAGCCATTGCCGGCTTCATTGAGGGCGCTGGCCAGGACTGGTGGCCGGATGGTACGGATTCCAG
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CACCTCCAAAGTGAACAACATCGTGGACAAGATGAACAAGCAGTACGAGATCATCGACCACGAGTTCA
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TACAAGGAGGAGGCCGCTGGAGGCCAGGGCAGCGCTACATCCAGAGGCCACGCGACGGACA
GGCCTATGTGCGCAAGGATGCCGAGTGGGTGCTGCTGAGCACCTCCTG **GGTCTGGCTCTGGTGAAG**
TGCAACTGCAACAAAGCGGCCAGAGCTGGTTAAGCCAGGTGCCAGTGTGAAGATGAGCTGCAAGGCC
AGCGGCTACACCTCACCAACTACGTGCTGCACTGGTCAAGCAGAACGCCCAAGGCCCTGGAATG
GATCGGCTACATCAACCCCTACAACGATGGCACCAAGTTCAACGAGAACGGCAAAGCCACGC
TGACCAGCGATACCAAGTAGCAGCACCCCTCATGGAACTGAGCAGCCTGACCTCCGAAGATAGCGCC
GTGTACTATTGCCGGCTGGCGATAATCTGCGCCCTACTACTTCGATTACTGGGCCAGGGAACGAC
CCTGACAGTTAGTCAGGTGGCGGAGGTAGCGGAGGTGGTGGATCAGGTGGTGGAAAGTGGTGGCG
GTGGATCCCAGATTGTGCTGACACACAGCCCCGCCATCATGAGTGCCTAGCCCAGGCAGAAAAGTGAC
ATGACATGCCAGTGCACGAGCAGCGTGTCTCATGTATTGGTATCAGCAAAGCCGGCAGCAGGCC
GCGTCTGCTGCTGTATGATAAGCTCCCTGAGCAGCGGAGTGCCCGTGCCTTACTGGAAAGCGGAT
CCGGAACAGCTACCCCTGACCATCAGTCGATGGAAGCCGAAGATGCCACGTACTACTGCCAG
CAGTGGTCCCGTTATCCGCCAACATTGGCGGAGGCACCAAGCTGGAATCAAG **GGTAAGCCTATCCC**
TAACCCCTCTCCCTCGGTCTCGATTCTACGCATCATCACCATCACCAT

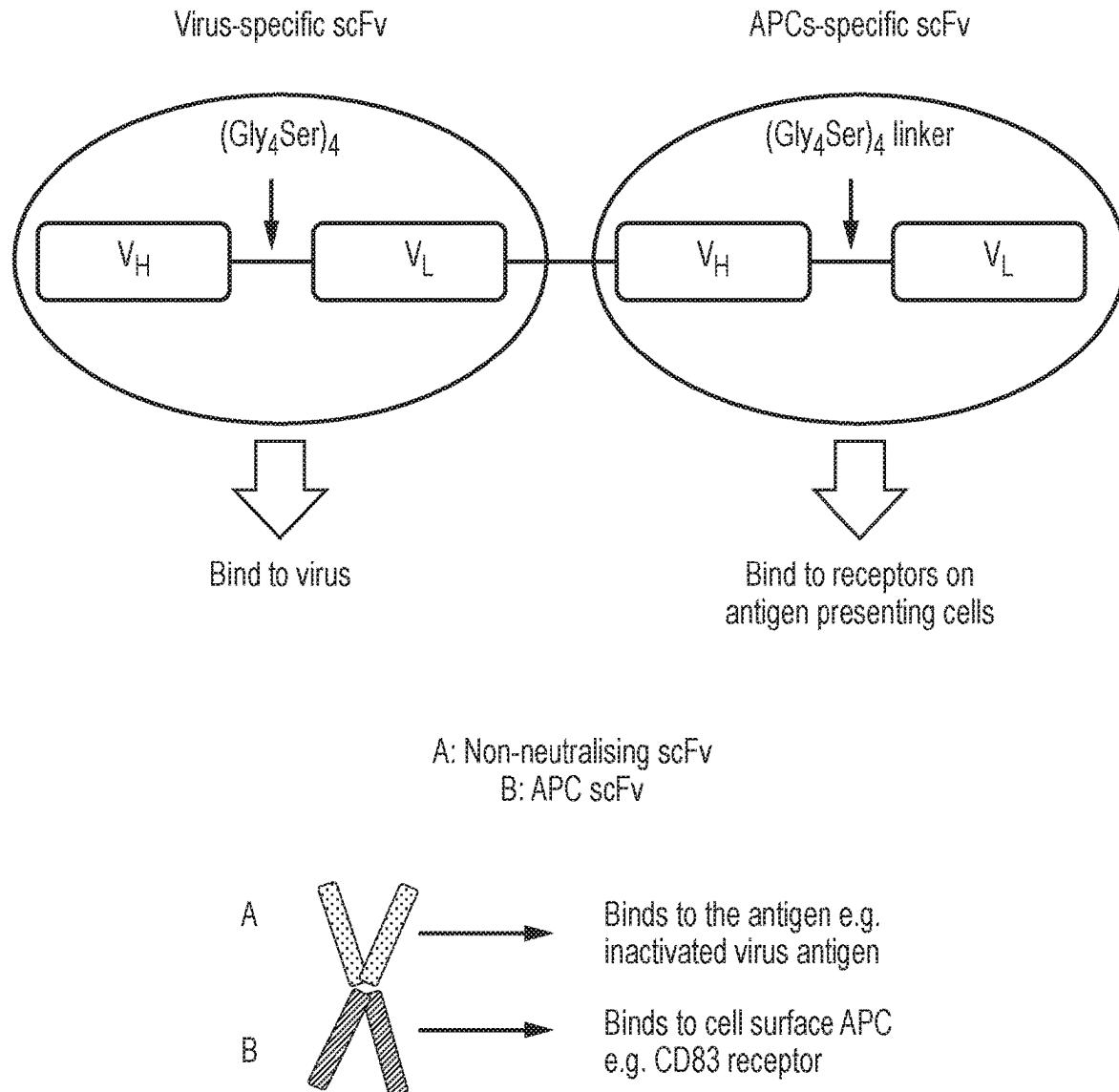


FIG. 18

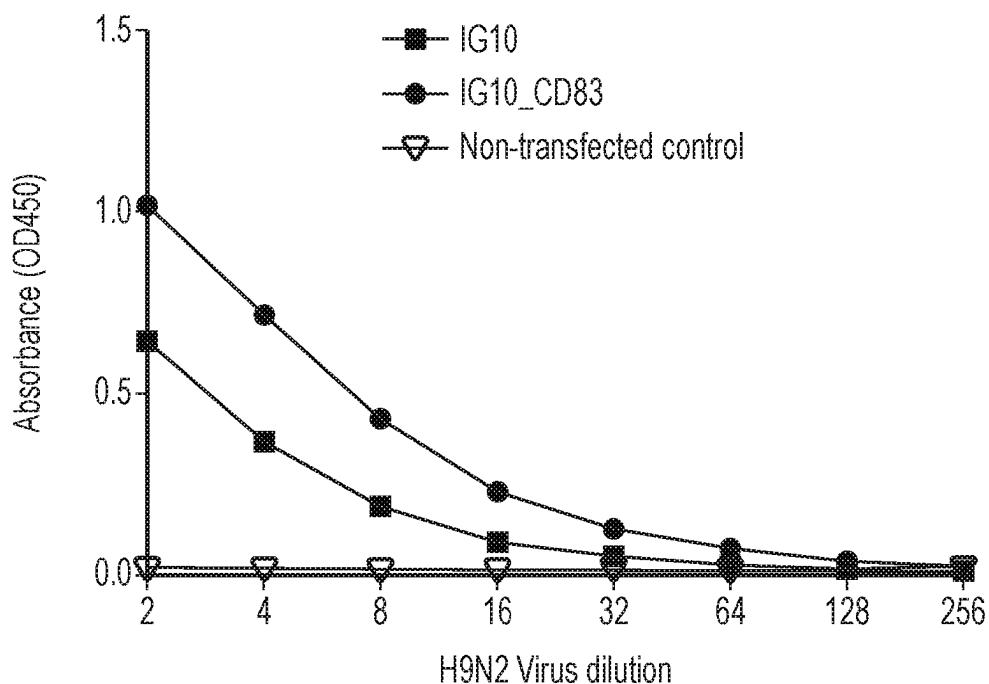


FIG. 19

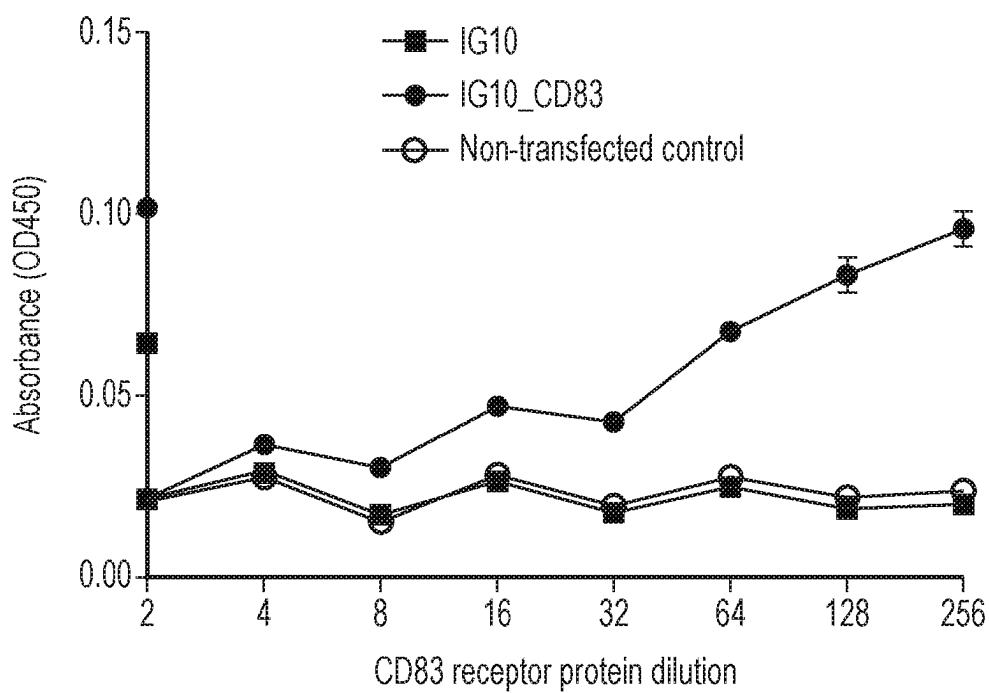


FIG. 20

CD33 SIGNAL-JG10 scFv-(Glycine₄Serine)₄ linker-CD83 scFv-Ctag (SEQ ID NO: 69)

ATGCCCTTGTGCTGCCACTGCTGTGGCCGGAGCCCTGGCATGGATGATATTCTGATGAC
TCAATGCCAAGCTCCATGAGTGTCTCGCTCGGCACACGGTAATAATAACTTGTCACGCTAGTCAGG
GCATTAGCAGTAACATAGGATGGCTCAGCAAAACCCGCAAGAGCTTAAGGGTTGATTTACAC
GCAACGAACCTGGAAGATGGAGTGCCAGCGATTAGTGGCGGAGGCAGCGGTGCCGATTACAGCTT
GACAATATCGAGTCTGGAATCGGAGGACTTGCCTATTATTGTGTACAGTACGGTCAGTTCCAT
TTACGTTCGGAAGCGGTACGAAGTTGGAGATAAAAGGCGGAGGCAGTGGCGGAGGAAGCGGA
GGCGGAGGATCCGAGGTTCAACTGCAACAATCGTTGCAGAATTGGTACGCCAGGAGCAAGTGTGAA
GCTGAGCTGCACGGCAGCGTTCAACATAAACACTTACATGCACTGGTGAAGCAACGCCAG
AGCAGGGCTTGGAGTGGATTGGACGCATTGATCCCGCCAACGGCAACACTAGGTACGCCGAAATT
CAAGGTAAAGCTACGATCACTGCAGATACTCGAGTAACACCCGCTACCTCCAACCTCGAGTCTCAC
TAGCGAGGATA CGGCATCTATTACTGCGCACGGTATTATTGGACCTGATTACTGGGTCAAGGAA
CGACTCTGACTGTATCCAGTGGCGAGGCAGTGGCGGAGGAAGTGGCGGAGGAAGCGGAGGCGAGGATCCGGC
GGAGGCGGCAGT GATATCGT GATGACCAGTCGCCAAGCAGTCTGGCTGTCCGTGGACAGAAAGT
GACCATGAGCTGCACCAGCAGCCAGGTGCTGCACAGCCCCAACAGAAGAATTACCTGGCTGGT
ATCAGCAGAACCCGCCAAGTCCGAAGCTGCTGGCTACTTTGCCACACCGCAGAGAGCCAGTG
CCAGATCGTTTACCGGAAGCGGCAGCGGACCGATTACCCCTGACAATTAGTAGCGTGCAGGCCGA
GGATCTGGCCGTGATTACTGCCAGCAGCACTACAGCACCCGCTGACATTGGCGCCGAACGAAGC
TGGAACTGAAAGCGGAGGTGGTAGGGTGGCGAGGATCAGGTGGTGGTCTGAAGTCAACTG
CAGCAGAGCGGCCAGAGCTGGTCAAACCAAGCAGGTGCCAGCGTGAAGATCAGCTGCAAGGCCAGCGGATA
CACCTCACCGATTACTACATCAACTGGTCAAGCAGAGCCACGGCAAGAGCCTGGAATGGATCGGCG
ATATCAACCCACCAACGGCGATAGCACCTACAGCCAGAAGTTCAAGGGCAAAGCCACGCTGACCGTG
GATAAGAGTAGCAGCACCGCCTACATGGAACTGGCGAGCCTGACAAGCGAAGTGTCCGCGTGTACTA
TTGCGCCCGTGATTACGCCATGGATTACTGGGACAGGGCACCAGTGTGACCGTTAGTAGTGAGCCAG
AGGCT

FIG. 21

FIGURE 22

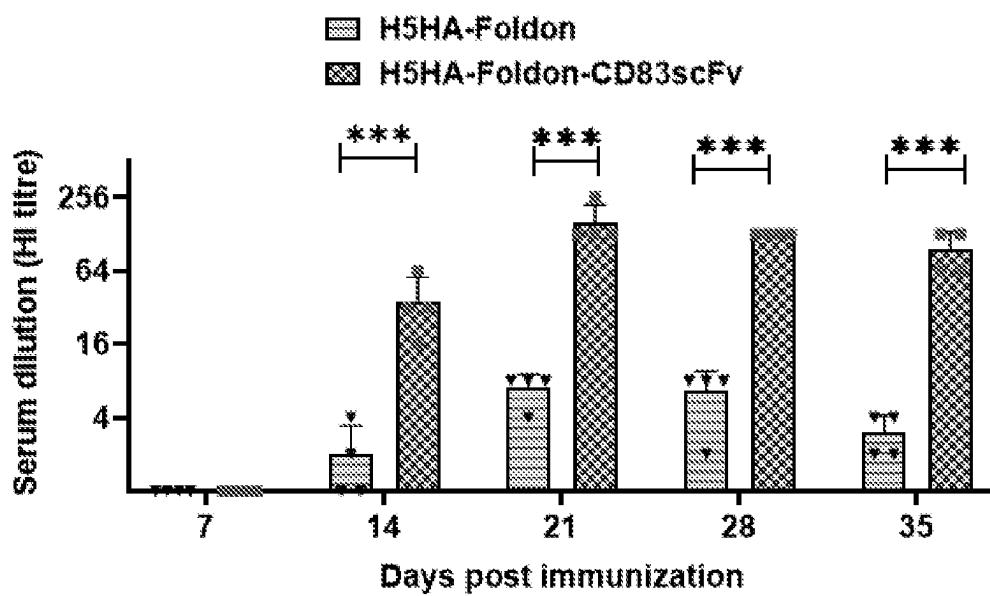


FIGURE 23

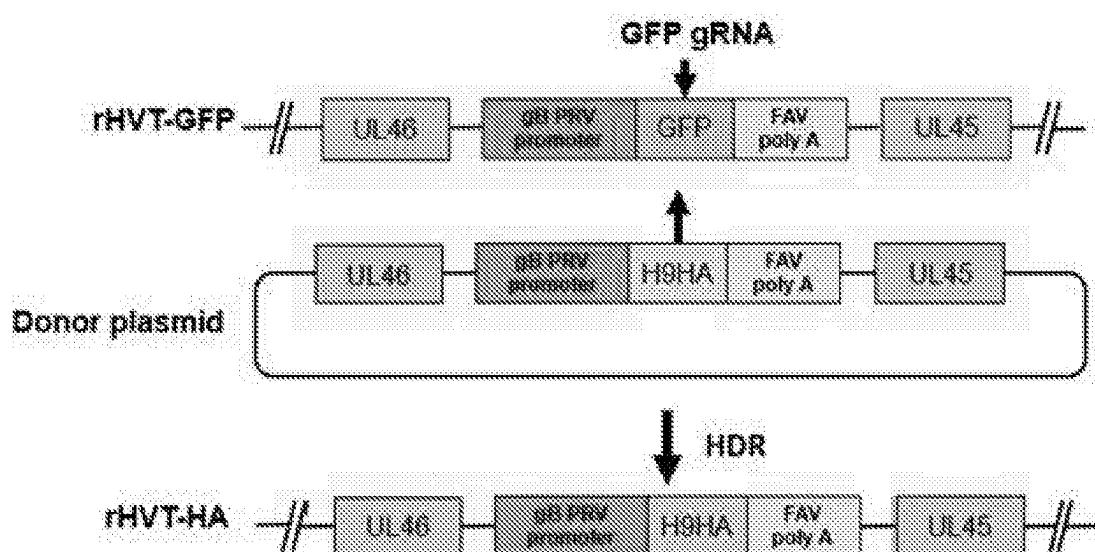


FIGURE 24

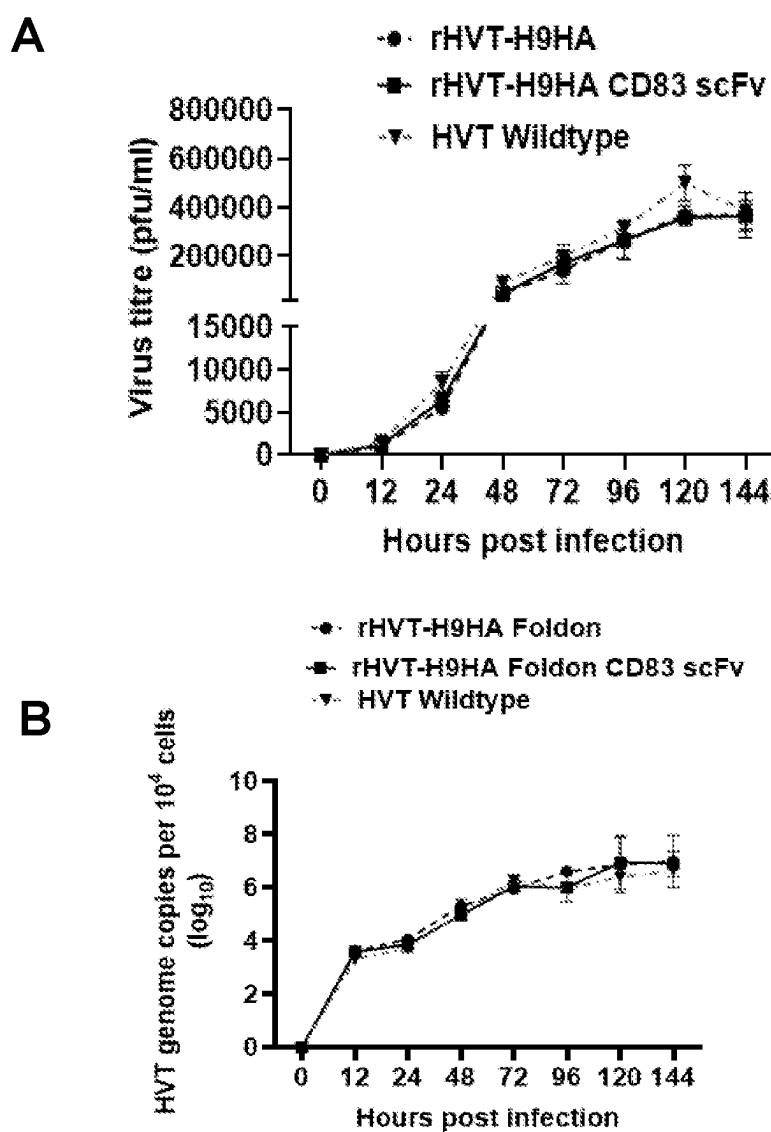


FIGURE 25

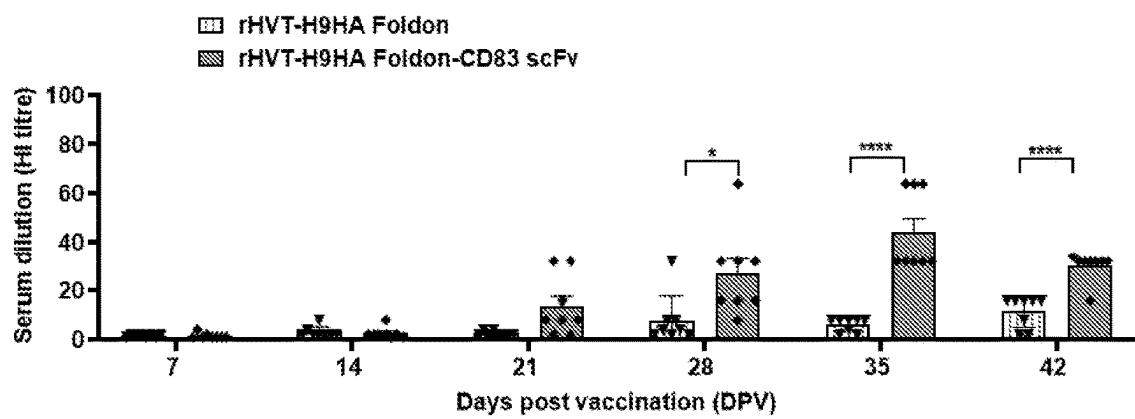


FIGURE 26

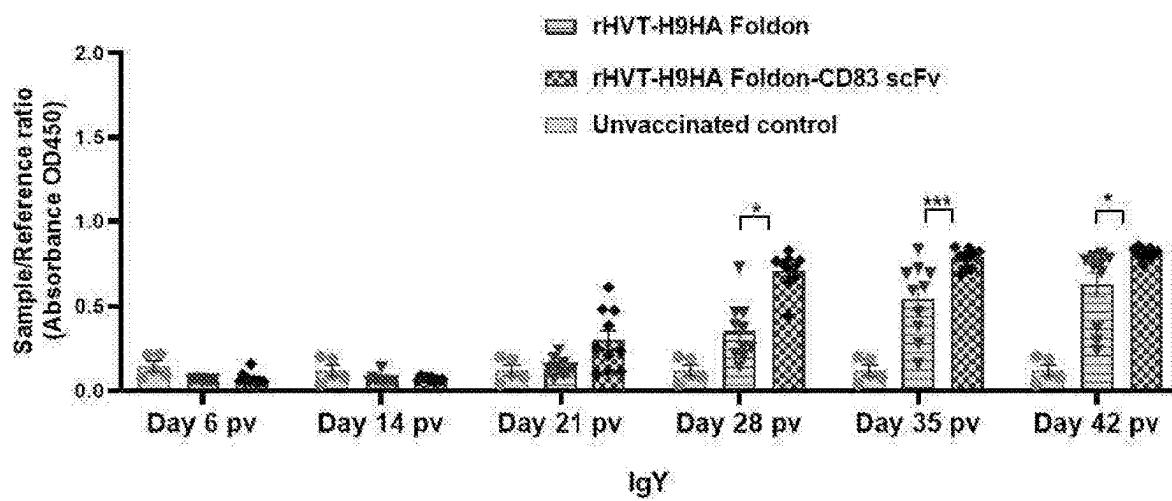


FIGURE 27

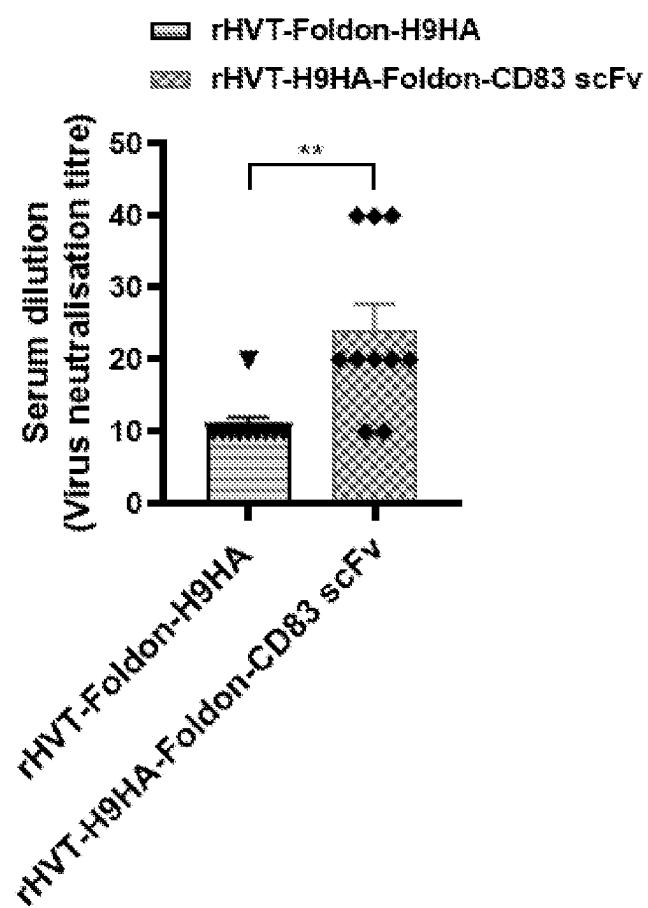


FIGURE 28

SEQ ID NO: 72 : BiP signal- **restriction site**-H5N8 HA ectodomain- **restriction site**-Foldon-
restriction site+Linker -CD83 scFv- **restriction site**-V5 tag **restriction site**-C tag -
Nucleotide sequence was codon optimised for expression in *Drosophila melanogaster* cells.

ATGAAGCTGTGCATCCTGCTGGCCGTGGTGGCCTTGTGGGATTGAGTTGGGCGTAGTCATGGCCCGGG
GATCAGATCTGTATTGGCTATCACGCCATAACAGCACCGAACAAAGTGCACACCATTATGGAAAAGAACGTAAC
CAGTCACGCATGCCAGGACATTCTCGAGAAAACCCAATGGCAAACCTGTGACTGAACGGCGTAAACACC
TCTCATACTGAAAGACTGTTGGCTCGCGGGCTGGCTCTCGGTATCCTATGTGCGACGAATTATCCGCGTAC
CTGAGTGGTCGTATATTGTTGAGCGGGCAAACCCGCAAATGATCTGCTATCCTGGAAGCCTAACGACTA
TGAAGAACTCAAACACCTCCTCGCGCATAAAATCACTCGAAAAAAACTCATACACCAAAGAGCAGTTGGC
CTAACACGAGACATCGCTGGCGTTCCGGCAGCCTGTCCCTATCAGGAACTCCATCGTTTCCGTAATGTA
GTATGGCTCATAAAAAGAATGACGCCATCCTACAATTAAAATTAGCTATAATAACACCCAATCGCGAAGACCT
CCTGATTATGTGGGTATTCACTCCAATAACGCCAAGAACAGACCAACTTGACAAAAACCCACTACCT
ATATAAGTGTGGGTACCACTGAAACCAAAGGCTGGTCAAAGATAGCTACTCGTAGCCAGGTTAATG
GTCAACGGGGACGTATGGACTCTTTGGACAATTGAAACCAAACGACGCAATACATTGAAAGCAATGG
TAACCTTATCGCCCCAGAGTACGCCATAGATCGTCAAGAACGGGAGACTCGACGATAATGAAGAGTGAAGT
AGAACATGGCCACTGCAATACGAAATGTCACGCCAGTCGGTGCCTAACAGCTCGATGCCGTTCCATAAC
ATCCATCCGCTGACGATTGGAGAGTGCCTGGAAATACGTAAGAGCAATAATTGGTCTGGCCACTGGCCTCC
GTAATAGTCCCCAGGGCGAGACACGGGTCTTGGTGCCTAGCTGGCTTATTGAGGGAGGTTGGCAGG
GTATGGTGGACGGATGGTACGGCTATCATAGTAATGAAACAGGGTAGTGGTTACGCAGCGGATAAGGAG
TCCACCCAGAAGGCTATCGATGGAGTGAACAAACAGGTGAACAGTATTGATAAAATGAACACCCAATTG
AAGCAGTGGGACGAGAGTTCAATAATCTGAACCGCGCATCGAAACACCTGAATAAAAGATGGAAGACGGA
TTCCGACGTCTGGACCTACAACGCTGAACCTGGTGTCTCATGGAAAACGAGCGGACGCTGATTCCACG
ATAGCAATGTTAAGAATCTGTATGACAAAGTCCGGCTCAATTGCGAGAACGCAAAGAACACTGGAAACG
GTTGTTTGAGTTCTATCATAATGTGATAACGAGTGCATGGAAAGTGTCCGAAATGGAACATACGATTACCC
ACAGTATTGGAGGAAGCTCGTTGAAAAGGGAGAAATATCCGGCGTAAACTCGAAAGTATCGAACCTA
TCAAGCGCCGCAGGCAGCGGCTACATCCCAGAGGCCCCACCGCACGGACAGGCTATGTGCGCAAGGATG
GCGAGTGGGTGCTGAGCACCTCTGTTAATTAAAGAATTGCGCCGCTCGAGGGAAAGTGGAAAGCGGA
GATATCGTGTGATGACCCAGTCGCCAACGAGCTCTGGCTGTGTCGGACAGAAAGTGCACCATGAGCTGCACC
AGCAGCCAGGTGCTGTCACAGCCCCAACAGAACGAAATTACCTGGCCTGGTATCAGCAGAACGCCCCGCAA
AGTCCGAAGCTGCTGGTCTACTTGCCAGCACCGCGAGAGCGGAGTGCAGATCGTTTACCGGAAGCGGC
AGCGGCACCGATTCCCTGACAATTAGTAGCGTGCAGGCCAGGATCTGGCGTGTATTACTGCCAGCAGC
ACTACAGCACCCCGCTGACATTGGCGCCGGAACGAAAGCTGAACTGAAAGGCCAGGGTGGTAGTGGTGGC
GGAGGATCAGGTGGTGGTGGTCTGGCGGTGGTGAAGTGAAGTGCACACTGCAGCAGAGCGGCCAGAGCT
GGTCAAACCAAGGTGCCAGCGTGAAGATCAGCTGCAAGGCCAGCGGATACACCTTCACCGATTACTACATA
CTGGGTCAAGCAGAGGCCAGGCCAGAGGCCAGGCTGGAATGGATCGCGATATCAACCCACCAACGGCGATAGCA
CCTACAGCCAGAAGTTCAAGGGCAAAGGCCAGCTGACCGTGGATAAGAGTAGCAGCACCGCCTACATGGAAAC
TGCAGCAGCTGACAAGCGAAGTGTCCGCCGTGTACTATTGCGCCCGTGAATTACGCCATGGATTACTGGGACA
GGGCACCAAGTGTGACCGTTAGTAGTTCTAGAGGGCCCTCGAAGGTAAGGCTATCCCTAACCCCTCCTCGGT
CTCGATTCTACGCGTAGGCGGCGAGCCAGAGGCTAA

SEQ ID NO: 73 : (BIP-H5HA-Foldon).

BiP signal- restriction site-H5N8 HA ectodomain- restriction site-Foldon- restriction site-V5- restriction site-Ctag - Nucleotide sequence was codon optimised for expression in *Drosophila melanogaster* cells).

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ATGAAGCTGTGCATCTGCTGGCCGTGGTGGCCTTGTGGGATTGAGTTGGACGTAGTCATGGCCCCGG
GATCAGATCTGTATTGGCTATCACGCCAATAACAGCACCGAACAGTGACACCATTATGGAAAAGAACGTA
CAGTCACGCATGCCAGGACATTCTGAGAAAACCCACAATGGCAAACCTCTGTACTTGAACGGCGTAAAC
TCTCATACTGAAAGACTGTTGGTCGCGGGCTGGCTCTCGGTAACTCTATGTGCGACGAATTATCCGCGTAC
CTGAGTGGCGTATATTGTTGAGCGGGCAAACCCGCAAATGATCTGTCTATCCTGGAAAGCCTCAACGACTA
TGAAGAACTCAAACACCTCCTCGCGCATAAATCACTCGAAAAAAACTCATACACCAAAAGAGCAGTTGGC
CTAACACGAGACATCGCTGGCGTTGGCAGCCTGTCCTATCAGGAACTCCATCGTTTCCGTAATGTA
GTATGGCTCATAAAAAGAATGACGCCTATCTACAATTAAATTAGCTATAATAACACCAATCGCGAAGACCT
CCTGATTATGTGGGGTATTCACTCCAATAACGCCAAGAACAGACCAACTGTACAAAAACCCACTACCT
ATATAAGTGTGGGTACCAAGTACACTGAACCAAAGGCTGGTCAAAGATAGCTACTCGTAGCCAGGTTAATG
GTCAACGGGGACGTATGGACTCTTGGACAATTGAAACCAAACGACGCAATACATTGAAAGCAATGG
TAACTTATCGCCCCAGAGTACGCCATAAGATGTCAGAAGAAGGGAGACTCGACGATAATGAAGAGTGAAGT
AGAACACGGCCACTGCAATACGAAATGTCACGCCAGTCGGTGCATCAACAGCTCGATGCCATCCATAAC
ATCCATCCGCTGACGATTGGAGAGTGCCGAAATACGTAAGAGCAATAATTGGCTTGGCCACTGGCCTCC
GTAATAGTCCCAGGGCAGACACGGGTCTTGGCTGCCATCGCTGGCTTATTGAGGGAGGTTGGCAGG
GTATGGTGGACGGATGGTACGGCTATCATAGTAATGAAACAGGGTAGTGGTTACGCAGCGGATAAGGAG
TCCACCCAGAAGGCTATGATGGAGTGACAAACAAAGGTGAACAGTATTATTGATAAAATGAACACCCAAATTG
AAGCAGTGGGACGAGAGTTCAATAATCTGAACGGCGCATGAAAACCTGAATAAAAGATGGAAGACGGA
TTCCTGACGTCTGGACCTACAACGCTGAACCTGTTGGTGCATGGAAAACGAGCGGACGCGCTGATTCCACG
ATAGCAATGTTAAGAATCTGTATGACAAAGTCCGGCTCAATTGCGAGACACGCCAAAGAACTGGAAACG
GTTTTGAGTTCTATCATAAAATGTGATAACGAGTGATGGAAAGTGTCCGAAATGGAACATACGATTACCC
ACAGTATTGAGGAGGAAAGCTGTTGAAAAGGGAAAGAAATATCCGGCGTAAACCTGAAAGTATCGAACCTA
TCAAGCGGCCCCGCAAGGCAGCGGCTACATCCAGAGGCCCCACGCCACGGACAGGCCTATGTGCGCAAGGATG
GCGAGTGGGTGCTGAGCACCTCCTGTCAGGAAAGGCTATCCCTAACCCTCTCCT
CGGTCTGATTCTACGCGTGAAGGCAGGCCAGAGGCTTAA

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SEQ ID NO: 74 : (H9HA-Foldon)

H9HA ectodomain-**Foldon-ctag**. Amino acid sequence (Nucleotide sequence was codon optimised for expression in *gallus gallus* cells).

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ATGGAGGCTATTAGTCTGATGATTATTCTGCTGGTGGTACAACCTCAAATGCCATAAAATTGATTGGACAT
CACTCTACTAACAGCACAGAGACTGTGGACACCCTGACAGAACTCAACATCCCCGTGACCCAGGAAAAGAGCTG
CTGCACACAGAACACAACGGGATGCTGTGGCCACCAATCTGGGAGGCCCTGATCTGGACACTGCAACCGTG
GAGGGCTGATCTACGGAAACCCCTAGCTGGATCTGCTGGCTGGCGGAAGAGAGTGGAGCTACATCGTGGAAAGG
CCCTCCGAGTGAATGGAACATGCTACCCGGAAAGTGGAAAATCTGGAGGAACCTGCGGATGCTGTTAGCTCC
GCCAGCTCTACCAAGAGAATCCAGATTTCCTGATGCTATCTGGAACGTGACCTACGACGGAACAAGCAAATCC
TGCAGCAACTCCTCTACAGAAATATGAGGTGGCTGACCCAGAAAGAACGGGAATTACCAATTGAGATGCTCAG
TACACAAACAATGGGGAAAGGACATCTGTTATTGGGGGATCCACCAACCCCCCTACTGATACCAACACAGACC
AACCTGTACACAAGAAACTGACACTACCACAAGCGTGAACCTGGAGAATCTGGATAGAACCTTCAAACCTCTGATC
GGCCCAAGGGCCCTGGTGAACGGCCTGATTGGACGCACTCAATTACTACTGGAGCGTGCTGAAGCCTGGACAGACT
CTGCGCGTGGTCCAACGGCAATCTGATGCCCATGGTACGGACACGTGCTGAGCGGAGAGTCCACGGAAAGG
ATTCTGAAGACCGACCTGAAATCCGGAACTGCGTGGTGCAGTCCAGACTGAAAAGGGGGCTGAACAGCACC
CTGCCATTCCACAATATCTCCAATACGCCCTCGGCACATGCCCAAGTACATGGAGTGAAGAGCCTGAAACTG
GCTATTGGCCTGCGCAACGTGCCAGCAAAGTCCAATGGGGCTGTTGGCGCAATTGCCGGTTATCGAGGG
GGATGGCCAGGCCTGGTGGATGGTACGGGTTCCAGCACAGAACGATAGGGAGTGGGAATGGCAGCTGAC
AGGGGAAGCACACAGAAGGCAGTGGATAAAATCACTCCAAGGTGAACAAACATCATCGATAAGATGAACAGGCAG
TACGAGATATTGACCAAGCAATTTCGAGATTGAAACACGCCCTGAATATGATTAACAACAGATCGATGACCGAG
ATTCAAGGACGTGTGGCTTACAACGCAAGAGCTGCTGGTGCCTGGAAAATCAGAAGACTCTGGATGAGCACGAC
GCCAACGTGAACAATCTGTACAATAAGGTGAAAAGAGCCCTGGGGAGCAACGCTATGGAGGATGGGAAGGCTGC
TTTGAACGTACCAAGTGCAGTACCGATGACCATGAAACAAATCAGGAACGGCACTTACAATAGAAGGAAGTAC
ACAGAGGAAAGCCGCTGGAGAGGCAGGGATCCGATGGTACGGGCTTCAACGAGGAAAGGCTACGTC
AGGAAGGATGGGAGTGGGTGCTGAGTACATTCTGGGGAAACCTGAGGCATAA

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SEQ ID NO: 75: (H9HA-Foldon-CD83scFv)

H9HA ectodomain-Foldon-CD83scFv-Ctag - Amino acid sequence. (Nucleotide sequence was codon optimised for expression in *gallus gallus* cells).

ATGGAGGCTATTAGTCGTGATTCTGCTGGTGGTACAAACCTCAAATGCCATAAAATTGTATTGGACATCAGTCT
ACTAACAGCACAGAGACTGTGGACACCCCTGACAGAATCCAACATCCCCGTGACCCAGGAAAAGAGCTGCTGCACACAG
AACACAACGGGATGCTGTGCGCCACCAATCTGGCAGGGCCCTGATCCTGGACACTTGACCGTGGAGGGCCTGATCTAC
GGAAACCCTAGCTGCGATCTGCTGCTGGCGGAAGAGAGTGGAGCTACATCGTGGAAAGGCCCTCCGAGTGAATGGA
ACATGCTACCCCTGGAACGTGGAAAATCTGGAGGAACCTGGATGCTTCACTCCGAGCTCTACCAGAGAACATCCA
GATTTTCTGATGCTATCTGGAACGTGACCTACGACGGAACAAGCAAATCTGCAGCAACTCTTACAGAAATATGAG
GTGGCTGACCCAGAAGAACGGGATTACCCAATTCAAGGATGCTCAGTACACAAACAATGGGGAAAGGACATCCTGTTT
ATTTGGGGATCCACCAACCCCCCTACTGATACCAACACAGACCAACCTGTACACAAGAACTGACACTACCACAAGCGTGA
ACCGAGAACATGGATAGAACATTCAAACCTCTGATCGGCCAACGGCCCTGGTGAACGGCCTGATTGGACGCATCAATT
CTACTGGAGCGTGTGAAGCCTGACAGACTCTGCGCGTCCACGGCAATCTGATGCCCATGGTACGGACAC
GTGCTGAGCGGAGAGTCCCACGGAAGGATTCTGAAGACCGACCTGAAATCCGGAACTGCGTGGTGCAGTGCAGACTG
AAAAAGGGGGCCTGAACAGCACCCGCCATTCAAAATCTCAAATACGCCCTGGCACATGCCCAAGTACATCGGA
GTGAAGAGCCTGAAACTGGCTATGGCCTGGCAACGTGCCAGCAAAGTCCAATGGGGGCTGTTGGCGCAATTGCCG
GGTTTATCGAGGGAGGATGGCCAGGCCCTGGTGGCTGGATGGTACGGGTTCCAGCACAGCAACGATCAGGGAGTGGAA
TGGCAGCTGACAGGGGAAGCACACAGAACGGCAGTGGATAAAATCACTCCAAGGTGAACAACATCATGATAAGATGAA
CAGGCAGTACGAGATCATTGACCACGAATTTCGAGATTGAAACACGCCCTGAATATGATTAACAACAAGATCGATGACC
AGATTCAAGACGTGTTACAACCGCAGAGCTGCTGGTCTGGAAAATCAGAAGACTCTGGATGAGCACGACGC
CAACGTGAACAATCTGTACAATAAGGTGAAAAGAGCCCTGGGGAGCAACGCTATGGAGGATGGAAAGGCTGTTGA
ACTGTACCAACAAGTGCATGACCGACTGCTGGTCTGGAAAATCAGAAGACTCTGGATGAGCACGACGC
AGCCGCCTGGAGAGGGCAGGGGATCCGGATACATTCTGAAGCACCGCAGTGGACAGGCCTACGTGAGGAAGGATGG
GGAGTGGGTGCTGAGTACATTCTGGGCCGGGATATTGTGATGACCCAGTCTCTAGTAGCCTGGCGTGTCC
GTGGGGCAGAAAGTGAACATGTTGACCTCTCTCAGGTGCTGCACTCCCCCTAACCAAGAAAAATTACCTGGCCTGG
TACCAAGCAGAAACCTGGCCAGGCCCTAACGCTGCTGGTGTACTCTGCCAGCACTAGAGAGTCCGGCGTGCAGATAGGTT
CACAGGATCCGGAGCGGGCACTGACTTTACCCCTGACAATCAGCTCCGTGCAAGGCCAGGAGATCTGGCGTGTACTACTGCC
AGCAGCACTACAGCACCCCCCTGACATTGGAGCAGGGACAAAAGTGGAACTGAAGGGCGGAGGAGATCCGGAGGAG
GAGGAAGCGGAGGAGGAGGGTCCGGCGGAGGAGGAAGCAGGTGCAGCTGCAGCAGAGCGGAGCAACTGGTGAA
ACCCGGAGCAAGCGTGAAAATCTCTGCAAGGCCAGCGATACTTACCCGATTACTACATTAACTGGGTGAAACAGT
CCCACGGGAAGAGCCTGGAATGGATGGCGATATTAAACCTACTAATGGAGACTCCACCTACAGCCAGAAGTTAAAGGC
AAGGCTACACTGACTGTGGACAAGAGCTCCAGCACCGCATACTGGAGCTGAGATCCCTGACAAGCGAAGTGTCCGCC
TGTACTACTGTGCCAGAGATTATGCTATGGACTATTGGGCCAGGGGACAAGCGTGAAGTGTGAGTTCCGAACCTGAGGC
ATAA

FIGURE 29

SEQ ID NO: 76:

BiP signal- restriction site-H5N8 HA ectodomain- restriction site-Foldon- restriction site+Linker -CD83 scFv- restriction site-V5 tag restriction site-C tag - Amino acid sequence. (Nucleotide sequence was codon optimised for expression in *Drosophila melanogaster* cells)

MKLCILLAVVAFVGLSLGRSPWPGDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLNGVKPLIL
KDCSVAGWLLGNPMCDDEFIRVPEWSYIVERANPANDLCYPGSLNDYEELKHLLSRINHFEKILIPKSSWPNHETSLG
VSAACPYQGTPSFFRNVVWLIKNDAYPTIKISYNNTNREDLLIMWGIGHHSNNAAEQTNLYKNPTTYISVGTSTLNQ
RLVPKIATRSQVNGQRGRMDFFWTLKPNDAIHFESNGNFIAPYAYKIVKKGDSTIMKSEVEYGHCNTKCQTPVG
AINSSMPFHNIHPLTIGECPKYVKSNLVLATGLRNSPQGETRGLFGAIAGFIEGGWQGMVDGWGYHHSNEQG
SGYAADKESTQKAIDGVTKVNSIIDKMNTQFEAVGREFNNLERRIENLNKMKMEDGFLDVWTYNAELLVMENER
TLDFHDSNVKNLYDKVRLQLRDNAELGNGCFEFYHKCDNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGT
YQAAAGSGGYIPEAPRDGQAYVRKDGEWVLLSTFL**LICKNSRPLEGSQSGDIVMTQSPSSLAVSGQKVTMSCSTSSQ**
VLLHSPNQKNYLAWYQQKPGQSPKLLVYFASTRESGPDRFTGSGSGTDFLTISSVQAEDLA^{VYYCQQHYSTPLTF}
GAGTKLELKG^{GGGSGGGGSGGGSGGGSEVQLQQSGPELVKPGASV}KISKASGYTFTDYYINWVKQSHGKSI
EWIGDINPTNGDSTYSOKFKGKATLTVDKSSSTAYMELRSLTSEVS^{AVYYCARDYAMDYWGQGTSVT}VSSSRGPFE
JKPIPPLLGLDSTREGGEPEA*****

SEQ ID NO: 77:

BiP signal- restriction site-H5N8 HA ectodomain- restriction site-Foldon- restriction site- restriction site-C tag - Amino acid sequence. (Nucleotide sequence was codon optimised for expression in *Drosophila melanogaster* cells).

MKLCILLAVVAFVGLSLGRSPWPGDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLNGVKPLIL
KDCSVAGWLLGNPMCDDEFIRVPEWSYIVERANPANDLCYPGSLNDYEELKHLLSRINHFEKILIPKSSWPNHETSLG
VSAACPYQGTPSFFRNVVWLIKNDAYPTIKISYNNTNREDLLIMWGIGHHSNNAAEQTNLYKNPTTYISVGTSTLNQ
RLVPKIATRSQVNGQRGRMDFFWTLKPNDAIHFESNGNFIAPYAYKIVKKGDSTIMKSEVEYGHCNTKCQTPVG
AINSSMPFHNIHPLTIGECPKYVKSNLVLATGLRNSPQGETRGLFGAIAGFIEGGWQGMVDGWGYHHSNEQG
SGYAADKESTQKAIDGVTKVNSIIDKMNTQFEAVGREFNNLERRIENLNKMKMEDGFLDVWTYNAELLVMENER
TLDFHDSNVKNLYDKVRLQLRDNAELGNGCFEFYHKCDNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGT
YQAAAGSGGYIPEAPRDGQAYVRKDGEWVLLSTFL**LICKITREGGEPEA***

SEQ ID NO: 78:

H9HA ectodomain-Foldon- restriction site-CD83 scFv-Ctag - Amino acid sequence.
(Nucleotide sequence was codon optimised for expression in *gallus gallus* cells).

MEAISLMIIILVVTTSNADKICIGHQSTNSTETVDTLTESNIPVTQAKELLHTEHNGMLCATNLGRPLILDCTVEGLIY
GNPSCDLLLGGREWSYIVERPSAVNGTCYPGNVENLEELRMLFSSASSYQRIQIFPDAIWNVTYDGTSKSCNSFYR
NMRWLTQKNGNYPIQDAQYTNNRGKDILFIWGIHHPPDTTQTNLYTRTDITTSVTENLDRTFKPLIGPRPLVNG
LIGRINYYSVLKPGQTLRVRSGNLIAPWYGHVLSGESHGRILKDLKSGNCVVCQCQTEKGLNSTLPFHNISKYA
FGTCPKYIGVKSLKLAIGLRNPVPAKSNRGLFGAIAGFIEGGWPGLVAGWYGFQHSNDQGVGMAADRGSTQKAVD
KITSKVNNIIDKMNRQYEIIDHEFSEIETRLNMINNKIDDQIQDVWAYNAELLVLENQKTLDEHDANVNNLYNKVK
RALGSNAMEDGKGCFELYHKCDDQCMETIRNGTYNRRKYTEESRLERQGSGYIPEAPRDGQAYVRKDGEWVLLST
FLGP^{GDIVMTQSPSSLAVSVGQKV}TMSCTSSQVLLHSPNQKNYLAWYQQKPGQSPKLLVYFASTRESGPDRFTG
SGSGTDFTLTISVQAEDLA^{VYYCQQHYSTPLTF}GAGTKLELKG^{GGGSGGGSGGGSGGGSEVQLQQSGPELV}

KPGASVKISCKASGYTFTDYYINWKQSHGKSLEWIGDINPTNGDSTYSQKFKGATLTVDKSSSTAYMELRSLTSE
VSAYYYCARDYAMDYWGGTTSVTVSSEPEA*

SEQ ID NO: 79:

H9HA ectodomain-Foldon-Ctag. Amino acid sequence (Nucleotide sequence was codon optimised for expression in *gallus gallus* cells).

MEAISLMIILLVVTTSNADKICIGHQSTNSTETVDTLTESNIPVTQAKELLHTEHNGMLCATNLGRPLIILDTCTVEGLIY
GNPSCDLLLLGGREWSYIVERPSAVNGTCYPGNVENLEELRMLFSSASSYQRIQIFPDAIWNVTYDGTSKSCNSFYR
NMRWLTQKNGNYPPIQDAQYTNNRGKDILFIWGIHHPPTDTTQTNLYTRTDITTSVTTENLDRTFKPLIGPRPLVNG
LIGRINYYWSVLKPGQTLRVRSGNLIAPWYGHVLSGESHGRILKDLKSGNCVVCQCQTEKGGLNSTLPFHNISKYA
FGTCPKYIGVKSLLAIGLRNVPAKSNRGLFGAIAGFIEGGWPGLVAGWYGFQHSNDQGVGMAADRGSTQKAVD
KITSKVNNIIDKMNRQYEIIDHEFSEIETRLNMINNKIDDQIQDVWAYNAELLVLENQKTLDEHDANVNNLYNKVK
RALGSNAMEDKGKGFELYHKCDDQCMETIRNGTYNRRKYTEESRLERQGSGYIPEAPRDGQAYVRKDGEVLLST
FLGEPEA*

FIGURE 30

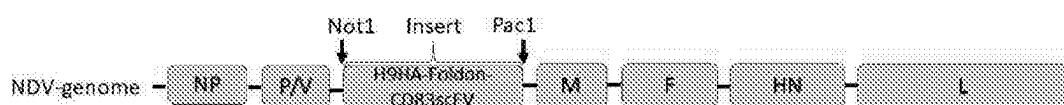
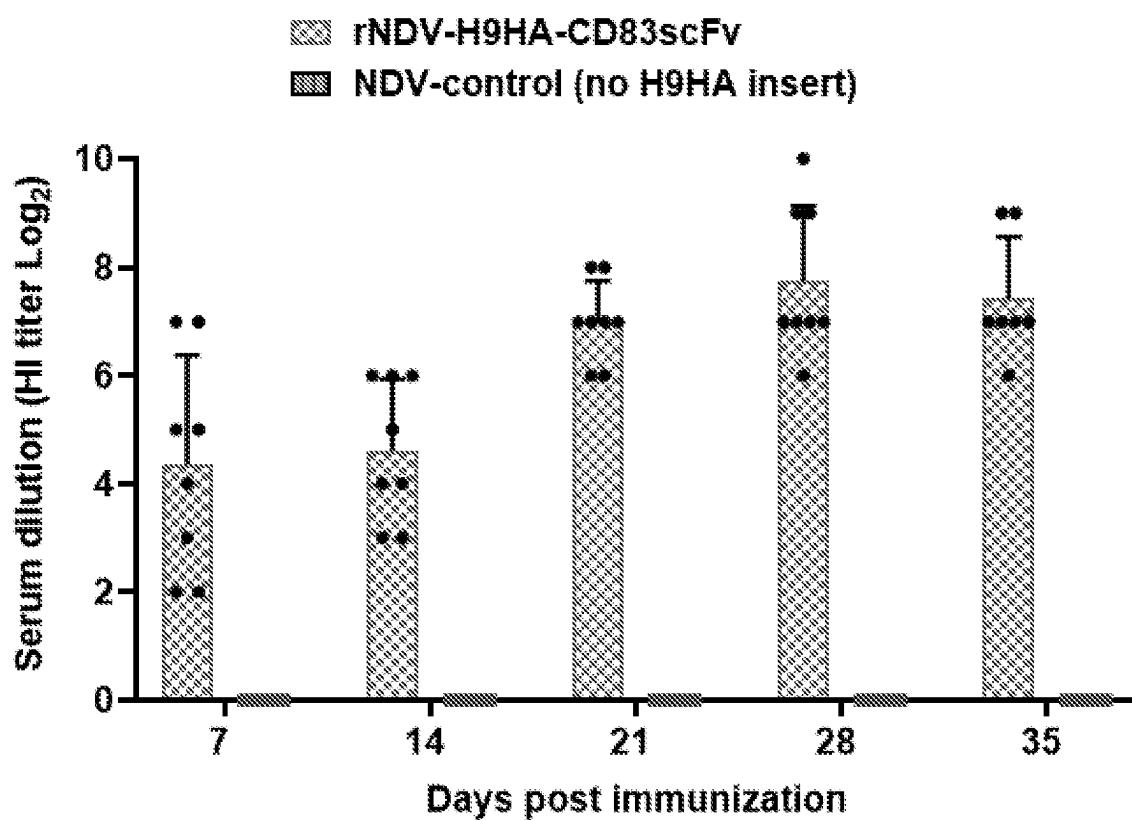


FIGURE 31



VACCINE

FIELD OF THE INVENTION

[0001] The present invention relates to engineered proteins for targeted antigen delivery to avian antigen presenting cells. The present invention also relates to avian vaccines which are targeted antigen delivery vaccines (TADV) or antigen targeted vaccines (ATV). The vaccines protect avian subjects against subsequent challenge with pathogen which comprises the at least one antigen. The present invention also relates to the use of such vaccines to treat and/or prevent disease in avian subjects.

BACKGROUND TO THE INVENTION

[0002] Vaccines are major tools to reduce the impact of disease, such as cancer or infectious disease, in farmed animals and humans.

[0003] Several approaches are employed in poultry vaccine design. Currently, three main formulations of vaccines for poultry diseases are practised. These include inactivated vaccines, live-attenuated vaccines and vectored vaccines.

[0004] The majority of licensed poultry vaccines are inactivated vaccines and largely produced in embryonated hen's eggs and after inactivation, these viruses are reconstituted with adjuvants. These vaccines have some inherent drawbacks including sub-optimal protection to vaccinated birds. For example, despite administration of multiple doses to individual birds (e.g. two to five doses to layers during one year), the infectious virus may continue to circulate in vaccinated flocks; vaccinated birds are not easy to distinguish from naturally infected birds by common serological tests; residual pathogenicity from inactivated vaccines can result in pathogens that can contribute to or exacerbate the outbreak; biohazards associated with manufacturing the vaccines; and low vaccine yield from embryonated hens' eggs when high pathogenicity strains are used as vaccine seed virus, has hindered their cost-effective production.

[0005] Many vaccines administered in multiple doses, inducing sub-optimal immunity that might protect from clinical disease and death, do not prevent shedding of infectious pathogens from vaccinated animals. Thus, the endemic cycle of disease continues.

[0006] In recent years, various strategies have been developed to enhance the immunogenicity of vaccines. One such strategy is ATV or recombinant TADV technology. This vaccine platform selectively delivers antigen to host immune cells known as antigen-presenting cells (APCs) that capture, process and present antigens for initiation and maintenance of immune responses whereby protective antigens are selectively delivered to professional APCs such as dendritic cells (DC), macrophages and B cells. These cells capture, process and present antigens to T lymphocytes for initiation and maintenance of immune responses. Previously, various studies explored antibody-based targeting to mammalian APCs via DC receptor for Endocytosis-205 (Dec205) with limited success. Dec205 is a C-type lectin endocytic receptor of the mannose receptor family and is shown to enhance antigen presentation via the Major Histocompatibility Complex II (MHCII) pathway. There are limited data available for targeting avian APCs for modulating immunogenicity of poultry vaccines. There is a need for improved vaccines to control disease and/or prevent the spread of disease in avian subjects.

SUMMARY OF THE INVENTION

[0007] Generally, the invention relates to directing delivery of antigens to avian immune cells and enhancing potency and efficacy of avian vaccines.

[0008] In one aspect, the present invention provides an engineered protein comprising: at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and at least one antigenic polypeptide.

[0009] In one aspect, the engineered protein is a genetically engineered protein.

[0010] Suitably, the antigenic polypeptide may comprise part or all of an antigen.

[0011] Suitably, the antigenic polypeptide may be part or all of an antigen (for example, the antigenic polypeptide may comprise or consist of one or more domains of the antigen).

[0012] In one aspect, the present invention provides an engineered protein (such as a genetically engineered protein) comprising: at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and at least one binding domain which is capable of binding to at least one antigenic polypeptide.

[0013] The at least one binding domain and at least one antigenic polypeptide or the at least two binding domains may be comprised in a single recombinant protein.

[0014] The at least one binding domain capable of binding to a cell surface protein may be operably linked to the at least one antigenic polypeptide or the at least one binding domain which is capable of binding to an antigen.

[0015] The antigen presenting cell may be at least one of a dendritic cell, macrophage, B cell, T cell or natural killer cell.

[0016] The avian antigen presenting cell may be selected from at least one of a dendritic cell, macrophage, B cell, T cell or natural killer cell.

[0017] The cell surface protein may be selected from an immunoglobulin family protein, an integrin family receptor or a C-type lectin.

[0018] The cell surface protein may be selected from CD83, CD11c or Dec205. The cell surface protein may be CD83.

[0019] The at least one antigenic polypeptide may be an avian influenza virus antigenic polypeptide, such as hemagglutinin antigenic polypeptide.

[0020] The engineered protein (such as a genetically engineered protein) according to the present invention may comprise a signal peptide.

[0021] The engineered protein (such as a genetically engineered protein) according to the present invention may comprise a domain which improves solubilisation, stabilization and/or folding of the engineered protein. In particular, the domain may improve solubilisation, stabilization and/or folding of the antigen.

[0022] The binding domain may be based on or may be the antigen binding site of an antibody or an antibody fragment such as a single-chain variable fragment (scFv), Fv, F(ab') or F(ab')2.

[0023] In another aspect, the present invention provides a nucleic acid construct which comprises a first polynucleotide which encodes at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and a second polynucleotide which

encodes at least one antigenic polypeptide or at least one binding domain which is capable of binding to at least one antigenic polypeptide.

[0024] In a further aspect, the present invention provides a vector which comprises a nucleic acid construct according to the present invention.

[0025] In another aspect, the present invention provides an engineered cell expressing an engineered protein according to the present invention, or comprising a nucleic acid construct according to the present invention, or comprising a vector according to the present invention.

[0026] In another aspect, the present invention provides an avian vaccine comprising an engineered protein (such as a genetically engineered protein) according to the present invention, a nucleic acid construct according to the present invention or a vector according to the present invention and a pharmaceutically acceptable carrier.

[0027] In one aspect, there is provided an avian vaccine according to the present invention for use in treating and/or preventing disease in a subject.

[0028] In another aspect, the present invention provides a method for treating and/or preventing a disease in a subject which comprises the step of administering to a subject an effective amount of a vaccine according to the present invention.

[0029] Suitably, administration of a vaccine according to the present invention may elicit a humoral and/or cellular immune response in the subject.

[0030] Suitably, administration of a vaccine according to the present invention may decrease the challenge pathogen load (such as viral load, bacterial load or parasitic load) in the subject.

[0031] Suitably, administration of a vaccine according to the present invention may elicit production of cross-reactive antibodies in the subject.

[0032] Suitably, the subject may be an avian subject.

[0033] Suitably the subject may be poultry, for example the subject may be selected from a chicken, turkey, duck, quail, pigeon or goose.

[0034] In another aspect, the present invention provides a method for the preparation of the vaccine according to the present invention, the method comprising the step of admixing a genetically engineered protein according to the present invention, a nucleic acid construct according to the present invention, and/or a vector according to the present invention, and a pharmaceutically acceptable carrier.

[0035] In a further aspect, the present invention provides the use of an engineered protein (such as a genetically engineered protein) according to the present invention, a nucleic acid construct according to the present invention and/or a vector according to the present invention in the manufacture of a medicament for the treatment and/or prevention of disease.

DESCRIPTION OF THE FIGURES

[0036] FIG. 1 shows schematic representations of scFv and H9HA ectodomain fused scFv antibody expression cassettes. (A) The scFv expression cassette includes a promoter and *Drosophila melanogaster* immunoglobulin heavy chain binding protein (BIP) secretion signal sequence at the 5' end followed by APCs-specific mAb variable light chain (vL), linker peptide (Gly₄Ser)₄ and variable heavy chain (vH). (B) The expression cassette includes a BIP secretion signal sequence at the 5' end followed by H9HA gene fused

with hemagglutinin trimerization signal (indicated as foldon), linked with APCs-specific mAb vL, linker peptide (Gly₄Ser)₄ and vH.

[0037] FIG. 2 shows schematic representations of recombinant hemagglutinin constructs. (A) Full length A/Chicken/Pakistan/UDL 01/2008 H9N2 hemagglutinin precursor (HA0:1-560 amino acids (aa)), HA1:19-338 aa, HA2: 339-560 aa) TM=transmembrane domain (510-550 aa) CT=cytosolic tail domain (550-560 aa). (B) Soluble A/Chicken/Pakistan/UDL 01/2008 H9N2 construct. Soluble H9HA construct was generated by removing the TM and CT domains (510-560 aa) and fusing the C-terminus of hemagglutinin to 30 aa long trimerization foldon sequence of the trimeric protein fibrin from T4.

[0038] FIG. 3 shows the results of His tag purification of the recombinant proteins. (A) His tag purification of scFv antibody. The size of the purified protein is about 30 kDa. Lane 1: Control supernatant from the untransfected cells; Lane 2: Dec205 scFv; Lane 3: CD11c scFv; Lane 4: CD83 scFv. (B) His tag purification of H9HA Foldon and H9HA Foldon-scFv. The sizes of the purified proteins are about 70 kDa and 100 kDa respectively. Lane 1: Control supernatant from the untransfected cells; Lane 2: H9HA Foldon; Lane 3: H9HA Foldon-Dec205 scFv; Lane 4: H9HA Foldon-CD11c scFv; Lane 5: H9HA Foldon-CD83 scFv.

[0039] FIG. 4 shows the results of crosslinking experiments to determine the oligomeric form of recombinant H9HA ectodomain with foldon. Lane 1: H9HA Foldon without the crosslinker bisulfosuccinimidyl suberate (BS3); Lane 2: H9HA Foldon with 10 mM BS3; Lane 3: H9HA Foldon-scFv without BS3; Lane 4: H9HA Foldon-scFv with 10 mM BS3. M=Monomer (70 kDa*100 kDa[^]) D=Dimer (140 kDa*200 kDa[^]) T=Trimer (210 kDa*300 kDa[^])*H9HA Foldon ^H9HA Foldon-scFv.

[0040] FIG. 5 shows Table 1; the results of a hemagglutination assay to test the activity of recombinant H9HA with foldon.

[0041] FIG. 6 shows characterization of scFv and H9HA Foldon-scFv. (A) Indirect ELISA for testing the binding and detection of scFv and H9HA Foldon-scFv. (B) Detection of CD11c receptor protein from the chicken splenocytes extract by H9HA Foldon-CD11c scFv. Lane 1 represents media only control; Lane 2 represents chicken splenocytes extract. The expected molecular weight of CD11c protein is 150 kDa (shown by the arrow).

[0042] FIG. 7 shows cytokine and chemokine production by splenocytes upon stimulation with scFv antibodies. (A) Cytokines (IFN γ , IL6, IL1 β , IL4 and IL18) and chemokine (CXCLi2) mRNA levels in splenocytes. Data were calculated using $2^{-\Delta\Delta CT}$ approach (n-fold change compared to the media only control group) and reported as values normalised to the expression level of a housekeeping gene ribosomal phosphoprotein lateral stalk subunit PO (RPLPO1). (B) IFN γ protein level upon stimulation of splenocytes. For both (A) and (B) data were analysed by one-way ANOVA followed by Tukey's multiple comparison test. Statistical significance between test scFv and control scFv have been shown with asterisks. *p<0.05.

[0043] FIG. 8 shows cytokine and chemokine production by splenocytes upon stimulation with H9HA Foldon and H9HA Foldon-scFv. (A) Cytokines (IFN γ , IL6, IL1 β , IL4 and IL18) and chemokine (CXCLi2) mRNA levels in splenocytes. Data were calculated using $2^{-\Delta\Delta CT}$ approach (n-fold change compared to the media only control group) and

reported as values normalised to the expression level of a housekeeping gene RPLPO1. (B) IFN γ protein level upon stimulation of splenocytes. For both panels (A) and (B) data were analysed by one-way ANOVA followed by Tukey's multiple comparison test. Statistical significance between H9HA Foldon-scFv vaccine (targeted) and H9HA Foldon vaccine (untargeted) groups have been shown with asterisks. ***p<0.001**p<0.01*p<0.05.

[0044] FIG. 9 shows Table 2; the results of a serum hemagglutination inhibition (HI) antibody titre assay. HI assay was carried out to determine HI antibody titre in the serum of the vaccinated chickens. HI titres are expressed as reciprocal of the highest dilution of antiserum that caused total inhibition of 4 units of virus hemagglutination activity. Average data is shown per (n=8). Figure legend: < indicates HI titres of less than 5; - indicates not applicable

[0045] FIG. 10 shows HA-specific IgY, IgM and IgA antibody levels in the serum of immunized chickens (35 μ g dose). The HA-specific isotypes of the antibodies were determined in 200-fold diluted sera collected at day 6, 14, 21 and 28 post primary vaccination by ELISA. Data is presented as mean \pm SD and analysed by one-way ANOVA followed by Tukey's multiple comparison test. Statistical significance between H9HA Foldon-scFv (targeted) and H9HA Foldon (untargeted) groups has been shown with asterisks ***p<0.0001***p<0.001**p<0.01*p<0.05. The key for treatment groups is provided from left to right.

[0046] FIG. 11 shows Table 3; the virus neutralizing antibody titre measured by virus microneutralization (MNT) assay in the serum of chickens immunized with either H9HA foldon, or H9HA foldon containing CD83 scFv, CD11c or Dec205, inactivated H9N2 virus and PBS control. MNT titres are expressed as reciprocal of the highest dilution of antiserum that blocked the virus infectivity in cultured cells inoculated with 150 TCID₅₀ (50% tissue culture infective dose). Average data is shown (n=8).

[0047] FIG. 12 shows survival and average weight gain of chickens after virus challenge. Panel A) shows percentage survival between vaccinated and PBS treated control chickens challenged with H9N2 virus. Survival curves between directly infected PBS control group (bottom line at days 4-8) and directly infected or contact vaccinated groups significantly different P value=<0.05 (Log rank (Mantel-Cox) test) are shown. Panel B) shows percentage average weight gain between vaccinated and PBS treated control chickens challenged with H9N2 virus. Data was analysed by one-way ANOVA followed by Tukey's multiple comparison test. Statistical significance between the vaccinated and PBS treated control groups is shown with asterisks ***p<0.0001.

[0048] FIG. 13 shows buccal shedding profiles of vaccinated and PBS treated control chickens challenged with H9N2 virus. Panel (A) shows the buccal shedding profile of chickens on different days post infection with each chicken represented by a single point. Panel (B) shows the average buccal shedding profile of at least 6 chickens per group for directly infected birds. Panel (C) shows the average buccal shedding profile of at least 6 chickens per group for contact birds. Data is presented as mean \pm SD and analysed by one-way ANOVA followed by Tukey's multiple comparison test for (A, B) and by unpaired t-test for (C). For panel (B) the asterisks represent significance difference between H9HA Foldon and H9HA Foldon-CD83 scFv (direct)

groups. ****p<0.0001***p<0.001**p<0.01*p<0.05. For panel (A) the key for treatment groups is provided from left to right.

[0049] FIG. 14 shows Table 4: results of a serum HI antibody titre assay. The HI assay was carried out using both vaccine virus strains UDL 01/08 and UAE/415 to determine HI antibody titre in the serum of the vaccinated chickens. HI titres are expressed as reciprocal of the highest dilution of antiserum that inhibited 4 units of virus hemagglutination activity. Average data is shown per (n=10). Figure legend < indicates HI titres of less than 5.

[0050] FIG. 15 shows nucleotide sequence SEQ ID NO: 65, which encodes amino acid SEQ ID NO: 62. SEQ ID NO: 65 comprises the following domains in order: BIP signal-H9HA ectodomain-Foldon-LINKER-Dec205 scFv-V5-His tag—nucleotide sequence.

[0051] FIG. 16 shows nucleotide sequence SEQ ID NO: 66 which encodes amino acid SEQ ID NO: 63. SEQ ID NO: 66 comprises the following domains in order: BIP signal-H9HA ectodomain-Foldon-LINKER-CD83 scFv-V5-His tag—nucleotide sequence.

[0052] FIG. 17 shows nucleotide sequence SEQ ID NO: 67 which encodes amino acid SEQ ID NO: 64. SEQ ID NO: 67 comprises the following domains in order: BIP signal-H9HA ectodomain-Foldon-LINKER-CD11c scFv-V5-His tag—nucleotide sequence.

[0053] FIG. 18 shows a schematic diagram of a genetically engineered protein comprising: at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and at least one binding domain which is capable of binding to an antigen, wherein the genetically engineered protein is a bispecific protein comprising a binding domain capable of binding to a virus antigen.

[0054] FIG. 19 shows ELISA results demonstrating binding of bispecific scFv antibodies to avian influenza virus. IG10 is non-neutralising scFv antibody that bind to hemagglutinin protein of A/CK/Pakistan/H9N2/UDL 01/08 avian influenza virus. IG10-CD83 is bispecific antibody made by recombinantly conjugating IG10 scFv with CD83 scFv.

[0055] FIG. 20 shows ELISA results demonstrating the binding of bispecific scFv antibodies to chicken CD83 receptor protein.

[0056] FIG. 21 shows nucleotide sequence SEQ ID NO: 69 which encodes amino acid SEQ ID NO: 68. SEQ ID NO: 69 comprises the following domains in order: CD33 SIGNAL-IG10 scFv-(Glycine₄Serine)₄ linker-CD83 scFv-Ctag.

[0057] FIG. 22 Analysis of HI antibody titres in serum from chicken vaccinated with H9HA-Foldon-CD83scFv and H9HA-Foldon. HI titres were expressed as the reciprocal of the highest dilution of serum causing the total inhibition of 4 HA units of virus haemagglutination activity. Data are presented as mean \pm SD and analysed by one-way ANOVA followed by unpaired student t-test. ***p<0.005.

[0058] FIG. 23 Schematic representation of generation of rHVT expressing H9HA-Foldon-CD83scFv and H9HA-Foldon antigens using HDR-CRISPR/Cas9 system.

[0059] FIG. 24 The growth kinetics of rHVT-Foldon-H9HA and rHVT-H9HA-Foldon-CD83scFv. Virus titres were measured at different time points after infection with 100 pfu of each virus by (A) plaque titration and by (B) qRT-PCR of HVT SORF1 gene on DNA extracted from infected chicken fibroblast cells (CEF). The virus growth was determined by calculating HVT genome copies per

10,000 CEF cells. The data are presented as mean \pm SD and analysed by one-way ANOVA followed by Tukey's multiple comparison test.

[0060] FIG. 25 Analysis of HI antibody titres in serum from chicken vaccinated with rHVT-H9HA-Foldon-CD83 scFv and rHVT-H9HA-Foldon. HI titres were expressed as the reciprocal of the highest dilution of serum causing the total inhibition of 4 HA units of virus haemagglutination activity. Data are presented as mean \pm SD and analysed by one-way ANOVA followed by unpaired student t-test. ***p<0.0001, *p<0.05

[0061] FIG. 26 HA-specific IgY antibodies in the serum of chickens vaccinated with rHVT-H9HA-Foldon and rHVT-H9HA-Foldon-CD83 scFv. Data are presented as mean \pm SD and analysed by one-way ANOVA followed by Tukey's multiple comparison test. ***p<0.0001***p<0.001**p<0.01*p<0.05

[0062] FIG. 27 Analysis of virus neutralising antibody titres in serum from chickens vaccinated with targeted (rHVT-H9HA-Foldon CD83scFv) and non-targeted (rHVT-H9HA-Foldon) vaccines. Data are presented as mean \pm SD and analysed by one-way ANOVA followed by unpaired student t-test. **p<0.01

[0063] FIG. 28 Nucleotide sequences of constructs

[0064] FIG. 29 Amino acid sequences of constructs

[0065] FIG. 30 Schematic representation of a rNDV-H9HA-Foldon-CD83scFv. The expression cassette (H9HA-Foldon-CD83scFv) indicated as "insert" was cloned into the modified NDV genome using Not1 and Pac1 restriction sites (SEQ ID NO: 75). The expression cassette includes HA protein ectodomain (amino acid from 1-509) of H9N2 virus (A/chicken/Pakistan/827/2016, accession no. MH180417.1). The C-terminus of HA was fused with a 30 amino acid sequence of T4 fibritin trimerization signal (indicated as foldon). This followed by 248 amino acid sequence of APCs-specific CD83scFv that composed of variable light chain (vL), linker peptide (Gly₄Ser)₄ and variable heavy chain (vH). This followed by 4 amino acid (EPEA) Ctag sequence.

[0066] FIG. 31 Analysis of H9HA-specific HI antibody titres in the serum of chickens vaccinated with rNDV-H9HA-Foldon-CD83scFv and NDV control (with no H9HA insert). HI titres were expressed as the reciprocal of the highest dilution of serum causing the total inhibition of 4 HA units of H9N2 virus haemagglutination activity. Data are presented as mean \pm SD.

DETAILED DESCRIPTION

[0067] The present invention provides an engineered protein which is capable of targeting a cargo to an avian antigen presenting cell. The engineered protein comprises at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell. The cargo is typically capable of eliciting an immune response, such as a humoral and/or cellular immune response in an avian subject. Therefore the engineered protein according to the present invention may be used in or as an avian vaccine. Suitably, the cargo may be at least one antigenic polypeptide, such as an antigen from an avian pathogen (for example, an antigen from a virus, a bacterium or a parasite). Suitably, the cargo may be at least one binding domain which is capable of binding to at least one antigenic polypeptide, such as an antigen from an avian pathogen (for example, an antigen from a virus, a bacterium or a parasite).

[0068] An "engineered protein" as used herein refers to both genetically engineered proteins and to chemically engineered proteins.

[0069] In one aspect, the present invention provides a genetically engineered protein.

[0070] In one aspect, the present invention provides a chemically engineered protein.

[0071] In one aspect, the engineered protein is one polypeptide.

Genetically Engineered Protein

[0072] A "genetically engineered protein" as used herein refers to a protein which has been designed and synthesised using recombinant technology.

[0073] In one aspect, the genetically engineered protein is a recombinant protein. In one aspect, the genetically engineered protein is one single recombinant protein. The genetically engineered protein may be designed, synthesized and fused using recombinant DNA technology.

[0074] Suitably, the genetically engineered protein may consist of domains which have been recombinantly fused together.

[0075] The present invention provides a genetically engineered protein comprising: at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and

[0076] a) at least one antigenic polypeptide; or

[0077] b) at least one binding domain which is capable of binding to at least one antigenic polypeptide.

[0078] In one aspect, the genetically engineered protein is encoded by a single open reading frame.

[0079] In one aspect, the genetically engineered protein is a single recombinant protein.

[0080] In some aspects, the genetically engineered protein has not been produced by chemical conjugation.

[0081] In other words, the genetically engineered protein comprises:

[0082] a) at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and

[0083] b) at least one antigenic polypeptide; or

[0084] c) at least one binding domain which is capable of binding to at least one antigenic polypeptide wherein a) and b) or a) and c) are joined by amino bonds.

[0085] Recombinantly engineered proteins may be more reproducible and scalable compared to other methods of producing antibody targeted vaccines. In some cases, chemical conjugation can reduce or eliminate the activity of a protein.

[0086] In one aspect, the at least one binding domain is operably linked to the at least one antigenic polypeptide; or to the at least one binding domain which is capable of binding to at least one antigenic polypeptide.

[0087] When delivered to a subject, the genetically engineered protein is capable of treating and/or preventing disease in said subject. The genetically engineered protein may be capable of eliciting a humoral and/or cellular immune response or reducing challenge pathogen load (such as viral load, bacterial load or parasitic load) when administered to a subject.

[0088] The term "operably linked" as used herein refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner.

[0089] For example, in the context of vectors or expression constructs, a promoter which is capable of driving the expression of a nucleic acid sequence is operably linked to said nucleic acid sequence.

[0090] For example, in the context of an engineered protein, a binding domain capable of binding to a cell surface protein on an avian antigen presenting cell which localises a) at least one antigenic polypeptide or b) at least one binding domain which is capable of binding to at least one antigenic polypeptide on the cell surface of an avian antigen presenting cell is operably linked to a) or b) respectively.

[0091] In one aspect, the genetically engineered protein is a fusion protein.

[0092] As used herein "fusion protein" or chimeric protein refers to a protein which comprises at least two domains which are natively encoded by separate genes but have been joined together so that they are transcribed and translated to produce a single polypeptide.

[0093] The fusion protein may be encoded by a nucleic acid in which the binding domain and the antigenic polypeptide are directly or indirectly attached. An example of an indirect attachment is the provision of a suitable spacer group, such as peptide linker, between the domains.

[0094] In one aspect the binding domain and the antigenic polypeptide are linked or joined by peptide bonds.

[0095] An example of a suitable spacer is a peptide linker. Peptide linkers can be classified into three groups, flexible, rigid and cleavable. Flexible linkers are generally composed of small, non-polar or polar residues such as Gly, Ser and Thr. For example, a flexible peptide linker may be a glycine and/or serine-rich peptide. The suitable peptide linker may comprise 4-20, 4-15, 4-10, 8-20 or 8-15 amino acids. Examples of suitable peptide linkers are known in the art and include, but are not limited to, GGSGGS (SEQ ID NO: 34), SGSGSGS (SEQ ID NO: 35), GGGGSGGGGS (SEQ ID NO: 36), GSGSGSGSGS (SEQ ID NO: 37), GGSGGSGGGSGS (SEQ ID NO: 38), and GGGGSGGGGSGGGGS (SEQ ID NO: 39). More rigid linkers may include proline residues or polyproline motifs such as proline-rich sequences. For example, a suitable spacer may include a proline rich sequence (XP)_n where X is any amino acid, preferably Ala, Lys or Glu or a proline and glycine rich linker (PGPG)_n. Rigid linkers may include alpha helix-forming linkers for example, comprising the sequence of (EAAAK)_n.

[0096] Cleavable linkers may include cyclopeptide linkers, in vivo cleavable disulphide linkers such as LEAGCKNFP*SFTSCGSLE (SEQ ID NO: 33) where * indicates the cleavage site. Other cleavable linkers may include tetra-peptides such as Gly-Phe-Leu-Gly (SEQ ID NO: 70) and Ala-Leu-Ala-Leu (SEQ ID NO: 71). Cleavable linkers allow for in vivo separation of domains.

[0097] Suitably, the engineered protein may be a single recombinant protein.

[0098] Suitably, the engineered protein may be encoded by a nucleic acid construct which comprises a first polynucleotide which encodes at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and a second polynucleotide which encodes at least one antigenic polypeptide; or a second binding domain which is capable of binding to at least one antigenic polypeptide.

[0099] In one embodiment, the present invention provides a genetically engineered protein comprising: at least one

binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and at least one binding domain which is capable of binding to an antigen.

[0100] In some aspects, the binding domain which is capable of binding to an antigen is non-neutralising.

[0101] Typically, the at least one binding domain which is capable of binding to an antigen will be capable of binding to an antigen from an avian pathogen. Suitably, the avian pathogen may be selected from any pathogen e.g. from viruses, bacteria or parasite.

[0102] In some aspects, the antigen may be present on the surface of a virus, such as an inactivated virus. In some aspects, the antigen may be present on the surface of a bacterium, such as an inactivated bacterium. In some aspects, the antigen may be present on the surface of a parasite, such as an inactivated parasite.

[0103] The antigen may be from a virus of the Orthomyxoviridae family. For example, Avian influenza virus (AIV). The antigen may be from a virus of the Paramyxoviridae family. For example, Newcastle disease virus (NDV). The antigen may be from a virus of the Coronaviridae family. For example, Infectious bronchitis virus (IBV). The antigen may be from a virus of the Birnaviridae family. For example, Infectious bursal disease virus (IBDV). The antigen may be from a virus of the Anelloviridae family. For example, Chicken anaemia virus (CAV). The antigen may be from a virus of the Reoviridae family. For example, avian reovirus (ARV). The antigen may be from a virus of the Adenoviridae family. For example Duck Adadenovirus A. or Fowl adenoviruses (FAdV's 2, 4, 8, 11).

[0104] Suitably, the at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and at least one binding domain which is capable of binding to an antigen are comprised in a single recombinant protein.

[0105] Suitably, at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell is operably linked to the at least one binding domain which is capable of binding to an antigen.

Antigen Presenting Cell

[0106] In one aspect, the present invention provides a genetically engineered protein comprising: at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and

[0107] a) at least one antigenic polypeptide; or

[0108] b) at least one binding domain which is capable of binding to at least one antigenic polypeptide.

[0109] The antigen presenting cell may be any avian antigen presenting cell.

[0110] In some aspects, the genetically engineered protein comprises at least one binding domain which is capable of binding to a cell surface protein on at least one of a dendritic cell, macrophage, B cell or natural killer cell. Suitably, the binding domain may be capable of binding to a dendritic cell. Suitably, the binding domain may be capable of binding to a macrophage. Suitably, the binding domain may be capable of binding to a B cell. Suitably, the binding domain may be capable of binding to a natural killer cell.

[0111] In some aspects, the binding domain may be capable of binding to two or more or three or more or all four cells selected from a dendritic cell, macrophage, B cell or natural killer cell.

Cell Surface Protein

[0112] In one aspect, the present invention provides an engineered protein comprising: at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and a) at least one antigenic polypeptide; or

[0113] b) at least one binding domain which is capable of binding to at least one antigenic polypeptide.

[0114] The cell surface protein may be any avian cell surface protein as described herein.

[0115] In one aspect, the cell surface protein selected from an immunoglobulin family protein, an integrin family receptor or a C-type lectin.

[0116] CD83 is a transmembrane glycoprotein belonging to the member of immunoglobulin (Ig) superfamily. CD83 is expressed on the surface of most DCs including thymic DCs, Langerhans cells in the skin, monocytes derived DCs following culture with GM-CSF and IL4, and interdigitating reticulum cells within the spleen. CD83 is also found on the surface of macrophages, neutrophils and NK cells. CD83 has been thought to be implicated in immune response, however its function on DC and T cells remains unclear. Based on the expression profile of CD83 and its structural similarity with B7 family members (CD80/CD86), CD83 is thought to play important roles during interactions between cells of the immune system.

[0117] In one aspect, the cell surface protein may be an immunoglobulin family protein, such as CD83. In one aspect, the cell surface protein is CD83.

[0118] CD11c is a beta2 (β_2) integrin expressed on variety of leukocytes including DCs, macrophages, NK cells, B and T cells. Integrins play an important role in innate immunity. They are involved in the interaction of phagocytic cells with endothelium and extracellular matrix, ingestion of complement-opsonized pathogens and cytokine production. In addition, they are also involved in the proliferation, survival and differentiation of lymphocytes during adaptive immunity.

[0119] In one aspect, the cell surface protein may be an integrin family receptor such as CD11c. In one aspect, the cell surface protein is CD11c.

[0120] C type lectin receptors (CLRs) belong to a large family of transmembrane and soluble receptors that can recognize wide variety of glycans on the pathogens in a calcium dependent manner.

[0121] Dec205 is a type I CLR which consists of a single polypeptide chain; the extracellular portion contains N-terminal cysteine-rich domain, a fibronectin type II domain and 10 carbohydrate recognition domains (CRDs). In mammals, the expression of Dec205 is primarily restricted to dendritic cells and thymic cortical epithelium, although human Dec205 can also be detected on peripheral T and B cells, natural killer (NK) cells and macrophages. In chickens, low-level expression of Dec205 has been detected in CD4+ve, CD8+ve and $\gamma\delta$ T lymphocytes, B lymphocytes and macrophages with most expression on dendritic cells. The function of Dec205 in antigen uptake, processing and presentation has been characterized.

[0122] In one aspect, the cell surface protein may be a C-type lectin such as Dec205. In one aspect, the cell surface protein is Dec205.

Chemically Engineered Proteins

[0123] In other aspects, the present invention provides chemically engineered proteins.

[0124] A “chemically engineered protein” as used herein refers to a protein which comprises domains which have been joined together by a non-amino group (or prosthetic group or cofactor). In other words, the protein comprises domains which are linked together without amino bonds.

[0125] The present invention provides a chemically engineered protein comprising: at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and

[0126] a) at least one antigenic polypeptide; or

[0127] b) at least one binding domain which is capable of binding to at least one antigenic polypeptide.

[0128] In other words, the chemically engineered protein comprises:

[0129] a) at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and

[0130] b) at least one antigenic polypeptide; or

[0131] c) at least one binding domain which is capable of binding to at least one antigenic polypeptide; wherein a) and b) or a) and c) have been chemically conjugated.

[0132] Methods for chemically conjugating proteins are known in the art and include for example the formation of a stable, covalent linkage between two or more separate proteins. Examples of chemical conjugation include the use of crosslinking reagents which typically contain two or more chemically reactive groups which will connect to the functional groups (e.g. primary amines, sulphydryls, carbonyls, carbohydrates or carboxylic acids) found in proteins. Cross-linkers may be homobifunctional, heterobifunctional or photoreactive.

[0133] In one aspect, the chemically engineered protein may be produced by crosslinking two or more proteins which comprise at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and at least one antigen.

[0134] For example, a chemically engineered protein may be produced by crosslinking using a heterobifunctional crosslinking reagent to crosslink amines-to sulphydryls e.g. using a thiolating agent followed by a Sulfo-SMCC (sulfo-succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) reagent.

Antigen Presenting Cell

[0135] In one aspect, the present invention provides a chemically engineered protein comprising: at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and

[0136] a) at least one antigenic polypeptide; or

[0137] b) at least one binding domain which is capable of binding to at least one antigenic polypeptide; wherein the avian antigen presenting cell is a macrophage, B cell or natural killer cell. Suitably, the binding domain may be capable of binding to a macrophage.

[0138] Suitably, the binding domain may be capable of binding to a B cell. Suitably, the cell the binding domain may be capable of binding to a natural killer cell.

[0139] In some aspects, the binding domain may be capable of binding to a cell surface protein which is present

on two or more or three or more or all four cells selected from a dendritic cell, macrophage, B cell or natural killer cells, the cell surface protein is found on dendritic cells and on macrophages.

Cell Surface Protein

[0140] In one aspect, the present invention provides a chemically engineered protein comprising: at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and

[0141] a) at least one antigenic polypeptide; or

[0142] b) at least one binding domain which is capable of binding to at least one antigenic polypeptide; wherein the cell surface protein is an immunoglobulin family protein or an integrin family receptor. Suitably, the cell surface protein may be an immunoglobulin family protein such as CD83. Suitably, the cell surface protein may be an integrin family receptor, such as CD11c.

[0143] In one aspect, the cell surface protein is CD83. In one aspect, the cell surface protein is CD11c.

Aspects of Engineered Proteins

Signal Peptide

[0144] In some aspects, the engineered protein (such as genetically engineered protein or chemically engineered protein) according to the invention comprises a signal peptide.

[0145] When a protein comprising a signal peptide is expressed in a cell, the nascent protein is directed to the endoplasmic reticulum and subsequently to the cell surface, where it is bound to the cell surface or secreted into the culture medium. Classical protein secretion may be predicted using Signal P and TargetP methods Nielsen, H., et al., (1997) Protein Eng., 10, 1-6; Emanuelsson, O., Nielsen, H., Brunak, S. and von Heijne, G. (2000) J. Mol. Biol., 300, 1005-1016), which are incorporated herein by reference.

[0146] Signal peptides are typically 16 to 30 amino acids in length and are usually found at the N-terminus of the newly synthesized protein.

[0147] An example of a signal peptide which may be used is the BIP secretion signal sequence such as SEQ ID NO: 1 below:

(SEQ ID NO: 1)
ILLAVVAPVGLSLG.

[0148] Another example of a signal peptide which may be used in the present invention is the CD33 signal peptide sequence. For example SEQ ID NO: 40 below:

(SEQ ID NO: 40)
MPLLLLLPLLWAGALAM.

[0149] Suitably, the engineered protein may comprise a signal peptide having the sequence SEQ ID NO: 1 or SEQ ID NO: 40, or variants thereof having at least 80% identity thereto which function as signal sequences.

Solubilisation/Stability and Folding Domain

[0150] In some aspects, the engineered protein (such as genetically engineered protein or chemically engineered

protein) according to the invention comprises a domain which improves solubilisation, stabilization and/or folding of the engineered protein. In particular, the domain may improve solubilisation, stabilization and/or folding of the engineered protein.

[0151] In particular, the domain may improve solubilisation, stabilization and/or folding of the antigen.

[0152] Methods for improving protein production in various systems are well known in the art and include molecular chaperones which act to preserve nascent proteins in a folding-competent conformation and prevent aggregation.

[0153] For example, various protein tags such as glutathione-S-transferase (GST), maltose-binding protein (MBP), small ubiquitin-related modifier (SUMO) and ubiquitin (UB) may be used for enhancing solubility and promoting the correct folding of recombinant proteins. Leucine zippers, GCN4 and GCN4pll may be used to facilitate oligomerization of proteins such as dimerization and trimerization.

[0154] An example of a domain which improves solubilisation, stabilization and/or folding of the engineered protein or antigen is a foldon. Suitably, the genetically engineered or chemically engineered protein may comprise a foldon sequence of the trimeric protein fibrin from bacteriophage T4, such as a 30 amino acid trimerization foldon sequence of the trimeric protein fibrin from bacteriophage T4.

[0155] The foldon domain can typically be used to improve solubilisation, stability and or folding of trimeric proteins. For example, the foldon domain may be used to improve solubilisation, stability and or folding of hemagglutinin. An exemplary sequence of a foldon domain is:

(SEQ ID NO: 2)
GSGYIPEAPRDGQAYVRKDGEWVLLSTFL

[0156] Suitably, an engineered protein according to the present invention may comprise a foldon domain having the sequence SEQ ID NO: 2, or variants thereof having at least 80% identity thereto which function to improve solubilisation, stabilization and/or folding of the engineered protein or antigen. Suitably, an engineered protein according to the present invention may comprise a foldon domain having the sequence SEQ ID NO: 2, or variants thereof having at least 15, at least 20, at least 25, at least 26, at least 27, at least 28 or at least 29 amino acids thereof.

Binding Domain

[0157] The engineered proteins (such as genetically engineered proteins and chemically engineered proteins) according to the invention comprise at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell.

[0158] In some aspects, the genetically engineered proteins and the chemically engineered proteins according to the invention further comprise at least one binding domain which is capable of binding to an antigen.

[0159] Suitably, the engineered protein may comprise at least two, at least three, at least four at least 5 or at least 6 binding domains. The binding domains may be capable of binding to two or more antigens from different strains (serotypes/genotypes) of one pathogen in order to immunize against one disease. The binding domains may be capable of binding to two or more antigens from two or more pathogens to immunize against two or more diseases. In other words,

the engineered protein may be bispecific, trispecific, tetraspecific, multispecific i.e. capable of binding to two or more antigens, three or more antigens or four or more antigens.

[0160] The at least one binding domain (or antigen-binding domain) which is capable of binding to a cell surface protein on an avian antigen presenting cell is the portion of the engineered protein which recognizes and binds to a cell surface protein on an antigen presenting cell.

[0161] The at least one binding domain (or antigen-binding domain) which is capable of binding to a capable of binding to an antigen is the portion of the engineered protein which recognizes and binds to an antigen, for example an antigen on an inactivated virus, inactivated bacterium or inactivated parasite.

[0162] Various binding domains are known in the art, including those based on the antigen binding site of an antibody, an antibody fragment, antibody mimetics, and T-cell receptors.

[0163] In some aspects, it may be advantageous to use antibody fragments in the engineered proteins to improve solubility and folding.

[0164] Examples of antibody fragments capable of binding to a selected target, include Fv, scFv, F(ab') and F(ab')2.

[0165] In one aspect, a binding domain is based on or is a single-chain variable fragment (scFv). The scFv may comprise a variable light chain and variable heavy chains which are joined together using a linker peptide, for example using a Glycine-serine linker such as (Gly₄Ser)₄. The binding domain may be a polypeptide having an antigen binding site which comprises at least one complementarity determining region (CDR). The binding domain may comprise 3 CDRs and have an antigen binding site which is equivalent to that of a domain antibody (dAb). The binding domain may comprise 6 CDRs and have an antigen binding site which is equivalent to that of a classical antibody molecule. The remainder of the polypeptide may be any sequence which provides a suitable scaffold for the binding site and displays it in an appropriate manner for it to bind the antigen. The binding domain may be part of an immunoglobulin molecule such as a Fab, F(ab')2, Fv, single chain Fv (scFv) fragment, Nanobody or single chain variable domain (which may be a VH or VL chain, having 3 CDRs). The binding domain may be avian. The binding domain may be chimeric.

[0166] The binding domain may comprise a binding domain which is not derived from or based on an immunoglobulin. For example, a number of "antibody mimetic" designed repeat proteins (DRPs) have been developed to exploit the binding abilities of non-antibody polypeptides. Such molecules include ankyrin or leucine-rich repeat pro-

teins e.g. DARPins (Designed Ankyrin Repeat Proteins), Anticalins, Avimers and Versabodies.

[0167] The binding domain may "specifically bind" to the cell surface protein or antigen as defined herein. As used herein, "specifically bind" means that the binding domain binds to the cell surface protein or antigen but does not bind to other proteins, or binds at a lower affinity to other proteins.

[0168] The binding affinity between two molecules, e.g. an antigen binding domain and an antigen, may be quantified, for example, by determination of the dissociation constant (KD) e.g. by a surface plasmon resonance (SPR) method (e.g. Biacore™) The binding domain may comprise a domain which is not based on the antigen binding site of an antibody. For example the antigen binding domain may comprise a domain based on a protein/peptide which is a soluble ligand for a cell surface receptor (e.g. a soluble peptide such as a cytokine or a chemokine); or an extracellular domain of a membrane anchored ligand or a receptor for which the binding pair counterpart is expressed on the cell.

[0169] The binding domain may be based on a natural ligand of the antigen.

[0170] The binding domain may comprise an affinity peptide from a combinatorial library.

[0171] In one aspect, the binding domain capable of binding to a cell surface protein on an avian antigen presenting cell binds to CD83.

[0172] In one aspect, the binding domain capable of binding to a cell surface protein on an avian antigen presenting cell is based on the anti-CD83 antibody clone F890/GE8.

[0173] Suitably, an engineered protein according to the present invention may comprise a binding domain based on or having the amino acid sequences set forth in SEQ ID NO: 5, 6, 7 and/or SEQ ID NO: 10, 11 or 12.

[0174] Suitably, an engineered protein according to the present invention may comprise a binding domain based on or having the amino acid sequences set forth in SEQ ID NO: 4 and/or SEQ ID NO: 9 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto). Suitably, the variant may comprise at least one, at least two, at least 3, at least 4, at least 5 at least 6 of the CDRs set forth in SEQ ID NO: 5, 6, 7 and/or SEQ ID NO: 10, 11 or 12.

[0175] Suitably, an engineered protein according to the present invention may comprise a binding domain encoded by the nucleotide sequences set forth in SEQ ID NO: 3 and/or SEQ ID NO: 8 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto).

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anti-CD83 antibody clone F890/GE8 heavy chain nucleotide sequence
(SEQ ID NO: 3)

GAGGTCCAGCTGCAACAATCTGGACCTGAGCTGGTGAAGCCTGGGCTTCAGTGAAGATATCCTGTAA

GGCTTCTGGATAACACGTTACTGACTACTACATAAACTGGGTGAAGCAGAGCCATGGAAAGAGCCTTG

AGTGGATTGGAGATAATCTACTAATGGTGAATTCTACCTACAGCCAGAAGTCAAGGGCAAGGCC

ACATTGACTGTAGACAAGTCCTCCAGCACAGCCTACATGGAGCTCCGCAGCCTGACATCTGAGGTCTC

TGCAGTCTATTACTGTGCAAGAGACTATGCTATGGACTACTGGGTCAAGGAACCTCAGTCACCGTCT

CCTCA

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anti-CD83 antibody clone F890/GE8 heavy chain amino acid sequence
 (SEQ ID NO: 4)
 EVQLQQSGPELVKPGASVKISCKASGYFTDYYINWVKQSHGKSLEWIGDINPTNGDSTYSQKEKGKA

TLTVVDKSSSTAYMELRSLTSEVSAVYYCARDYAMDYWGQGTSVTVSS

anti-CD83 antibody clone F890/GE8 heavy chain amino acid CDR-H1
 (SEQ ID NO: 5)
 DYYIN

anti-CD83 antibody clone F890/GE8 heavy chain amino acid CDR-H2
 (SEQ ID NO: 6)
 DINPTNGDSTYSQKFKG

anti-CD83 antibody clone F890/GE8 heavy chain amino acid CDR-H3
 (SEQ ID NO: 7)
 DYAMDY

anti-CD83 antibody clone F890/GE8 light chain nucleotide sequence
 (SEQ ID NO: 8)
 GACATTGTGATGACCCAGTCCTCCATCCTCCCTGGCTGTGTCAGTCGGACAGAAGGTCACTATGAGCTG

CACGTCCAGTCAGGTCTTTACATAGTCCAATCAAAGAACATTTGGCCTGGTACCGAGCAGAAC

CAGGACAGTCCTAAACTCTGGTATACTTGCATCCACTAGGGAACTCTGGGTCCCTGATCGCTTC

ACAGGGCAGTGGATCTGGGACAGATTCACTTACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGT

TTATTACTGTCAAGAACATTATAGCACTCCGCTCACGTTCCGGTGCTGGGACCAAGCTGGAGCTGAAA

anti-CD83 antibody clone F890/GE8 light chain amino acid sequence
 (SEQ ID NO: 9)
 DIVMTQSPSSLAVSVGQKVMTSCTSSQVLLHSPNQKNYLAWYQQKPGQSPKLVYFASTRESGVPDFR

TGSGSGTDFLTLSVQAEDLAVYYCQQHYSTPLTFGAGTKLELK

anti-CD83 antibody clone F890/GE8 light chain amino acid CDR-L1
 (SEQ ID NO: 10)
 TSSQVLLHSPNQKNYLA

anti-CD83 antibody clone F890/GE8 light chain amino acid CDR-L2
 (SEQ ID NO: 11)
 FASTRES

anti-CD83 antibody clone F890/GE8 light chain amino acid CDR-L3
 (SEQ ID NO: 12)
 QQHYSTPLT

[0176] In one aspect, the binding domain is based on the anti-CD11c antibody clone 8F2.

[0177] Suitably, an engineered protein according to the present invention may comprise a binding domain based on or having the amino acid sequences set forth in SEQ ID NO: 15, 16, 17 and/or SEQ ID NO: 20, 21 or 22.

[0178] Suitably, an engineered protein according to the present invention may comprise a binding domain based on or having the amino acid sequences set forth in SEQ ID NO: 14 and/or SEQ ID NO: 19 or variants thereof having at least

80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto). Suitably, the variant may comprise at least one, at least two, at least 3, at least 4, at least 5 at least 6 of the CDRs set forth in SEQ ID NO: 15, 16, 17 and/or SEQ ID NO: 20, 21 or 22.

[0179] Suitably, an engineered protein according to the present invention may comprise a binding domain based encoded by the nucleotide sequences set forth in SEQ ID NO: 13 and/or SEQ ID NO: 18 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto).

anti-CD11c antibody clone 8F2 heavy chain nucleotide sequence
 (SEQ ID NO: 13)
 GAGGTCCAGTCGACAGTCCTGGACCTGGTAAAGCTGGGCTTCAGTGAAGATGTCCTGCAA
 GGCTTCTGGATACACATTCACTAACTATGTTCTGCACTGGGTGAAGCAGAACCTGGCAGGGCCTTG
 AGTGGATTGGATATTAATCCTTACAATGATGGTACTAAGTTCAATGAGAAGTTCAAAGGCAAGGCC
 ACACTGACTTCAGACACATCCTCCAGCACAGCCTCATGGAACCTCAGCAGCCTGACCTCTGAGGACTC
 TGCGGTCTATTACTGTGCAAGAGGAGATAATCTACGGCCCTACTACTTTGACTACTGGGGCCAAGGCA
 CCACTCTCACAGTCTCCTCA

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anti-CD11c antibody clone 8F2 heavy chain amino acid sequence
 (SEQ ID NO: 14)
 EVQLQQSGPELVKPGASVKMSCKASGYTFTNYVLHWVKQKPGQGLEWIGYINPYNDGKENEKFKGKA
 TLTSDTSSSTAPMELSSLTSEDAVYYCARGDNLRPYYFDYWQGTTLTVSS

anti-CD11c antibody clone 8F2 heavy chain amino acid CDR-H1
 (SEQ ID NO: 15)
 NYVLH

anti-CD11c antibody clone 8F2 heavy chain amino acid CDR-H2
 (SEQ ID NO: 16)
 YINPYNDGKFNFKFG

anti-CD11c antibody clone 8F2 heavy chain amino acid CDR-H3
 (SEQ ID NO: 17)
 GDNLRPYYEDY

anti-CD11c antibody clone 8F2 light chain nucleotide sequence
 (SEQ ID NO: 18)
 CAAATTGTTCTCACCCATTCTCCAGCAATCATGTCTGCATCTCCAGGGAGAAGTCACCATGACCTG
 CAGTGCCAGCTCAAGTGTAAAGTTCATGTACTGGTACCCAGCAGAAGCCAGGATCCTCCCCCGACTCC
 TGCTTTATGACACATCCAGCCTGCTTCTGGAGTCCCTGTTGCTTCAGTGGCAGTGGCTCTGGGACC
 TCTTACTCTCTACAATCAGCGAATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTGGAG
 TCGTTACCCACCGACGTTGGAGGACCAAGCTGGAAATCAA

anti-CD11c antibody clone 8F2 heavy chain amino acid sequence
 (SEQ ID NO: 19)
 QIVLTHSPAIMSASPGEKVTMTCSASSVS FMYWYQQKPGSSPRLLYDTSSLSSGVFVRFSGSGSGT
 SYSLTISRMEAEDAATYYCQQWSRYPPT FGGGTKLEIK

anti-CD11c antibody clone 8F2 heavy chain amino acid CDR-L1
 (SEQ ID NO: 20)
 SASSSVSEMY

anti-CD11c antibody clone 8F2 heavy chain amino acid CDR-L2
 (SEQ ID NO: 21)
 DTSSLSS

anti-CD11c antibody clone 8F2 heavy chain amino acid CDR-L3
 (SEQ ID NO: 22)
 QQWSRYPPT

[0180] Suitably, the binding domain may be produced against or may recognise carbohydrate recognition domains 4, and/or 5, and/or 6 of the chicken Dec205 receptor.

[0181] In one aspect, the binding domain is not produced against or does not recognise carbohydrate recognition domain 2 of chicken Dec205 receptor.

[0182] In one aspect, the binding domain is based on the anti-Dec205 antibody clone F887/AD6. Suitably, an engineered protein according to the present invention may comprise a binding domain based on or having the amino acid sequences set forth in SEQ ID NO: 25, 26, 27 and/or SEQ ID NO: 30, 31 or 32.

[0183] Suitably, an engineered protein according to the present invention may comprise a binding domain based on

or having the amino acid sequences set forth in SEQ ID NO: 24 and/or SEQ ID NO: 29 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto). Suitably, the variant may comprise at least one, at least two, at least 3, at least 4, at least 5 at least 6 of the CDRs set forth in SEQ ID NO: 25, 26, 27 and/or SEQ ID NO: 30, 31 or 32.

[0184] Suitably, an engineered protein according to the present invention may comprise a binding domain encoded by the nucleotide sequences set forth in SEQ ID NO: 23 and/or SEQ ID NO: 28 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto).

anti-DEC205 antibody clone F887/AD6 heavy chain nucleotide sequence
 (SEQ ID NO: 23)
 GAGGTGCAACTGGTGGAGTCTGGGGAGACTTAGTGAAGCCTGGAGGGTCCCTGAAACTCTC
 CTGTGCAGCCTCTGGATTCACTTCAGTAGCTATGGCATGTCTGGTTCGCCAGACTCCAG
 ACAAGAGGCTGGAGTGGGTGCAACCATTAGTAGTGGTGGTAGTTACACCTACTATCCAGAC

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AGTGTGAAGGGCGATTCAACCATTCCAGAGACAATGCCAAGAACATCCTGTATCTGCAAAT
GAGCAGTCTGAAGTCTGAAGACACAGCCATGTATTACTGTGCAAGACTTCAACCTGGACT
GGTACTTCGATGTCTGGGCACAGGGACCACGGTCACCGTCTCCTCA
anti-DEC205 antibody clone F887/AD6 heavy chain amino acid sequence
(SEQ ID NO: 24)
EVQLVESGGDLVKPGGSLKLSCAASGTFSSYGMSWVRQTPDKRLEWVATISSGGSYTYPD
SVKGRFTISRDNAKNILYLQMSSLKSEDTAMYYCARLSTWDWYFDVWGTGTTVTVSS
anti-DEC205 antibody clone F887/AD6 heavy chain amino acid CDR-H1
(SEQ ID NO: 25)
SYGMS
anti-DEC205 antibody clone F887/AD6 heavy chain amino acid CDR-H2
(SEQ ID NO: 26)
SSGGSYTYYPDSDKGRF
anti-DEC205 antibody clone F887/AD6 heavy chain amino acid CDR-H3
(SEQ ID NO: 27)
LSTWDWYFDV
anti-DEC205 antibody clone F887/AD6 light chain nucleotide sequence
(SEQ ID NO: 28)
GAAATTGTGCTACCCAGTCTCCAGCACTCATGGCTGCATCTCCAGGGAGAAGGTCACCAT
CACCTGCAGTGTCAAGTATAAGTTCCGGCAACTTTCACTGGTACCAAGCAGAAGTCAG
GAACCTCCCCAAACTCTGGATTATGGCACATCCAACCTGGCTCTGGAGTCCCTGTTCGC
TTCAGTGGCAGTGGATCTGGGACCTCTTATTCTCTCACAACTCAGCAGCATGGAGGCTGAAGA
TGCTGCCACTTAACTGTCAACAGTGGAGTAGTTACCCATTACGTTGGCTCGGGACAA
AGTTGGAAATAAAA
anti-DEC205 antibody clone F887/AD6 light chain amino acid sequence
(SEQ ID NO: 29)
EIVLTQSPALMAASPGEKVITCSVSSSISSGNFHWWQQKSGTPLWIYGTSNLASGVPV
FSGSGSGTSYSLTISSSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
anti-DEC205 antibody clone F887/AD6 amino acid CDR-L1
(SEQ ID NO: 30)
SVSSSISSGNFH
anti-DEC205 antibody clone F887/AD6 amino acid CDR-L2
(SEQ ID NO: 31)
GTSNLAS
anti-DEC205 antibody clone F887/AD6 amino acid CDR-L3
(SEQ ID NO: 32)
QQWSSYPFT

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[0185] In one aspect the present invention provides an antibody or antigen-binding fragment thereof which binds to CD83, such as avian CD83, in particular to poultry and/or chicken CD83.

[0186] In one aspect the present invention provides an antibody or antigen-binding fragment thereof having the CDRs set forth in SEQ ID NO: 5, 6, 7 and/or SEQ ID NO: 10, 11 or 12.

[0187] Suitably, the antibody may comprise sequences set forth in SEQ ID NO: 4 and/or SEQ ID NO: 9 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto). Suitably, the variant may comprise at least one, at least two, at least 3, at least 4, at least 5 at least 6 of the CDRs set forth in SEQ ID NO: 5, 6, 7 and/or SEQ ID NO: 10, 11 or 12.

[0188] Suitably, the antibody may comprise a binding domain encoded by the nucleotide sequences set forth in

SEQ ID NO: 3 and/or SEQ ID NO: 8 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto). In one aspect the present invention provides an antibody or antigen-binding fragment thereof which binds to CD11c, such as avian CD11c, in particular to poultry and/or chicken CD11c. In a further aspect the present invention provides an antibody or antigen-binding fragment thereof having the CDRs set forth in SEQ ID NO: 15, 16, 17 and/or SEQ ID NO: 20, 21 or 22.

[0189] Suitably, the antibody may comprise sequences set forth in SEQ ID NO: 14 and/or SEQ ID NO: 19 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto). Suitably, the variant may comprise at least one, at least two, at least 3, at least 4, at least 5 at least 6 of the CDRs set forth in SEQ ID NO: 15, 16, 17 and/or SEQ ID NO: 20, 21 or 22.

[0190] Suitably, the antibody may comprise a binding domain based encoded by the nucleotide sequences set forth in SEQ ID NO: 13 and/or SEQ ID NO: 18 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto).

[0191] In one aspect the present invention provides an antibody or antigen-binding fragment thereof which binds to Dec205, such as avian Dec205, in particular to poultry and/or chicken Dec205.

[0192] In a further aspect the present invention provides an antibody or antigen-binding fragment thereof having the CDRs set forth in SEQ ID NO: 25, 26, 27 and/or SEQ ID NO: 30, 31 or 32.

[0193] Suitably, the antibody may comprise the amino acid sequences set forth in SEQ ID NO: 24 and/or SEQ ID NO: 29 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto). Suitably, the variant may comprise at least one, at least two, at least 3, at least 4, at least 5 at least 6 of the CDRs set forth in SEQ ID NO: 25, 26, 27 and/or SEQ ID NO: 30, 31 or 32.

[0194] Suitably, the antibody may comprise a binding domain encoded by the nucleotide sequences set forth in SEQ ID NO: 23 and/or SEQ ID NO: 28 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto).

[0195] In one aspect, the binding domain capable of binding to an antigen binds to hemagglutinin of an avian influenza virus, such as hemagglutinin antigen of H9N2 avian influenza virus.

[0196] In one aspect, the binding domain is based on the anti-CD83 antibody clone F955/IG10.

[0197] Suitably, the engineered protein according to the present invention may comprise a binding domain based on or having the amino acid sequences set forth in SEQ ID NO: 44, 45, 46 and/or SEQ ID NO: 49, 50 or 51.

[0198] Suitably, the engineered protein according to the present invention may comprise a binding domain based on or having the amino acid sequences set forth in SEQ ID NO: 43 and/or SEQ ID NO: 48 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto). Suitably, the variant may comprise at least one, at least two, at least 3, at least 4, at least 5 at least 6 of the CDRs set forth in SEQ ID NO: 44, 45, 46 and/or SEQ ID NO: 49, 50 or 51.

[0199] Suitably, the engineered protein according to the present invention may comprise a binding domain encoded by the nucleotide sequences set forth in SEQ ID NO: 42 and/or SEQ ID NO: 47 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto).

anti-hemagglutinin antibody clone F955/IG10 heavy chain nucleotide sequence
(SEQ ID NO: 42)

GAGGTTCACTGCAGCAGTCTGTGGCAGAGCTTGTGAGGCCAGGGCCTCAGTCAGTTGTCCTGCAC

AGCTTCTGGCTTCAACATTAACACACCTATATGCACTGGGTGAAGCAGAGGCCGAAACAGGGCCTGG

AGTGGATTGGAAGGATTGATCCTCGGAATGGTAATACTAGGTATGCCCGAAGTTCAGGGCAAGGCC

ACTATAACTGCAGACACATCCTCCAACACAGCCTACCTGCAGCAGCCTGACATCTGAGGACAC

TGCCATCTATTACTGTGCCCGTTATTACTTCGGTCTGACTACTGGGCAAGGCCACTCTCACAG

TCTCCTCA

anti-hemagglutinin antibody clone F955/IG10 heavy chain amino acid sequence
(SEQ ID NO: 43)

EVQLQQSVAELVRPGASVQLSCTASGFNIKNTYMHVKQRPEQGLEWIGRIDPANGNTRYAPKPQGKA

TITADTSSNTAYLQLSSLTSEDTAIYYCARYYFGPDYWGQGTTLVSS

anti-hemagglutinin antibody clone F955/IG10 heavy chain amino acid CDR-H1
(SEQ ID NO: 44)

NTYMH

anti-hemagglutinin antibody clone F955/IG10 heavy chain amino acid CDR-H2
(SEQ ID NO: 45)

RIDPANGNTRYAPKFQG

anti-hemagglutinin antibody clone F955/IG10 heavy chain amino acid CDR-H3
(SEQ ID NO: 46)

YYFGPDY

anti-hemagglutinin antibody clone F955/IG10 light chain nucleotide sequence
(SEQ ID NO: 47)

GACATCCTGATGACCCAATCTCCATCCTCATGTCTGTATCTCTGGGAGACACAGTCATCATCATTG

CCATGCAAGTCAGGGCATTAGCAGTAATATAGGGTGGTTGCAGCAGAAACCAGGGAAATCATTAAAGG

GCTGTGATCTATCATGCAACCAACTTGGAGATGGAGTTCCATCAAGGTTCAAGTGGCGGTGGATCTGGA

GCAGATTATTCTCACCATCAGCAGCCTGGAATCTGAAGATTTGCAGACTATTACTGTGTACAGTA

TGGTCAGTTCCATTACGTTGGCTCGGGACAAAGTTGGAAATAAAA

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anti-hemagglutinin antibody clone F955/IG10 light chain amino acid sequence
 (SEQ ID NO: 48)
 DILMTQSPSSMSVSLGDTVIITCHASQGISSNIGWLQQKPGKSFKGLIYHATNLEDGVPSRFSGGGSG

ADYSLTIISSLESEDFA^DYYCVQYGQFPFTFGSGTKLEIK

anti-hemagglutinin antibody clone F955/IG10 light chain amino acid CDR-L1
 (SEQ ID NO: 49)
 HASQGISSNIG

anti-hemagglutinin antibody clone F955/IG10 light chain amino acid CDR-L2
 (SEQ ID NO: 50)
 HATNLED

anti-hemagglutinin antibody clone F955/IG10 light chain amino acid CDR-L3
 (SEQ ID NO: 51)
 VQYGQFPFT

[0200] In one aspect, the binding domain capable of binding to an antigen binds to hemagglutinin of an avian influenza virus, such as hemagglutinin antigen of H9N2 avian influenza virus.

[0201] In one aspect, the binding domain is based on the anti-CD83 antibody clone F955/HD8.

[0202] Suitably, the engineered protein according to the present invention may comprise a binding domain based on or having the amino acid sequences set forth in SEQ ID NO: 54, 55, 56 and/or SEQ ID NO: 59, 60 or 61.

[0203] Suitably, the engineered protein according to the present invention may comprise a binding domain based on

or having the amino acid sequences set forth in SEQ ID NO: 53 and/or SEQ ID NO: 58 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto). Suitably, the variant may comprise at least one, at least two, at least 3, at least 4, at least 5 at least 6 of the CDRs set forth in SEQ ID NO: 54, 55, 56 and/or SEQ ID NO: 59, 60 or 61.

[0204] Suitably, the engineered protein according to the present invention may comprise a binding domain encoded by the nucleotide sequences set forth in SEQ ID NO: 52 and/or SEQ ID NO: 57 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto).

anti-hemagglutinin antibody clone F955/HD8 heavy chain nucleotide sequence
 (SEQ ID NO: 52)
 GAGGTTCTAGCTGCAGCAGTCAGTCTGGCAGAGCTTGAGGCCAGGGGCTCAGTCAGATTGTCCTGCAC

AGCTTCTGGCTTCAACATTAACACCTATATGCACGGGTGAAGCAGAGGCCAACAGGGCTGG

AGTGGATTGGAAGGATTGATCCTGCGAATGGTAATACTAGATATGCCCAAGGGCTACCTGCAGCTCAGCAGCCTGACATCTGACGACAC

ACTATAACTGCAGACACATCCTCAAACACAGCCTACCTGCAGCTCAGCAGCCTGACATCTGACGACAC

TGCCATCTATTACTGTGGTAGGACAGAGTCAGGAATGCTATGGACTACTGGGGCTAACAGGAACCTCAG

TCACCGTCTCCTCA

anti-hemagglutinin antibody clone F955/HD8 heavy chain amino acid sequence
 (SEQ ID NO: 53)
 EVQLQQSVAELVRPGASVQLSCTASGFNIKNTYMHWVKQRPEQCLEWIGRIDPANGNTRYAPKFQGKA

TITADTSSNTAYLQLSSLTSDDTAIYYCGRTEFRNAMDYWGQGTSVTVSS

anti-hemagglutinin antibody clone F955/HD8 heavy chain amino acid CDR-H1
 (SEQ ID NO: 54)
 NTYMH

anti-hemagglutinin antibody clone F955/HD8 heavy chain amino acid CDR-H2
 (SEQ ID NO: 55)
 RIDPANGNTRYAPKFQG

anti-hemagglutinin antibody clone F955/HD8 heavy chain amino acid CDR-H3
 (SEQ ID NO: 56)
 TEFRNAMDY

anti-hemagglutinin antibody clone F955/HD8 light chain nucleotide sequence
 (SEQ ID NO: 57)
 GACATCCAGATGACTCAGTCAGCCTCCCATCTGCCATCTGGGGAGAAACTGTCACCATGACATG

TCGAGCAAGTGAGAATTTACAGTAATTAGCATGGTATCAGCAGAACAGGGAAAATCTCCTCAGC

TCCTGGTCTATGCTGCAACAAACTTAGCAGATGGTGTGCCATCAAGGTTCAAGTGGCAGTGGATCAGG

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ACACAGTTTCTCTGAAGATCAACAGCCTGCAGCCTGAAGATTGGAAATTATTACTGTCAACATTT

TTATAATACTCCGTACACGTTGGAGGGGGACCAAGCTGGAAATAAAA

anti-hemagglutinin antibody clone F955/HD8 light chain amino acid sequence
(SEQ ID NO: 58)

DIQMTQSPASLSPSVEGETVMTCRASENIYSNLAWYQQKQGKSPQLLVYATNLADGVPSRFSGSGSG

TQFSLKINSNLQPEDFGNYYCQHFYNTPYTFGGGTKEIK

anti-hemagglutinin antibody clone F955/HD8 light chain amino acid CDR-L1
(SEQ ID NO: 59)

RASENIYSNLA

anti-hemagglutinin antibody clone F955/HD8 light chain amino acid CDR-L2
(SEQ ID NO: 60)

AATNLAD

anti-hemagglutinin antibody clone F955/HD8 light chain amino acid CDR-L3

(SEQ ID NO: 61)

QHFYNTPYT

[0205] In one aspect the present invention provides an antibody or antigen-binding fragment thereof which binds to hemagglutinin of an avian influenza virus, such as hemagglutinin antigen of H9N2 avian influenza virus.

[0206] In one aspect the present invention provides an antibody or antigen-binding fragment thereof having the CDRs set forth in SEQ ID NO: 44, 45, 46 and/or SEQ ID NO: 49, 50 or 51.

[0207] Suitably, the antibody may comprise sequences set forth in SEQ ID NO: 43 and/or SEQ ID NO: 48 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto). Suitably, the variant may comprise at least one, at least two, at least 3, at least 4, at least 5 at least 6 of the CDRs set forth in SEQ ID NO: 44, 45, 46 and/or SEQ ID NO: 49, 50 or 51.

[0208] Suitably, the antibody may comprise a binding domain encoded by the nucleotide sequences set forth in SEQ ID NO: 42 and/or SEQ ID NO: 47 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto).

[0209] In one aspect the present invention provides an antibody or antigen-binding fragment thereof having the CDRs set forth in SEQ ID NO: 54, 55, 56 and/or SEQ ID NO: 59, 60 or 61.

[0210] Suitably, the antibody may comprise sequences set forth in SEQ ID NO: 53 and/or SEQ ID NO: 58 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto). Suitably, the variant may comprise at least one, at least two, at least 3, at least 4, at least 5 at least 6 of the CDRs set forth in SEQ ID NO: 54, 55, 56 and/or SEQ ID NO: 59, 60 or 61.

[0211] Suitably, the antibody may comprise a binding domain encoded by the nucleotide sequences set forth in SEQ ID NO: 52 and/or SEQ ID NO: 57 or variants thereof having at least 80% identity (at least 85%, at least 90%, at least 95%, at least 97%, at least 99% identity thereto).

Cell Surface Proteins

[0212] The engineered proteins (such as genetically engineered proteins and chemically engineered proteins) accord-

ing to the invention comprise at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell.

[0213] As used herein, the “cell surface protein” refers to a protein which is expressed on the surface of a cell. In other words, at least part of the protein is exposed to the extracellular space.

[0214] The cell surface protein may be a plasma membrane protein which has at least part of a domain exposed to the extracellular space or the exoplasmic surface of the plasma membrane.

[0215] The cell surface tissue antigen may be an integral (or intrinsic) membrane protein. Integral membrane proteins are permanently attached to the membrane and have one or more domains which are embedded in the phospholipid bilayer. Examples of integral membrane proteins include transporters, channels, receptors and cell adhesion proteins.

[0216] The cell surface tissue antigen may be a transmembrane protein. Transmembrane proteins span the lipid bilayer. Transmembrane proteins may be single or multi-pass membrane proteins. For example, the transmembrane protein may be a member of the immunoglobulin superfamily. The cell surface protein may be an integral monotropic protein, associated with one side of the lipid bilayer and do not span the lipid bilayer.

[0217] The cell surface protein may be a peripheral membrane protein. Peripheral membrane proteins do not interact with the hydrophobic core of the phospholipid bilayer. Peripheral membrane proteins are typically bound to the membrane indirectly by interactions with integral membrane proteins or directly by interactions with lipid polar head groups.

[0218] Peripheral proteins may be localized to the outer surface of the plasma membrane. The cell surface protein may be a peripheral exoplasmic membrane protein.

[0219] The cell surface tissue antigen may be anchored to the plasma membrane e.g. covalently attached to lipids embedded within the cell membrane (such as via a glycosylphosphatidylinositol (GPI) anchor).

[0220] The cell surface membrane protein may be a GPI-anchored protein.

[0221] Numerous methods exist for determining the sub-cellular localisation of proteins and include for example: electron microscopy; confocal microscopy using co-locali-

sation with known membrane proteins; immuno-fluorescence; and flow cytometry with fluorescently tagged antibodies.

[0222] The engineered proteins (such as genetically engineered proteins and chemically engineered proteins) according to the invention may comprise at least one binding domain which binds to a cell surface protein selected from CD83, CD11c, Dec205, BU-1, CD28, CD40, CD14, CD80, CD86, MRC1L, CD25, CD45, MHVII or CD44.

[0223] The genetically engineered proteins and the chemically engineered proteins according to the invention may comprise at least one binding domain which binds to a cell surface protein listed in Table 5 below:

TABLE 5

Cell surface proteins on avian antigen presenting cells for targeted delivery of antigens by binding domains such as scFv monoclonal antibodies surface		
Cell surface protein	Expression on antigen presenting cells	Exemplary antibody
1 BU-1	B cells	Southern Biotech, Cat no: 8395-02, Clone: AV20
2 CD28	T cells	Southern Biotech, Cat no: 8260-01, Clone: AV7
3 CD40	B-cells, Macrophages, Monocytes	Bio-Rad Cat no: MCA2836, Clone AV79
4 CD14	Macrophage	Bio-Rad, Cat no: MCA5926GA, Clone: AV141
5 CD80	B cells, DCs, Macrophages	Bio-Rad Cat no: MCA2837
6 CD86	B cells, DCs, Macrophages	Bio-Rad Cat no: MCA2838, Clone: IAH/F853/AG2
7 CD25	T cells, Monocytes, Macrophages	Bio-Rad, Cat no: MCA5925GA, Clone: AV142
8 CD45	B cells, T cells	Bio-Rad, Cat no: MCA2413GA, Clone UM16-6
9 MHCII	DCs, Macrophages, B cells	Southern Biotech, Cat no: 8350-01, Clone: 2G11
10 CD44	B cells, T cells, Monocytes	Southern Biotech, Cat no: 8400-01, Clone: AV6

Antigen

[0224] In some aspects, the engineered proteins (such as genetically engineered proteins and chemically engineered proteins) according to the invention comprise at least one antigenic polypeptide.

[0225] In one aspect, the antigenic polypeptide is at least part of the antigen.

[0226] In other words, the engineered protein may comprise the amino acid sequence of at least part of the antigen which is antigenic. For example, the engineered protein may comprise part of a domain or of one or more domains of the antigen.

[0227] In one aspect, the engineered protein comprises the entire antigen.

[0228] Typically, the antigen or part thereof or antigenic polypeptide will be capable of inducing specific neutralizing antibodies against a pathogen e.g. virus, bacteria or parasite.

[0229] Without wishing to be bound by theory, the antigenic polypeptide will be recognised by the subject's immune system and will elicit a humoral and/or cellular immune response.

[0230] Suitably, the at least one antigenic polypeptide may be from an avian pathogen.

[0231] As used herein, "antigen or antigenic polypeptide from an avian pathogen" refers to an antigen or antigenic polypeptide from a pathogen found in an avian subject. For

example the antigen or antigenic polypeptide may be associated with disease in the avian subject and may not be expressed or may be expressed at a low level in healthy avian subjects.

[0232] Suitably, the antigen may be expressed at a level which is at least 20% higher, at least 30% higher, at least 40% higher, at least 50% higher, at least 60% higher, at least 70% higher, at least 80% higher, at least 90% higher than in a corresponding healthy avian subject.

[0233] Suitably, the antigen may be disease-specific.

[0234] As used herein "disease-specific" means that the antigen is not expressed or is expressed at a lower amount in a healthy avian subject. The level of expression may be

calculated using methods known in the art, for example ELISA. A comparison may be made between samples taken from a subject known to be infected with a disease and subjects known to be healthy.

[0235] The antigen may be from any pathogen. In one aspect the antigen is a viral antigen. In another aspect, the antigen is a bacterial antigen.

[0236] For example, the viral antigen may be an antigen from AIV, NDV, aMPV, IBV, IBD, IBDV), CAV, ARV, or AdV.

[0237] In one aspect, the antigenic polypeptide may comprise or consist of an immunogenic peptide epitope. The antigenic polypeptide may compromise or consist of a peptide immunogenic epitope from an avian pathogen.

[0238] An "immunogenic peptide epitope" as used herein refers to a peptide which is recognised by either a T cell receptor (TCR) or a B cell receptor (BCR)/antibody.

[0239] "Peptides" are short chains of two or more amino acids. Typically peptides consist of up to around twenty amino acids.

[0240] Suitably, the immunogenic peptide epitope may be a T cell epitope or a B cell epitope.

[0241] In one aspect the immunogenic peptide epitope is a T cell epitope.

[0242] In an adaptive immune response, T cells are capable of recognising internal epitopes of a protein antigen.

APCs take up protein antigens and degrade them into short peptide fragments. A peptide may bind to a major histocompatibility complex (MHC class II) inside the cell and be carried to the cell surface. When presented at the cell surface in conjunction with an MHC molecule, the peptide may be recognised by a T cell (via the TCR), in which case the peptide is a T cell epitope.

[0243] Suitably, an immunogenic peptide epitope may be capable of being recognised by a TCR when presented in the context of a MHC. Peptides that bind to MHC class I are typically 7 to 13, more usually 8 to 10 amino acids in length. The binding of the peptide is stabilised at its two ends by contacts between atoms in the main chain of the peptide and invariant sites in the peptide-binding groove of all MHC class I molecules. There are invariant sites at both ends of the groove which bind the amino and carboxy termini of the peptide. Variations in peptide length are accommodated by a kinking in the peptide backbone, often at proline or glycine residues that allow flexibility.

[0244] Peptides which bind to MHC class II molecules are typically between 8 and 20 amino acids in length, more usually between 10 and 17 amino acids in length, and can be longer (for example up to 40 amino acids). These peptides lie in an extended conformation along the MHC II peptide-binding groove which (unlike the MHC class I peptide-binding groove) is open at both ends. The peptide is held in place mainly by main-chain atom contacts with conserved residues that line the peptide-binding groove.

[0245] A T cell epitope may thus be a peptide derivable from an antigen which is capable of binding to the peptide-binding groove of an MHC molecule and being recognised by a T cell.

[0246] The minimal epitope is the shortest fragment derivable from an epitope, which is capable of binding to the peptide-binding groove of an MHC class I or II molecule and being recognised by a T cell. For a given immunogenic region, it is typically possible to generate a "nested set" of overlapping peptides which act as epitopes, all of which contain the minimal epitope but differ in their flanking regions.

[0247] Thus, it is possible to identify the minimal epitope for a particular MHC molecule: T cell combination by measuring the response to truncated peptides. For example, if a response is obtained to the peptide comprising residues 1-15 in the overlapping library, sets which are truncated at both ends (i.e. 1-14, 1-13, 1-12 etc. and 2-15, 3-15, 4-15 etc.) can be used to identify the minimal epitope.

[0248] Bioinformatics methods for predicting T cell epitopes from a protein are known in the art and include, but are not limited to, EpiDOCK, MotifScan, Rankpep, SYFPEITHI, MAPPP, PREDIVAC, PEPVAC, EPISOPT, Vaxign, MHCPred, EpiTOP, BIMAS, TEPIPOPE, Propred, EpiJen, IEDB-MHCI, IEDB-MHCl, MULTIPRED2, MHC2PRED, NetMHC, NetMHCI, NetMHCpan, NetMHCIpan, nHLApred, SVMHC, SVRMHC, NetCTL and WAPP.

[0249] In one aspect the immunogenic peptide epitope is a B cell epitope. A B cell epitope refers to a peptide which is capable of binding to a B cell receptor (BCR)/antibody. B cell epitopes are generally divided into two categories, conformational epitopes and linear epitopes, based on their structure and interaction with the antibody. A conformational epitope is formed by the 3D conformation adopted by the interaction of discontinuous amino acid residues. In

contrast, a linear epitope is formed by the 3D conformation adopted by the interaction of contiguous amino acid residues.

[0250] Suitably, the peptide epitope may be a linear B cell epitope when provided in the linked antigenic peptide construct.

[0251] Bioinformatics methods for predicting B cell epitopes from a protein are known in the art and include, but are not limited to, linear B cell epitope predictors such as PEOPLE, BepiPred, ABCpred, LBtope, BCPREDS and SVMtrip and conformational B cell epitope predictors such as CEP, DiscoTope, ElliPro, PEPITO, SEPPA, EPITOPIA, EPSVR, EPIPRED, PEASE, MIMOX, PEPITOPE, EpiSearch, MIMOPRO and CBTOPE.

[0252] The present engineered protein may comprise at least one B cell epitope and at least one T cell epitope as defined herein.

[0253] Suitably, the antigenic polypeptide may comprise at least one immunogenic B cell epitope. The peptide epitope(s) may independently be at least 7, at least 10, at least 15, at least 20, at least 30, at least 40, at least 50 or at least 100 amino acids in length.

[0254] The peptide epitope(s) may independently be about 7 to about 100, about 7 to about 50, or about 7 to about 20 amino acids in length.

[0255] The antigenic polypeptide induces an immune response when administered to a subject.

[0256] The antigenic polypeptide may induce a cytotoxic T cell response and/or a humoral immune response in a subject. Preferably, the antigenic polypeptide may induce a memory humoral immune response in a subject.

[0257] Methods for determining if a peptide is immunogenic are known in the art and include, for example, immune cell activation assays using CD4+ and/or CD8+ T cells or B cells.

[0258] Suitable markers for activation may include T cell proliferation and/or expression of cytokines (e.g. IFN γ , IL6, IL1 β , IL4 and CXCL12 (IL8) using methods such as quantitative PCT, ELISA and/or ELISpot.

[0259] T cell epitopes may be determined using the above assays, and/or MHC binding assays. Suitably, B cell epitopes may be identified from epitopes bound by antibodies isolated from a subject previous infected/recovered from a pathogen infection and/or previously vaccinated with an antigen from the pathogen. Methods for determining the epitope bound by an antibody (i.e. a B cell epitope) include, but are not limited to, X-ray crystallography, cryogenic electron microscopy, array-based oligo-peptide screening, site-directed mutagenesis mapping, high-throughput mutagenesis mapping, hydrogen-deuterium exchange, cross-linking-coupled mass spectrometry.

[0260] For example, the antigen may be an antigen from AIV, NDV, aMPV, IBV, IBDV, CAV or ARV.

[0261] Suitably, the antigen may be: hemagglutinin from avian influenza (for example, GenBank accession number: ACP50708.1, HA1: 19-349 and HA2: 1-174); the fusion protein (F) protein from NDV (for example GenBank accession number: AAK55550.1); Haemagglutinin-neuramidase (HN) from NDV (for example GenBank accession number: MH1614933.1); VP2 protein from IBDV (for example GenBank accession number: KX827589.1); spike protein from IBV (for example GenBank accession number:

AAA66578.1); or VP1 and/or VP2 protein from CAV (for example GenBank accession number: AQM56826.1 and AF313470.1).

[0262] In one aspect, the antigen is an avian influenza antigen and the vaccine of the invention treats and/or prevents avian influenza. For example, the antigen may be hemagglutinin.

[0263] In some aspects, the hemagglutinin may be synthetically produced using a consensus sequence. The hemagglutinin may be from any subtype. For example, the hemagglutinin may be selected from any of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, H17 or H18, suitably, the hemagglutinin may be selected from an avian influenza virus.

[0264] In some aspects, the antigen may have about 90%, (such as about 95%, such as about 96%, such as about 97%, such as about 98%) amino acid sequence similarity to the hemagglutinin ectodomain of A/Chicken/Pakistan/UDL 01/2008 H9N2 virus (GenBank accession number: ACP50708.1, HA1: 19-349 and HA2: 1-174) virus or the H5HA antigen of avian influenza H5N8 virus strain (A/duck/Egypt/SS19/2017, accession no. MH893738.1). Suitably, the antigen may be soluble. Suitably, the antigen may be a secreted protein.

[0265] In some aspects, the antigen is engineered to improve solubility and/or secretion. For example, the antigen may be engineered to remove any transmembrane domains which are typically present. The antigen may be engineered to comprise a signal peptide which allows secretion from cells in the subject.

[0266] In some aspects, the genetically engineered protein or chemically engineered protein may comprise an ectodomain of the H9HA gene that lacks both the hemagglutinin gene signal peptide and the transmembrane domain, replaced with a 30 amino acid trimerization foldon sequence of the trimeric protein fibritin from bacteriophage T4.

[0267] Suitably, the H9HA ectodomain for use in the present invention may comprise the amino acid sequence set forth in SEQ ID NO: 41:

(SEQ ID NO: 41)

```
DKICIGHQSTNSTETVDTLTETNVPVTHAKELLHTEHNGMLCATNLGHP
LILDTCTIEGLIYGNPSCDLLGGREWSYIVERPSAVNGTCYPGNVENL
EELRTLFSSSSYQRQIFPDITIWNVTYTGTSKCSDSFYRNMRWLTQK
SGLYPVQDAQYTNRNGKDILFWVGIIHHPPDTAQTNLYTRTDTSVTT
ENLDRTFKPVIGPRPLVNGLIGRINYYWSVLKPGQTLRVRNSGNLIAWP
YGHVLSGESHGRIKTLNNSGNCCVQCQTEKGGLNSTLPFHNIKYAFG
NCPKYIGVKSLKLAIGLRNVPARSSRGLFGAIAGFIEGGWPGLVAGWYG
FQHSNDQGVGMAADRDSTQKAVDKITSKVNNIVDKMNKQYEIIDHEFSE
VETRINMINNKIDDQIQDVWAYNAELLVLLENQKTLDEHDANVNNLYNK
VKRALGSNAMEDGKGCPELYHKCDDQCMETIRNGTYNRRKYKEESRLER
Q
```

[0268] In one aspect, the antigen comprises or consists of the sequence SEQ ID NO: 41; or a variant thereof having at least 80% identity thereto (such as at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99% identity thereto).

[0269] In some aspects, the genetically engineered protein or chemically engineered protein may comprise an ectodomain of the H5N8 gene that lacks both the hemagglutinin gene signal peptide and the transmembrane domain, replaced with a 30 amino acid trimerization foldon sequence of the trimeric protein fibritin from bacteriophage T4.

[0270] Suitably, the H5N8 ectodomain for use in the present invention may comprise the amino acid sequence set forth in SEQ ID NO: 80:

```
(SEQ ID NO: 80)
DQICIGYHANNSTEQVDTIMEKNVTVTQAQDILEKTHNGKLCDLNGVKP
LILKDCSVAGWLLGNPMCDEFIRVPEWSYIVERANPANDLCYPGSLNDY
EELKHLLSRINHFKEKILIIPKSSWPNHETSLGVSAACPYQGTPSFFRNV
VWLKKNDAYPTIKISYNNTNRDILLIMWGIHHSNNAAEQTNLYKNPTT
YISVGTSTLNQRLVPKIATRSQVNGQRGRMDFFWTILKPNDAIHFESNG
NFIAPEYAYKIVKKGDSTIMKSEVEYGHCNTKCQTPVGAINTSSMPFHNI
HPLTIGECPKYVKSINKLVLATGLRNSPQGETRGLEGAIAGFIEGGWQGM
VDGWYGYHHSNEQGSGYAADKESTQKAIDGVINKVNSIIDKMNTQFEAV
GREFNNLERRIENLNKKMEDGELDWVTTYNAELLVLMENERTLDFHDSNV
KNLYDKVRLQLRDNNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQYSE
EARLKREEISGVKLESIGTYQ
```

[0271] In one aspect, the antigen comprises or consists of the sequence SEQ ID NO: 80; or a variant thereof having at least 80% identity thereto (such as at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99% identity thereto).

[0272] With respect to function, the variant should be capable of inducing an immune response. By way of example, the induction of an immune response may be determined by the demonstration of a recall response in peripheral immune cells or splenocytes isolated from a subject previously immunised with the polypeptide or immunogenic fragment thereof. For instance, a recall response may be demonstrated by interferon production (e.g. IFN γ) and/or a proliferative response following an in vitro challenge with an antigen or polypeptide previous used to immunise a subject. (An exemplary assay is provided in Example 1.) Preferably, the variant should be capable of inducing a protective immune response in a subject, against subsequent challenge with avian influenza virus.

Exemplary Architecture

[0273] Examples of engineered protein architecture according to the present invention and for use in vaccines according to the present invention include those listed in Table 6 below; typically the engineered protein will comprise a binding domain as described herein and an antigen as defined herein and may optionally comprise a signal peptide and/or a solubilisation/stabilization or folding domain.

TABLE 6

Exemplary engineered protein architecture						
Engineered protein	Cell surface protein	Binding domain	Binding domain structure	Antigen	Signal peptide (optional)	Solubilisation/stabilization/folding domain (optional) Subject
Genetically engineered or chemically engineered	CD83	Based on antibody clone F890/GE8	May be based on an antibody fragment such as scFv, Fv, F(ab') or F(ab')2	HA	BiP	Foldon Avian subject e.g., chicken, turkey, duck, quail, pigeon or goose
Genetically engineered or chemically engineered	CD11c	Based on antibody clone 8F2	May be based on an antibody fragment such as scFv, Fv, F(ab') or F(ab')2	HA	BiP	Foldon Avian subject e.g., chicken, turkey, duck, quail, pigeon or goose
Genetically or chemically engineered	Dec205	Based on antibody clone F877/AD6	May be based on an antibody fragment such as scFv, Fv, F(ab') or F(ab')2	HA	BiP	Foldon Avian subject e.g., chicken, turkey, duck, quail, pigeon or goose

[0274] An example of domain architecture which may be used in the present invention is, in the following order (SEQ ID NO: 62):
BIP signal-1H9HA ectodomain-Foldon-LINKER-CD83 scFv-V5-His tag

binding to a cell surface protein on an avian antigen presenting cell. The BIP signal domain is a signal peptide, the foldon domain is a domain which improves solubilisation, stabilization and/or folding, V5 and the His tag are used for purifying the engineered protein. It will be appreciated that

(SEQ ID NO: 62)
MKLCILLAVVAFVGLSLGDKICIGHQSTNSTETVDTLTETNVPVTHAKELLHTEHNGMLCATNLGHPL
ILDCTCTIEGLIYGNPSCDLLLGGREWSYIVERPSAVNGTCYPGNVENLEELRTLFSSSSSYQRIQIFF
DTIWNVTYTGTSKCSDSFYRNMRWLTQKSGLYPVQDAQYTNNRGKDILFVWGIHHPPDTDATQTNLYT
RTDTTSVTENLDRTFKPVIGPRPLVNLIGRINYYWSVLPGQTLRVRNSGNLIAWPYGHVLSGES
HGRILKTDLNSNCVVQCQTEKGGLNSTLPFHNI SKYAFGNCPKYIGVKSLKLAIGLRNVPARSRGL
FGAIAGFIEGGWPGLVAGWYGFQHSNDQGVGMAADRDS TQAKVDKITSKVNNIVDKMNQYEIIDHEF
SEVETRLNMINNKIDDQIQDWWAYNAELLVLLENQKTLDEHDANVNNLYNKVKRALGSNAMEDGKCF
ELYHKCDDQCMETIRNGTYNRRKYKEESRLERQGSGYIPEAPRDRGQAYVRKDGEWVLLSTFLGSGSGD
IVMTQSPSSLAVSVGQKVTMSCTSSQVLLHSPNQKNYLAWYQQKPGQSPKLLVYFASTRESGVPDFRT
GSGSGTDFTLTISSVQAEDLAVYYCQQHYSTPLTFGAGTKLELKGGGGSGGGGGGGGGSEVQL
QQSGPELVKPGASVKISCKASGYTFTDYYINWVKQSHGKSLEWIGDINPTNGDSTYSQPKKGKATLTV
DKSSSTAYMELRSLTSEVSAYYCARDYAMDYWGQGTSVTVSSGKPIPNPLLGLDST~~HHHHHH~~

wherein the H9HA ectodomain is the at least one antigen and 0083 scFv is the at least one binding domain capable of

the BIP signal, foldon and linker domains shown in SEQ ID NO: 42 are optional. The nucleotide sequence, (SEQ ID NO:

65) codon optimised for *Drosophila melanogaster* which encodes the amino acid sequence SEQ ID NO: 62 is given in FIG. 15.

[0275] An example of domain architecture which may be used in the present invention is, in the following order (SEQ ID NO: 63):

BIP signal-H9HA ectodomain-Foldon-LINKER-CD11c scFv-V5-His tag

(SEQ ID NO: 63)
MKLCILLAVVAFVGLSLGDKICIGHQSTNSTETVDTLTETNVPVTHAKELLHTEHNGMLCATNLGHPL
ILDTCIEGLIYGNPSCDLLLLGGREWSYIVERPSAVNGTCYPGNVENLEELRTLFSSSSSYQRIQIFP
DTIWNVTYTGTGTSKSCSDSFYRNMRWLTKSGLYPVQDAQYTNNRGKDILFVWGIHHPPDTAQTNLYT
RTDTTSVTENLDRTFKPVIGPRPLVNGLIGRINYWSVLPGQTLRVRSGNLIAPWYGHVLSGES
HGRILKTDLNSNCVVQCOTEKGGLNSTLPFHNISKYAFGNCPKYIGVSKSLKLAIGLRNPARSSRGL
FGAIAGFIEGGWPGLVAGWYGPQHSNDQGVGMAADRDSTQKAVDKITSKVNNIVDKMNKQYEIIDHEF
SEVETRLNMINNKIDDQIQDVWAYNAELLVLENQTLDEHDANVNNLYNKVKRALGSNAMEDGKGCE
ELYHKCDDQCMETIRNGTYNRRKYKEESRLERQGSGYIPEAPRDGQAYVRKDGEWLLSTFLGSGSGE
VQLQQSGPELVKPGASVMSCKASGYFTNYVHLWVKQKPGQGLEWIGYINPYNDGKNEFKKGKAT
LTSDTSSSTAFMELSSLTSEDSAVYYCARGDNLRPYYFDYWQGTTLTVSSGGGSGGGGSGGGSGG
GGSQIVLTHSPAIMSASPGEKVTMTCSASSVSFMYWYQQKPQSSPRLLLVDTSSLSSGPVRFSGSG
SGTSYSLTISRMEAEDAATYYCQQWSRYPPTFGGGTKLEIKGKPIPNPLLGLDST|||||||,

wherein the H9HA ectodomain is the at least one antigen and CD11 scFv is the at least one binding domain capable of binding to a cell surface protein on an avian antigen presenting cell. The BIP signal domain is a signal peptide, the foldon domain is a domain which improves solubilisation, stabilization and/or folding, V5 and the His tag are used for purifying the engineered protein. It will be appreciated that the BIP signal, foldon and linker domains shown in SEQ ID NO: 63 are optional. The nucleotide sequence, (SEQ ID NO:

66) codon optimised for *Drosophila melanogaster* which encodes the amino acid sequence SEQ ID NO: 63 is given in FIG. 16.

[0276] An example of domain architecture which may be used in the present invention is, in the following order (SEQ ID NO: 64):

BIP signal-H9HA ectodomain-Foldon-LINKER-Dec205 scFv-V5-His tag

(SEQ ID NO: 64)
MKLCILLAVVAFVGLSLGDKICIGHQSTNSTETVDTLTETNVPVTHAKELLHTEHNGMLCATNLGHPL
ILDTCIEGLIYGNPSCDLLLLGGREWSYIVERPSAVNGTCYPGNVENLEELRTLFSSSSSYQRIQIFP
DTIWNVTYTGTGTSKSCSDSFYRNMRWLTKSGLYPVQDAQYTNNRGKDILFVWGIHHPPDTAQTNLYT
RTDTTSVTENLDRTFKPVIGPRPLVNGLIGRINYWSVLPGQTLRVRSGNLIAPWYGHVLSGES
HGRILKTDLNSNCVVQCOTEKGGLNSTLPFHNISKYAFGNCPKYIGVSKSLKLAIGLRNPARSSRGL
FGAIAGFIEGGWPGLVAGWYGPQHSNDQGVGMAADRDSTQKAVDKITSKVNNIVDKMNKQYEIIDHEF
SEVETRLNMINNKIDDQIQDVWAYNAELLVLENQTLDEHDANVNNLYNKVKRALGSNAMEDGKGCE
ELYHKCDDQCMETIRNGTYNRRKYKEESRLERQGSGYIPEAPRDGQAYVRKDGEWLLSTFLGSGSGE
IVLTQSPALMAASPGEKVTITCSVSSISSGNPHWYQQKSGTSPKLWYGTSNLASGPVRFSGSGSG
TSYSLTISSMEAEDAATYYCQQWSYPPTFGSGTKLEIKGGGGGGGGGGGGGGSEVQLVESGG
DLVKPGGSLKLSCAASGFTSSYGMWSVRQTPDKRLEWVATISSGGSYTYPDSVKGRFTISRDNAKN
ILYLQMSSLKSEDTAMYYCARLSTWDWFDWVWTGTTVSSGKPIPNPLLGLDST|||||||,

wherein the H9HA ectodomain is the at least one antigen and Dec205 scFv is the at least one binding domain capable of binding to a cell surface protein on an avian antigen presenting cell. The BIP signal domain is a signal peptide, the foldon domain is a domain which improves solubilisation, stabilization and/or folding, V5 and the His tag are used for purifying the engineered protein. It will be appreciated that the BIP signal, foldon and linker domains shown in SEQ ID NO: 44 are optional. The nucleotide sequence, (SEQ ID NO: 67) codon optimised for *Drosophila melanogaster* which encodes the amino acid sequence SEQ ID NO: 64 is given in FIG. 17.

[0277] In one embodiment, the present invention provides a genetically engineered protein comprising: at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and at least one binding domain which is capable of binding to at least one antigenic polypeptide.

[0278] An example of domain architecture which may be used in the present invention is, in the following order (SEQ ID NO: 68):

CD33 SIGNAL-IG10 scFv-(Glycine₄Serine)₄linker-CD83 scFv-Ctag

(SEQ ID NO: 68)
 MPLLLLLLPLLWAGALAMDIILMTQSPSSMSVSLGDTVIITCHASQGISSNIGWLQQKPGKSEKGLIYHA
 TNLEDGVPSRFSGGGSGADYSILTISSESEDFADEYYCVQYGQFPFTFGSGTKLEIKGGGGSGGGGSGG
 GGSEVQLQQSVAELVRPGASVQLSCTASGFNIKNTYMHWWVKQRPEQGLEWIGRIDPANGNTRYAPKFQ
GKATITADTSSNTAYLQLSSLTSEDTAIYYCARYYFGPDYWGQGTTLTVSSGGGGSGGGGGGGGGGG
GGSDIVMTQSPSSLAVSVGQKVMTMSCTSSQVLHSPNQKNYLAWYQQKPGQSPKLLVYFASTRESGP
DRFTGSGSGTDFTLTISSVQAEDLAVYYCQQHYSTPLTFGAGTKLELKGGGGSGGGGGGGSEVQLQ
QSGPELVKPGASVKISCKASGYFTDYYINWVKQSHGKSLEWIGDINPTNGDSTSQKFKGKATLTVD
KSSSTAYMELRSLTSEVSAYYYCARDYAMDYWQGTSVTVSSEPEA;

wherein the IG10 scFv is the at least one binding domain which is capable of binding to at least one antigenic polypeptide and CD83 scFv is the at least one binding domain capable of binding to a cell surface protein on an avian antigen presenting cell. The CD33 signal domain is a signal peptide, Glycine₄Serine₄ is a linker and the C tag is used for purifying the engineered protein. It will be appreciated that the signal, linker and purification tag domains shown in SEQ ID NO: 68 are optional. The nucleotide sequence, (SEQ ID NO: 69) which encodes the amino acid sequence SEQ ID NO: 68 is given in FIG. 21.

[0279] Further illustrative embodiments are shown as SEQ ID NO: 72 (nucleotide sequence) and SEQ ID NO: 76 (amino acid sequence). The construct of the invention may comprise or consist of any of the above mentioned sequences, or a variant with at least 80%, 85%, 90%, 95% or 99% sequence identity thereto. The construct of the invention may comprise or consist of SEQ ID NO: 72 or 76, or a variant with at least 80%, 85%, 90%, 95% or 99% sequence identity thereto.

Nucleic Acids/Nucleic Acid Constructs

[0280] As used herein, the terms "polynucleotide" and "nucleic acid" are intended to be synonymous with each other. The nucleic acid sequence may be any suitable type of

nucleotide sequence, such as a synthetic RNA/DNA sequence, a cDNA sequence or a partial genomic DNA sequence.

[0281] It will be understood by a skilled person that numerous different polynucleotides and nucleic acids can encode the same polypeptide as a result of the degeneracy of the genetic code. In addition, it is to be understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the polypeptide sequence encoded by the polynucleotides described here to reflect the codon usage of any particular host organism in which the polypeptides are to be expressed.

[0282] The present invention provides a polynucleotide which encodes an engineered protein according to the present invention. The present invention provides a polynucleotide which encodes an engineered protein comprising: at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and at least one antigen.

[0283] Nucleic acids encoding an engineered protein according to the present invention may comprise DNA or RNA. They may be single-stranded or double-stranded. They may also be polynucleotides which include within

them synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes of the use as described herein, it is to be understood that the polynucleotides may be modified by any method available in the art. Such modifications may be carried out in order to enhance the *in vivo* activity or life span of polynucleotides of interest.

[0284] The polynucleotide may be in isolated or recombinant form. It may be incorporated into a vector and the vector may be incorporated into a host cell. Such vectors and suitable hosts form yet further aspects of the present invention.

[0285] The polynucleotide which encodes the engineered protein according to the present invention may be codon optimised. Different cells differ in their usage of particular codons. This codon bias corresponds to a bias in the relative abundance of particular tRNAs in the cell type. By altering the codons in the sequence so that they are tailored to match with the relative abundance of corresponding tRNAs, it is possible to increase expression. Suitably, the polynucleotide may be codon optimised for expression in a specific avian subject.

[0286] In one embodiment, there is provided a nucleic acid construct which comprises a first polynucleotide which encodes at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell as defined herein; and a second polynucleotide which encodes at least one antigenic polypeptide as defined herein.

[0287] In one embodiment, the first and second polynucleotides are operably linked to the same promoter. Suitably, a nucleic acid construct according the present invention may encode a genetically engineered protein such as a fusion protein, wherein the first and second polynucleotides are operably linked to the same promoter. In some aspects, the nucleic acid construct encodes at least two, at least three, at least four or at least five antigens. In this way, a single vaccine will express antigens derived from multiple antigenically divergent viruses and afford protection against multiple pathogens and/or antigenically divergent variants within virus subtypes.

[0288] In some aspects, the nucleic acid construct comprises a domain which allows purification of the engineered protein such as an affinity tag. Numerous tags suitable for protein purification are known in the art and include, for example, His tag, polyHis tag, polycysteine tag, FLAG epitope, histidine affinity tag, bacteriophage T7 epitope and other epitope tags.

Vector

[0289] The present invention also provides a vector comprising at least one nucleic acid construct according to the present invention.

[0290] The term “vector” as used herein includes an expression vector, i.e., a construct enabling expression of an engineered protein according to the present invention.

[0291] Suitably the expression vector enables expression of an engineered protein according to the present invention.

[0292] An “expression cassette” comprises a gene of interest (open reading frame (ORF)) and one or more regulatory sequences enabling expression of the gene of interest. Typically expression cassettes comprise a promoter, a gene of interest and a terminator.

[0293] In some embodiments, the vector is a multivalent vector. The multivalent vector comprises multiple expression cassettes enabling expression of more than one engineered protein according to the present invention.

[0294] In some embodiments, the vector is a cloning vector.

[0295] Suitable vectors may include, but are not limited to, plasmids, viral vectors, transposons, nucleic acid complexed with polypeptide or immobilised onto a solid phase particle.

[0296] Viral delivery systems include but are not limited to adenovirus vector, an adeno-associated viral (AAV) vector, a herpes viral vector (such as turkey herpes virus (HTV/HVT), retroviral vector, lentiviral vector, baculoviral vector).

[0297] Retroviruses are RNA viruses with a life cycle different to that of lytic viruses. In this regard, a retrovirus is an infectious entity that replicates through a DNA intermediate. When a retrovirus infects a cell, its genome is converted to a DNA form by a reverse transcriptase enzyme. The DNA copy serves as a template for the production of new RNA genomes and virally encoded proteins necessary for the assembly of infectious viral particles.

[0298] There are many retroviruses, for example murine leukemia virus (MLV), human immunodeficiency virus (HIV), equine infectious anaemia virus (EIAV), mouse mammary tumour virus (MMTV), Rous sarcoma virus (RSV), Fujinami sarcoma virus (FuSV), Moloney murine leukemia virus (Mo-MLV), FBR murine osteosarcoma virus (FBR MSV), Moloney murine sarcoma virus (Mo-MSV), Abelson murine leukemia virus (A-MLV), Avian myelocytomatosis virus-29 (MC29), and Avian erythroblastosis virus (AEV) and all other retroviridae including lentiviruses.

[0299] A detailed list of retroviruses may be found in Coffin et al., (“Retroviruses” 1997 Cold Spring Harbour Laboratory Press Eds: J M Coffin, S M Hughes, H E Varmus pp 758-763), incorporated herein by reference.

[0300] Lentiviruses also belong to the retrovirus family, but they can infect both dividing and non-dividing cells (Lewis et al., (1992) EMBO J. 3053-3058), incorporated herein by reference.

[0301] The vector may be capable of transferring a polynucleotide the invention to a cell, for example a host cell as defined herein. The vector should ideally be capable of sustained high-level expression in host cells, so that the VH and/or VL domain are suitably expressed in the host cell.

[0302] The vector may be a retroviral vector. The vector may be based on or derivable from the MP71 vector backbone. The vector may lack a full-length or truncated version of the Woodchuck Hepatitis Response Element (WPRE).

[0303] Examples of viral vectors which may be particularly suitable as vectors for use according to the present invention include a herpes viral vector (such as turkey herpes virus (HTV/HVT), Newcastle disease virus (NDV), Duck enteritis virus (DEV), Avian infectious laryngotracheitis (ILT), Fowl Adenovirus, Marek disease virus (MDV) and Infectious bursal disease virus (IBDV), Infectious bronchitis virus (IBV)).

[0304] In particular, examples of vectors which may be used in the present invention are HTV/HVT and NDV.

Cell

[0305] The present invention further provides an engineered cell comprising an engineered protein according to the present invention (such as a genetically engineered protein or a chemically engineered protein according to the present invention). In one aspect, the engineered cell may comprise a nucleic acid construct or vector which encodes an engineered protein according to the present invention. The engineered cell may be any cell which can be used to express and produce an engineered protein according to the present invention. Suitably, the cell may be an avian cell. Suitably, the cell may be from a chick, or chicken, turkey, duck, quail, pigeon or goose. Suitably, the cell may comprise viral vector according to the present invention.

Vaccine/Pharmaceutical Composition

[0306] The present invention also provides a vaccine comprising at least one engineered protein(s) of the invention, nucleic acid construct(s) according to the invention, or vector(s) according to the invention or engineered cell(s) according to the invention.

[0307] The term “vaccine” as used herein refers to a preparation which, when administered to a subject, induces or stimulates a protective immune response. A vaccine can render an organism immune to a particular disease, for

example avian influenza. The vaccine of the present invention thus induces an immune response in a subject which is protective against subsequent pathogen challenge e.g. viral, bacterial or parasite challenge. A vaccine of the invention may be capable of inducing a cross-protective immune response against a plurality of virus genotypes. In an embodiment a vaccine of the invention of a single genotype may be capable of inducing a cross-protective immune response against a plurality of pathogen serotypes, subtypes and genotypes.

[0308] Suitably, the vaccine may be a recombinant subunit vaccine.

[0309] The vaccine may comprise a plurality of components such as engineered protein(s) according to the invention, nucleic acid construct(s) according to the invention or vector(s) according to the invention and a pharmaceutically acceptable carrier. The plurality of components may correspond to a plurality of different isolates, for example, different isolates of high or unknown virulence. Such a vaccine may be capable of inducing a cross-protective immune response against a plurality of virus genotypes.

[0310] In some embodiments, the vaccine is a monovalent vaccine. Suitably, a monovalent vaccine may immunize the subject against a single antigen. In some embodiments, the vaccine is a multivalent or polyvalent vaccine. Suitably, a multivalent or polyvalent vaccine may immunize the subject against two or more antigens from different strains (serotypes/genotypes) of one pathogen in order to immunize against one disease. In some embodiments, the vaccine is a multi-disease or multi-pathogen vaccine. Suitably a multi-disease or multi-pathogen vaccine may comprise protective antigens from two or more pathogens to immunize against two or more diseases.

[0311] In some aspects, the vaccine encodes at least two, at least three, at least four at least five different antigens. The antigens may be from divergent variants within a subtype (e.g. different isolates of avian influenza virus) or viruses or may be from divergent viruses (e.g. from avian influenza virus, Newcastle Disease virus, infectious bursal disease virus and infectious bronchitis virus). In this way, a single vaccine may express antigens derived from multiple antigenically divergent pathogens, viruses and/or bacteria and afford protection against multiple pathogens and/or antigenically divergent variants within virus subtypes.

[0312] The vaccine may be useful in preventing disease(s). Accordingly, the invention provides a vaccine of the invention for use in treating and/or preventing disease(s).

[0313] The present invention also provides a pharmaceutical composition which comprises at least one engineered protein of the invention, nucleic acid construct according to the invention or vector according to the invention. The pharmaceutical composition may be used for treating or preventing disease in a subject.

[0314] The vaccine or pharmaceutical composition may optionally comprise one or more adjuvants, excipients, carriers and diluents. The choice of pharmaceutical excipient, carrier or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as (or in addition to) the carrier, excipient or diluent, any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s) and other carrier agents. The pharmaceutical compositions typically should be sterile and stable under the conditions of manufacture and

storage. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, nanoparticles and implantable sustained-release or biodegradable formulations. Sterile injectable formulations may be prepared using a non-toxic parenterally acceptable diluent or solvent. A pharmaceutical composition of the present invention may include pharmaceutically acceptable dispersing agents, wetting agents, suspending agents, isotonic agents, coatings, antibacterial and antifungal agents, carriers, excipients, salts, or stabilizers which are nontoxic to the subjects at the dosages and concentrations employed. Preferably, such a composition can further comprise a pharmaceutically acceptable carrier or excipient for use in the treatment of disease that is compatible with a given method and/or site of administration, for instance for parenteral (e.g. sub-cutaneous, intradermal, or intravenous injection) administration.

[0315] The vaccine or pharmaceutical composition may comprise one or more engineered protein(s) of the invention, nucleic acid construct(s) according to the invention or vector(s) according to the invention in an effective amount.

[0316] In an embodiment the invention provides engineered protein(s) of the invention, nucleic acid construct(s) according to the invention or vector(s) according to the invention which when administered to a subject induces an immune response which is protective against subsequent challenge with pathogens. In an embodiment the invention provides engineered protein(s) of the invention, nucleic acid construct(s) according to the invention or vector(s) according to the invention which when administered to a subject induces an immune response which is protective against subsequent challenge with a pathogen of a different subtype or genotype to the pathogen of the vaccine.

Methods of Prevention/Treatment

[0317] The present invention also provides a method of preventing and/or treating a disease in a subject by administration to the subject of an effective amount of an engineered protein of the invention, a nucleic acid construct according to the invention, a vector according to the invention or an avian vaccine according to the invention.

[0318] The term "preventing" is intended to refer to averting, delaying, impeding or hindering the contraction of disease. For example, by delivering disease-specific antigens to professional antigen presenting cells, the subject's immune system may be enabled to recognise and eliminate infective cells by a humoral or cellular immune response.

[0319] The term "treating" is intended to refer to reducing or alleviating at least one symptom of an existing disease or infection.

[0320] Suitably, the challenge pathogen load (such as viral load, bacterial load or parasitic load) in the subject may be decreased by administration to the subject of an effective amount of an engineered protein of the invention, a nucleic acid construct according to the invention, a vector according to the invention or an avian vaccine according to the invention.

[0321] Suitably, administration to the subject of an effective amount of an engineered protein of the invention, a nucleic acid construct according to the invention, a vector according to the invention or an avian vaccine according to the invention may elicit production of cross-reactive antibodies.

[0322] A vaccine according to the invention may elicit production of antibodies which are capable of targeting two or more antigenically variant antigenic polypeptides from different strains of pathogen e.g. a virus. For example, a vaccine designed to target H9 influenza viruses may provide protection against antigenically variant H9 variants.

[0323] Suitably, administration of the vaccine according to the invention elicits a humoral and/or cellular immune response in the subject. Suitably, administration of the vaccine induces an increased humoral and/or cellular immune response relative to the administration of corresponding control. For example, a suitable control may be identical to the vaccine according to the invention except it lacks the at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell. In other words, the corresponding control may essentially consist of antigen.

[0324] Suitably, the administration of the vaccine according to the invention may elicit a faster humoral and/or cellular immune response in the subject compared with a corresponding control. For example, the vaccine according to the invention may elicit a humoral and/or cellular response within 6 days post primary vaccination (ppv).

[0325] Typically, the humoral immune response is mediated by antibody molecules that are secreted by plasma cells of vaccinated or infected host immune cells such as B cells. These antibody molecules bind to the pathogen (viruses) and neutralize their ability to infect and cause disease (for example, by blocking the ability of the virus to bind to host cells or enter into host cells).

[0326] Suitably, humoral immune response may be measured by determining the titre of antibodies produced in response to immunization and comparing this to a suitable control. For example, a vaccine according to the invention may elicit a higher humoral immune response when compared with a suitable control. Suitably, antigen specific antibodies in sera of immunized subjects may be measured by ELISA. (Example 1 describes the measurement of antigen specific immunoglobulins (Ig) IgY (mammalian IgG equivalent), IgA and IgM antibody levels in the sera were determined by ELISA assay.) In the case of viruses which have the hemagglutinin glycoprotein on their surface, a hemagglutination inhibition assay (HI) may be used to quantify the relative concentration of antibodies.

[0327] Typically, cellular immune responses or cell-mediated immunity refers to the activation of host immune cells such as T-cells, macrophages and natural killer cells and the secretion of cytokines that directly or indirectly destroy or block invading pathogens and protect the host from infection.

[0328] The production of cytokines and chemokines is an integral part of the cellular immune response. Suitably, the cellular immune response may be determined by measuring the production of inflammatory cytokines such as interferon gamma (IFNy), interleukin 6 (IL6), interleukin 1 β (IL1 β), CXCLi2 (IL8) and interleukin 4 (IL4).

[0329] Suitably, the cellular immune response (for example measured by IFNy, IL6, IL1 β , CXCLi2 (IL8) and/or IL4) may be increased by at least 2 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 30 fold, at least 50 fold, at least 75 fold, at least 100 fold relative to a corresponding control.

[0330] The “challenge pathogen load” refers to the value given to the quantity of challenge pathogen (for example, a viral pathogen, bacterial pathogen or parasitic pathogen) in a given volume of fluid.

[0331] The “challenge pathogen” refers to the pathogen which the vaccine is directed to.

[0332] The “viral load” refers to the value given to the quantity of virus in a given volume of fluid. A higher viral burden usually correlates with the severity of an active viral infection.

[0333] The challenge pathogen load in subjects may be determined by performing a plaque assay or quantification of amount of viral RNA using quantitative polymerase chain reaction (qPCR).

Subject

[0334] In one embodiment, the subject may be any avian subject. The subject may be poultry. The subject may be selected from a chicken, turkey, duck, quail, pigeon or goose.

[0335] Suitably, the subject vaccinated according to the present invention may be domestic poultry. The subject may be a domestic chicken, turkey, duck, quail, pigeon or goose.

[0336] In one embodiment the subject is a chicken and the disease is avian influenza (caused by avian influenza virus).

Disease

[0337] The vaccine of the invention may treat and/or prevent disease in a subject.

[0338] The vaccine of the invention may treat and/or prevent disease caused by any pathogen e.g. from viruses, bacteria or parasite.

[0339] In some embodiments, the vaccine may treat and/or prevent one or more viral diseases. In some embodiments, the vaccine may treat and/or prevent one or more bacterial diseases. In some embodiments, the vaccine may treat and/or prevent one or more viral diseases and one or more bacterial diseases.

[0340] The viral disease may be caused by viruses from the Orthomyxoviridae family. For example, the viral disease may be AIV.

[0341] The viral disease may be caused by viruses from the Paramyxoviridae family. For example, the viral disease may be NDV. For example, the viral disease may be caused by aMPV.

[0342] The viral disease may be caused by viruses from the Coronaviridae family. For example, the viral disease may be Infectious bronchitis caused by IBV.

[0343] The viral disease may be caused by viruses from the Birnaviridae family. For example, the viral disease may be IBD, caused by IBDV.

[0344] The viral disease may be caused by viruses from the Anelloviridae family. For example, the viral disease may be Chicken anaemia, caused by CAV.

[0345] The viral disease may be caused by viruses from the Reoviridae family. For example, the viral disease may be caused by ARV.

[0346] The viral disease may be caused by viruses from the Adenoviridae family. For example, the viral disease may be Egg drop syndrome '76, caused by Duck Adenovirus A. The viral disease may be caused by Fowl adenoviruses (FAdV's 2, 4, 8, 11) In one embodiment, the vaccine of the invention treats and/or prevents avian influenza.

Administration

[0347] The vaccine of the invention may be administered by any convenient route, such as by injection e.g. intramuscular or subcutaneous. Other suitable routes of administration include intranasal, topical ocular, oral or transdermal. In one embodiment, oral administration comprises adding the vaccine to animal feed or drinking water. In another embodiment, the vaccine may be added to bait for a wild animal, for example bait suitable for wild aquatic birds or wildfowl which may infect domestic poultry and other bird and animal species.

[0348] The dose for immunisation may be around 1 µg to around 100 µg. For example the dose may be around 1 µg to around 90 µg, around 1 µg to around 80 µg, around 1 µg to around 70 µg, around 1 µg to around 60 µg, around 1 µg to around 50 µg, around 1 µg to around 40 µg, around 1 µg to around 35 µg, around 1 µg to around 30 µg, around 1 µg to around 25 µg, around 1 µg to around 20 µg, around 1 µg to around 15 µg, around 1 µg to around 10 µg, or around 1 µg to around 5 µg.

[0349] The dose for immunisation may be around 2 µg to around 100 µg. For example the dose may be around 2 µg to around 90 µg, around 2 µg to around 80 µg, around 2 µg to around 70 µg, around 2 µg to around 60 µg, around 2 µg to around 50 µg, around 2 µg to around 40 µg, around 2 µg to around 35 µg, around 2 µg to around 30 µg, around 2 µg to around 25 µg, around 2 µg to around 20 µg, around 2 µg to around 15 µg, around 2 µg to around 10 µg, or around 2 µg to around 5 µg. The dose may be around 2 µg to around 35 µg. For example the dose may be around 20 µg to around 35 µg.

[0350] The dose may be determined by a veterinary practitioner within the scope of sound veterinary judgment. For example, taking into account dose sparing e.g. the ability to reduce dose without compromising treatment.

[0351] The vaccine may be administered following a prime-boost regime. For example, after the first inoculation, the subjects may receive a second boosting administration some time (such as about 6, 7, 14, 21 or 28 days) later. In some aspects, the boosting administration may be at the same dosage as the priming administration. In other aspects, the boosting administration may be at a higher dose than the priming administration.

[0352] This disclosure is not limited by the exemplary methods and materials disclosed herein, and any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of this disclosure. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, any nucleic acid sequences are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively.

[0353] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise.

[0354] The terms "comprising", "comprises" and "comprised of" as used herein are synonymous with "including", "includes" or "containing", "contains", and are inclusive or open-ended and do not exclude additional, non-recited members, elements or method steps. The terms "comprising", "comprises" and "comprised of" also include the term "consisting of".

[0355] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that such publications constitute prior art to the claims appended hereto.

EMBODIMENTS

[0356] The following embodiments of the present invention, presented as numbered paragraphs, may be used in combination with the other embodiments described herein:

[0357] 1. In one embodiment, the present invention provides an engineered protein comprising: at least one binding domain which is capable of binding to a cell surface protein on an antigen presenting cell; and

[0358] a) at least one antigenic polypeptide; or

[0359] b) at least one binding domain which is capable of binding to at least one antigenic polypeptide.

[0360] 2. In one embodiment, the present invention provides an engineered protein comprising: at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and

[0361] a) at least one antigenic polypeptide; or

[0362] b) at least one binding domain which is capable of binding to at least one antigenic polypeptide.

[0363] 3. An engineered protein according to paragraph 1 or 2, wherein said engineered protein comprises at least one antigenic polypeptide.

[0364] 4. An engineered protein according to paragraph 1 or 2, wherein said engineered protein comprises at least one binding domain which is capable of binding to at least one antigenic polypeptide.

[0365] 5. An engineered protein according to any preceding paragraph, wherein said engineered protein is genetically engineered.

[0366] 6. An engineered protein according to any preceding paragraph, wherein said engineered protein is chemically engineered.

[0367] 7. An engineered protein according to any of paragraphs 1 to 5, wherein the at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and a) at least one antigenic polypeptide; or b) at least one binding domain which is capable of binding to at least one antigenic polypeptide are comprised in a single recombinant protein.

[0368] 8. An engineered protein according to any preceding paragraph, wherein the at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and a) at least one antigenic polypeptide; or b) at least one binding domain which is capable of binding to at least one antigenic polypeptide are operably linked.

[0369] 9. An engineered protein according to any preceding paragraph, wherein the avian antigen presenting cell is at least one of a dendritic cell, macrophage, B cell or natural killer cell.

[0370] 10. An engineered protein according to any preceding paragraph, wherein the cell surface protein is selected from an immunoglobulin family protein, an integrin family receptor or a C-type lectin.

[0371] 11. An engineered protein according to any preceding paragraph, wherein the cell surface protein is selected from CD83, CD11c or Dec205.

- [0372] 12. An engineered protein according to any preceding paragraph, wherein the cell surface protein is CD83.
- [0373] 13. An engineered protein according to any of paragraphs 1 to 11, wherein the cell surface protein is CD11c.
- [0374] 14. An engineered protein according to any of paragraphs 1 to 11, wherein the cell surface protein is Dec205.
- [0375] 15. An engineered protein according to any preceding paragraph, wherein the at least one antigen is an avian influenza virus antigen, such as hemagglutinin.
- [0376] 16. An engineered protein according to any of paragraphs 1 to 3 or 5 to 15 wherein the at least one antigenic polypeptide is an avian influenza virus antigenic polypeptide, such as a hemagglutinin antigenic polypeptide.
- [0377] 17. An engineered protein according to any preceding paragraph, further comprising a signal peptide.
- [0378] 18. An engineered protein according to any preceding paragraph, further comprising a domain which improves solubilisation, stabilization and/or folding of the engineered protein.
- [0379] 19. An engineered protein according to any of paragraphs 1 or 4 to 18, wherein the at least one binding domain which is capable of binding to an antigen is capable of binding to an avian antigen. Suitably, the antigen from an avian pathogen may be present on the surface of any pathogen e.g. avian pathogen. For example, the antigen may be present on the surface of a virus, on the surface of a bacterium or on the surface of a parasite. Suitably, the antigen may be present on the surface of an inactivated virus, on the surface of an inactivated bacterium or on the surface of an inactivated parasite.
- [0380] 20. An engineered protein according to any preceding paragraph, wherein at least one binding domain is based on the antigen binding site of an antibody or an antibody fragment such as a single-chain variable fragment (scFv), Fv, F(ab') or F(ab')².
- [0381] 21. A nucleic acid construct which comprises a first polynucleotide which encodes at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell as defined in any of paragraphs 1 to 20; and a second polynucleotide which encodes at least one antigenic polypeptide or at least one binding domain which is capable of binding to at least one antigenic polypeptide as defined in any of paragraphs 1 to 20.
- [0382] 22. A vector which comprises a nucleic acid construct according to paragraph 21.
- [0383] 23. A vector according to paragraph 22 which is a herpes viral vector (such as turkey herpes virus (HTV/HVT) or Newcastle disease virus (NDV) vector.
- [0384] 24. An engineered cell expressing an engineered protein according to any of paragraphs 1 to 20, or comprising a nucleic acid construct according to paragraph 21, or comprising a vector according to paragraph 22 or 23.
- [0385] 25. An avian vaccine comprising a genetically engineered protein according to any of paragraphs 1 to 20, a nucleic acid construct according to paragraph 21 or a vector according to paragraph 22 or 23 and a pharmaceutically acceptable carrier.
- [0386] 26. An avian vaccine according to paragraph 24 for use in treating and/or preventing disease in a subject.
- [0387] 27. Use of a genetically engineered protein according to any of paragraphs 1 to 20, a nucleic acid construct according to paragraph 21, and/or a vector according to paragraph 22 or 23 in the manufacture of a medicament for the treatment and/or prevention of disease.
- [0388] 28. A method for treating and/or preventing a disease in a subject which comprises the step of administering to a subject an effective amount of a vaccine according to paragraph 25 or 26.
- [0389] 29. A vaccine for use according to paragraph 26, or a method according to paragraph 28, wherein administration of said vaccine elicits a humoral and/or cellular immune response in the subject.
- [0390] 30. A vaccine for use, use of a vaccine or a method according to paragraphs 26 to 29, wherein administration of said vaccine decreases the challenge pathogen load in the subject.
- [0391] 31. A vaccine for use, use of a vaccine or a method according to paragraphs 26 to 30, wherein administration of said vaccine elicits production of cross-reactive antibodies.
- [0392] 32. A vaccine for use, use of a vaccine or a method according to paragraphs 26 to 31, wherein the subject is an avian subject.
- [0393] 33. A vaccine for use, use of a vaccine or a method according to paragraphs 25 to 32, wherein the subject is poultry, for example the subject may be selected from a chicken, turkey, duck, quail, pigeon or goose.
- [0394] 34. A method for the preparation of the vaccine according to paragraph 25, the method comprising the step of admixing a genetically engineered protein according to any of paragraphs 1 to 20, a nucleic acid construct according to paragraph 21, and/or a vector according to paragraph 22 or 23, and a pharmaceutically acceptable carrier.
- [0395] The invention will now be further described by way of Examples, which are meant to serve to assist one of ordinary skill in the art in carrying out the invention and are not intended in any way to limit the scope of the invention.

EXAMPLES

Example 1—Antibody Targeted Vaccines Induce Faster and Stronger Immunity and Protection in Chickens

Materials and Methods

Viruses and Cells

[0396] A/Chicken/Pakistan/UDL 01/2008 H9N2 virus was propagated in 10-day old specific pathogen free (SPF) embryonated chicken eggs and titrated by plaque assay or TCID₅₀ on Madin-Darby canine kidney (MDCK) cells. The virus was inactivated chemically using Beta-propiolactone (BPL) and purified by ultracentrifugation through a continuous 30-60% w/v sucrose gradient.

[0397] MDCK cells were maintained in Dulbecco modified Eagle medium (DMEM) supplemented with 10% foetal bovine serum (FBS) and 0.1% penicillin and streptomycin at 37° C., 5% CO₂. *Drosophila* Schneider 2 (S2) cells were obtained from Invitrogen and maintained in Schneider's insect medium supplemented with 10% FBS at 25° C.

Construction of scFv and H9HA Fused scFv Protein Expressing Plasmids

[0398] The gene sequences comprising the vL chain and vH chain of chicken Dec205 mAb (clone: IAH F877: AD6), putative chicken CD11c mAb (clone: IAH 8F2) and chicken CD83 scFv mAb (clone: IAH F890:GE8) derived from mouse hybridoma were analyzed commercially (Absolute Antibody Ltd, UK). Synthetic cDNA containing the vL and vH sequences of APC-mAb were joined by (Gly₄Ser)₄ linker peptide sequence and manufactured commercially by Geneart (Thermo Fisher Scientific). The respective individual vL-Linker-vH cDNA were then cloned into *Drosophila melanogaster* expression vector (pMT/BIP/V5-His version A, Thermo Fisher Scientific) using the Not I and Xba I restriction sites (FIG. 1A). The resultant vector named pMT-BIP-scFv-V5-His was used to insert an ectodomain of the H9HA gene that lacks both hemagglutinin gene signal peptide and the TM domain, replaced with a 30 amino acid trimerization foldon sequence of the trimeric protein fibrin from bacteriophage T4, using Kpn I and Pac I restriction sites (FIG. 1B). The hemagglutinin used in this study was synthetically produced incorporating consensus sequence of hemagglutinin of H9N2 viruses derived from analysis of over 2000 H9HA sequences (from the public database) of G1-like H9 virus lineage using Minimum Sphere Consensus (MScon) method. However, this synthetic hemagglutinin has about 98% amino acid sequence similarity to hemagglutinin ectodomain of A/Chicken/Pakistan/UDL 01/2008 H9N2 virus (GenBank accession number: ACP50708.1, HA1: 19-349 and HA2: 1-174) virus. FIG. 1 shows a schematic representation of an expression cassette and a fusion construct.

Expression and Purification of scFv and H9HA Fused scFv Proteins

[0399] Recombinant proteins were produced and purified using the *Drosophila* Expression System (DES®, Life technologies). Briefly, pMT/BIP/scFv/V5-His or pMT/BIP/H9HA Foldon scFv/V5-His plasmids were co-transfected into *Drosophila* S2 cells together with a hygromycin B resistance plasmid (pCoHYGRO, Life technologies). Antibiotic selection was carried out for four weeks using hygromycin B at a concentration of 250 µg/ml and single cell clones were obtained via the limiting dilution. Recombinant proteins were secreted into culture supernatant after CuSO₄ (500 µM) induction and then purified by Profinity™IMAC uncharged column (Bio-Rad). Concentration of purified recombinant proteins was determined by Bradford assay and the purity was assessed by SDS-PAGE and Western Blot.

Characterization of scFv and H9HA Foldon-scFv Proteins

[0400] Indirect Enzyme Linked Immunosorbent Assay (ELISA) was carried out to examine if Dec205/CD83 scFv and H9HA Foldon-Dec205/CD83 scFv proteins can detect and bind to their respective receptor proteins. The coding sequences of chicken CD83 ectodomain and Dec205 C-type lectin domains 4-5-6 (Staines et al., PLOS One 2013, 8 (1), e51799) were cloned into pMT/BIP/His vector for expression in *Drosophila melanogaster* S2 cells. Briefly, 8 µg of the respective receptor proteins were added onto the first

well of 96 well maxisorp ELISA plates (Thermo Fisher Scientific). Then, two-fold dilutions of the respective receptor proteins were made. The plates were incubated at 4° C. overnight. For detection, the plates were incubated with equimolar concentration of the respective scFv and H9HA Foldon-scFv for two hours at 4° C. This was followed by further incubation with horseradish conjugated (HRP) anti-V5 secondary antibodies. The colorimetric detection was carried out by adding Tetramethylbenzidine (TMB) substrate (Thermo Fisher Scientific) and read at wavelength 450 nm in ELx808 Absorbance Microplate Reader (BioTek).

[0401] For the characterization of H9HA Foldon-CD11c scFv, the detection of the corresponding CD11c receptor molecules present in the chicken splenocytes extract was analysed using Western Blot analysis. Briefly, chicken splenocytes were solubilized with 400 µl lysis buffer (NP40) for 30 minutes on ice. During the incubation with the lysis buffer, the lysate was vortexed every 10 minutes. The lysate was then centrifuged at 12,000 rpm for 10 minutes and the resultant supernatant was harvested. The chicken splenocytes extract was run on 10% SDS-PAGE. The protein from the SDS-PAGE gel was blotted onto nitrocellulose membrane and incubated with H9HA Foldon-CD11c scFv overnight at 4° C. This was followed by the incubation with anti-V5 HRP secondary antibody for one hour at room temperature. 3,3'-diaminobenzidine (DAB) substrate (Thermo Fisher Scientific) was then added for detection.

Flow Cytometry

[0402] The binding of the recombinant scFv antibodies to the chicken splenocytes was further investigated using flow cytometry. Briefly, 1×10⁵ chicken splenocytes were stimulated with 200 ng/ml Lipopolysaccharides (LPS, Sigma) for 24 hours. The splenocytes were centrifuged and resuspended in 50 µl of FACS buffer (PBS with 1% Bovine Serum Albumin (BSA) containing 3 µg of respective scFv proteins for 45 minutes at 4° C. After the incubation with scFv proteins, the splenocytes were washed with 150 µl of FACS buffer and resuspended in 100 µl of FACS buffer containing secondary antibody (FITC conjugated anti-V5 tag, 1:100 dilution, Bio-Rad Antibodies) and incubated in the dark for 30 minutes at 4° C. This was followed by fixing of labelled splenocytes with 50 µl of 1% Paraformaldehyde (PFA) for 20 minutes in the dark. The plates were read the next day using MACSQuant flow cytometer and analysed with FCS Express 6 software.

Bis[Sulfosuccinimidyl] Suberate (BS3) Crosslinking

[0403] To determine the oligomeric structure of the recombinant H9HA containing trimerization foldon domain, cross linking was performed using BS3 (Thermofisher Scientific). Briefly, 15 µg recombinant protein was incubated at room temperature in the presence of BS3 (final concentration 10 mM) for one hour. Crosslinking was stopped by the addition of 1M Tris-HCl pH 8.0 to a final concentration of 50 mM. The cross-linked products were separated on SDS gel under reducing conditions, blotted and immunodetected using an anti-H9HA monoclonal antibody.

Preparation and Stimulation of Chicken Splenocytes

[0404] Splenocytes were prepared from the spleens of 3 weeks old unvaccinated Specified Pathogen Free (SPF) chickens via density gradient centrifugation by using his-

topaque 1083 (Sigma) according to the manufacturer's instructions. About 2×10^6 cells were plated on each well of 24 well plate suspended in 300 μl of complete Roswell Park Memorial Institute 1640 (RPMI) medium containing 10% FBS and 0.1% penicillin and streptomycin. Cells were treated with 10 μg of H9HA Foldon or 14 μg of H9HA Foldon-scFv (containing 10 μg H9HA Foldon according to the molecular weight) or 10 μg of scFv. Cells were also stimulated with Phorbol Myristate Acetate (PMA)/lonomycin (final concentration 10 $\mu\text{g}/\text{ml}$) as a positive control for IFN γ cytokine production. All cells were stimulated for 5 hours, 22 hours, 30 hours and 45 hours in vitro at 41° C.

RNA Extraction and Quantitative Reverse Transcription PCR (qRT-PCR) of Cytokines and Chemokines

[0405] RNA was extracted from the stimulated splenocytes using Rneasy™ kit (Qiagen) according to the manufacturer's protocol. To perform RNA quantification, single-step real time reverse transcription PCR was done using Superscript™ III Platinum One-Step qRT-PCR kit (LifeTechnologies) as per the manufacturer's protocol in 7500 fast real-time PCR machine (Applied Biosystems). Cycling conditions are as follows: i) 5 min hold step at 50° C. ii) a 2 min hold step at 95° C. iii) 40 cycles of 3 sec at 95° C., 30 sec annealing and extension at 60° C. Data were calculated using $2^{-\Delta\Delta CT}$ approach (n-fold change compared to the media only control group) and reported as values normalized to the expression level of a housekeeping gene RPLPO1. Out of the three reference genes (RPLPO-1, RPL13 and 28S) selected for normalization, RPLPO1 was the most stable gene across the samples hence, chosen for normalization.

IFN γ Sandwich ELISA

[0406] Supernatants from the stimulated splenocytes were harvested and examined by sandwich ELISA. Briefly, anti-chicken IFN γ (1:150 dilution, Invitrogen) was coated onto 96-well maxisorp ELISA plates (Thermo Fisher Scientific). The coated plates were blocked at room temperature with PBS containing 3% BSA for 1 hour. 1:2 dilution of the supernatants was made in PBS buffer containing 3% BSA. The plates were then incubated with the diluted supernatants for two hours at room temperature. Detection was carried out using biotinylated anti-chicken IFN γ detection antibody (1:600 dilution, Invitrogen) for 1 hour at room temperature followed by HRP conjugated streptavidin (1:1000 dilution, Amersham) for another 1 hour at room temperature. 100 μl of Tetramethylbenzidine (TMB) substrate (BD biosciences) was added for 10 minutes. The reaction was stopped using 2M H₂SO₄ and read at wavelength 450 nm in ELx808 Absorbance Microplate Reader (BioTek).

Hemagglutination Assay (HA) and Hemagglutination Inhibition (HI) Assay

[0407] World Health Organisation guidelines were followed for HI assay (World Health Organization. WHO Global Influenza. Surveillance Network. WHO Global Influenza Surveillance Network: Manual for the Laboratory Diagnosis and Virological Surveillance of Influenza. 2011, 153) and HA assay was performed as previously described (Walker, J. M. In *Animal Influenza Virus*, 2nd ed.; Spackman, Erica.; Springer Science+Business Media: New York, USA, 2014; pp. 6-10, ISBN 978-1-4939-0757-1). For HI assay 4

HA units of virus was used. Both assays utilized 50 μl of 1% chicken red blood cells (RBCs).

Chicken Vaccination and Blood Sample Collection

[0408] Groups of 7 days old SPF chickens (n=8) were immunized with vaccine dose of containing 2.8 μg , 28 μg and 49 μg of recombinant H9HA Foldon-Dec205 scFv, H9HA Foldon-CD11c scFv or H9HA Foldon-CD83 scFv proteins equivalent to 2 μg , 20 μg and 35 μg of recombinant H9HA Foldon (equimolar concentration). The proteins were formulated in montadine ISA 71 VG (Sippic) adjuvant. The ratio of protein to adjuvant volume was 1:3. The vaccine dose (0.2 ml) was administered subcutaneous, delivered at the back of the neck. Control groups were immunized with PBS or montadine adjuvant. Additionally, one group of chickens (n=8) was vaccinated with inactivated H9N2 virus (A/Chicken/Pakistan/UDL01/2008, 1024 HAU/dose). All vaccinated groups received a booster dose at 14 days old. In all cases, blood samples were collected from the wing vein 6, 14, 21 and 28 days post first vaccination.

Viral Challenge and Swab Sample Collection

[0409] For the viral challenge study, SPF chickens (n=7) were divided into four groups: PBS control, Inactivated H9N2 virus (A/Chicken/Pakistan/UDL 01/2008), H9HA Foldon (25 $\mu\text{g}/\text{dose}$) and H9HA Foldon CD83 scFv (25 $\mu\text{g}/\text{dose}$ equivalent of H9HA Foldon). The PBS control and H9HA Foldon-CD83 scFv groups were further divided into two subgroups: Direct virus inoculated and contacts. The chickens were vaccinated at 7 and 14 days old. All chickens except the contact groups were challenged with 1×10^6 plaque forming units (PFU)/100 μl of A/Chicken/Pakistan/ UDL 01/2008 H9N2 virus intranasally at day 22 old (one week after the boost vaccination). Chickens were monitored daily for clinical signs and weight changes throughout the experiment.

[0410] Swab samples from buccal and cloacal cavities were collected daily until day 7 post infection with the last sampling performed on day 10 post infection. Sterile polyester tipped swabs were transferred into the virus transport media, vortexed and centrifuged for 10 min at 4500 rpm to clarify the medium. Samples were stored at -80° C. until further analysis.

Measurement of Serum IgY, IgA and IgM Anti-HA Antibody Levels

[0411] Antigen specific IgY (mammalian IgG equivalent), IgA and IgM antibody levels in the sera were determined by ELISA assay. Briefly, flat bottom 96 well maxisorp ELISA plates (Thermo Fisher Scientific) were coated with 1 μg of recombinant H9HA Foldon protein diluted in 50 μl of carbonate buffer (pH 9.6) overnight at 4° C. Protein coated plates were blocked at room temperature with 5% milk powder (Marvel) in PBS-tween 0.1% (PBS-T) for 1 hour. Plates were washed thrice with wash buffer PBS-T. 1:200 dilution of chicken sera was made in PBS-T buffer with 1% milk powder. The plates were then incubated with 50 μl of the diluted sera at room temperature for 1 hour. Plates were again washed thrice and incubated for 1 hour at room temperature with 50 μl of goat anti-chicken IgY, IgA and IgM antibody conjugated to HRP (Abcam) diluted 1:3000 in PBST buffer with 1% milk powder. Plates were washed $\times 4$ with PBS-T then 100 μl of TMB substrate (BD biosciences)

was added for 10 minutes. The reaction was stopped using 2M H₂SO₄ and read at wavelength 450 nm in ELx808 Absorbance Microplate Reader (BioTek). A standard serum (serum collected from 35-day old chicken challenged with A/Chicken/Pakistan/UDL 01/2008 (H9N2) virus) was included in all assays. The amount of anti-HA IgY, IgM or IgA antibodies were expressed as sample to reference ratio (relation of absorbance of tested serum sample to absorbance of the reference serum).

Plaque Assay

[0412] The virus titre from the allantoic fluid or swab samples was obtained using plaque assay. Pre-seeded 12 well plates with MDCK cells were inoculated with 10-fold serially diluted samples and left for 1 h at 37° C. Cells were washed with PBS and overlaid with DMEM (1×MEM, 0.21% BSA, 1 mM L-glutamate, 0.15% sodium bicarbonate, 10 mM Hepes, 0.1% penicillin G/streptomycin) containing 0.6% purified agar (Oxoid) and 2 µg/ml TPCK trypsin. Cells were left at 37° C. for 72 hours. After 3 days medium was removed, and cells were stained in crystal violet solution for 30 minutes.

Virus Microneutralization (MNT) Assay

[0413] MDCK cells were pre-seeded into the 96 well plates to reach 90-95% confluence. The immunized chicken sera were inactivated at 56° C. for 30 minutes. Then, 1:200 dilution of the inactivated serum was carried out. This was followed by two-fold serial dilution in triplicates and mixed with 90 µl of A/Chicken/Pakistan/UDL 01/2008 (H9N2) virus containing 150 TCID₅₀. The sera-virus mixture was incubated at 37° C. for 1 hour. Cells were washed with PBS and inoculated with sera-virus mixture for 1 hour at 3TC. After the incubation, cells were washed once again with PBS and serum free DMEM containing 2 µg/ml TPCK trypsin was added, cells were left at 37° C. for 72 hours. After 3 days medium was removed, and cells were stained in crystal violet solution for 30 minutes.

Statistical Analysis

[0414] Results are expressed as the mean±standard deviation (SD). Statistical significance (p-values) was determined using a one-way ANOVA, Log rank (Mantel-Cox) test, unpaired Student's t-test and Tukey's multiple comparison test using Prism™ 8.3.0 (GraphPad Software). Differences were considered statistically significant if P<0.05.

Results

Expression and Purification of the Recombinant Proteins

[0415] To create a soluble H9HA protein, the TM domain of H9HA was replaced with 30 amino acid long foldon of the trimeric protein fibritin from bacteriophage T4 (FIG. 2). Furthermore, the soluble H9HA protein was recombinantly fused with scFv antibodies targeting Dec205, CD11c and CD83 receptor proteins on chicken APCs. The scFv and H9HA Foldon-scFv proteins were successfully expressed in *Drosophila* S2 cells. Subsequent purification of the recombinant proteins by His-tag affinity chromatography produced proteins with the expected molecular weights of about 30 kDa for scFv, 70 kDa for H9HA Foldon and 100 kDa for H9HA Foldon-scFv (FIG. 3). Based on recovered purified

proteins, it was estimated that expression levels of recombinant proteins range from 10-20 mg/litre of culture supernatants.

H9HA Ectodomain Fused to T4 Bacteriophage Foldon can Trimerize and Retain the Hemagglutination Activity

[0416] The oligomerization state of soluble H9HA protein with trimerization foldon was determined by cross-linking using BS3. Multimeric proteins exposed to this cross-linker will have each subunit crosslinked together with the formation of amide bonds. This provides direct evidence for their close proximity. This also helps to stabilize the structure of oligomers, allowing them to be analysed on SDS denaturing gels for Western blot analysis. This method was used to confirm the native structure of the protein. Recombinant H9HA Foldon and H9HA Foldon-scFv proteins were exposed to BS3 cross linker and the cross-linked products were separated on SDS gel under reducing and denaturing conditions, blotted and immunodetected using anti-H9HA monoclonal antibody. The result is shown in FIG. 4. Without BS3 crosslinking, three species; monomer, dimer and trimer are observed (major band at monomer; Lane 1 and Lane 3 corresponding to about 70 kDa and 100 kDa for H9HA Foldon and H9HA Foldon-scFv respectively). With cross-linking, stable trimeric form is observed (Lane 2 and Lane 4 corresponding to about 210 kDa and 300 kDa for H9HA Foldon and H9HA Foldon-scFv respectively). This indicates that the native structure of recombinant H9HA protein with foldon is a trimer.

[0417] Next, the biological activity of soluble H9HA Foldon and H9HA Foldon scFv proteins was tested using HA assay (FIG. 5). The soluble H9HA Foldon protein, both on its own and when fused to scFv antibodies was able to agglutinate chicken RBC's retaining its hemagglutination activity. Furthermore, on average soluble H9HA Foldon and H9HA Foldon scFv showed hemagglutination upto 0.14 µg/ml. Lower concentrations of soluble H9HA Foldon and H9HA Foldon scFv and PBS control showed no hemagglutination activity. Results are shown in FIG. 5.

The Fusion of scFv Antibodies to H9HA Foldon Protein does not Affect their Function

[0418] The specificity of scFv antibodies was demonstrated by binding to chicken splenocytes (FIG. 6A). Chicken splenocytes were stained by all the scFv antibodies. In order to determine if the scFv antibodies can retain their activity after being fused to H9HA Foldon protein, indirect ELISA was carried out. Both Dec205/CD83 scFv and H9HA Foldon fused Dec205/CD83 scFv antibodies were able to detect and bind to their respective receptor proteins and as expected the ability of the scFv antibody to bind to their receptor protein decreased with the fusion to hemagglutinin protein. Since the coding sequence of chicken CD11c receptor protein was publicly unavailable, we were unable to express chicken CD11c receptor protein and use it to characterize H9HA Foldon-CD11c scFv. However, for testing the activity of CD11c scFv fused to H9HA Foldon, we carried out western blot analysis. H9HA Foldon-CD11c scFv was able to detect 150 kDa CD11c protein in the chicken splenocytes extract (FIG. 6B).

Activation of Chicken Splenocytes by scFv, H9HA Foldon and H9HA Foldon-scFv Proteins Ex Vivo

[0419] Splenocytes were isolated from unvaccinated SPF chickens and treated with scFv, H9HA Foldon and H9HA Foldon-scFv proteins for 5 hours, 22 hours, 30 hours and 45

hours in vitro. We investigated the production of cytokines IFN γ , IL6, IL1 β , IL4, IL18 and chemokine CxCLi2. With regards to stimulation by scFv, CD83 scFv and CD11c scFv were able to induce expression of IL6 (~5-15 fold, with CD11c scFv ~13 fold, p<0.05 at 5 hours post stimulation), IL1 β (~5-10 fold) and CxCLi2 (~5-20 fold) compared to the control scFv (FIG. 7A). There was little or no expression of IL4 and IL18 cytokines. There was a low expression of IFN γ at 45 hours post stimulation by CD11c scFv and CD83 scFv. This was verified by IFN γ ELISA (FIG. 7B).

[0420] Interestingly, H9HA Foldon-CD83 scFv and H9HA Foldon-CD11c scFv were able to induce significantly higher levels of pro-inflammatory cytokines IFN γ (~50-180 fold), IL6 (~50-115 fold) and IL1 β (~30-45 fold) compared to H9HA Foldon (FIGS. 8A and 8B). IL6 and L1P cytokines were induced earlier (5 hours post stimulation) whereas IFN γ was induced later (after 22 hours post stimulation) by H9HA Foldon-CD83 scFv and H9HA Foldon-CD11c scFv. There was no expression of IL18 cytokine. In addition, H9HA Foldon-CD83 scFv and H9HA Foldon-CD11c scFv induced higher levels of IL4 (~15 fold) and chemokine CxCLi2 (~15-55 fold) at 22 hours and 30 hours post stimulation (not significant) compared to H9HA Foldon.

Immunization with H9HA Foldon-scFv Proteins Induces Faster and Higher Humoral Response

[0421] A standard HI assay was conducted to test the ability of H9HA Foldon-scFv to generate HI antibodies. HI antibody titres were measured at 6, 14, 21 and 28 days post primary vaccination. With 20 μ g and 35 μ g dose of H9HA-scFv we were able to detect HI antibodies as early as day 6 post primary vaccination (ppv) (FIG. 9, Table 2). 2 μ g dose of H9HA-scFv was found insufficient to induce earlier antibody response like that induced by 20 μ g and 35 μ g doses. However, after 21 days ppv even the 2 μ g H9HA Foldon-scFv vaccine group had HI antibody titre either higher or similar to that of inactivated vaccine group. Furthermore, with 20 μ g and 35 μ g doses of H9HA Foldon-CD83 scFv and H9HA Foldon-CD11c scFv, the titre of HI antibodies produced was significantly higher on all days tested compared to the H9HA Foldon. However, with H9HA-Dec205 scFv significantly higher HI titre was produced only with 35 μ g dose compared to H9HA Foldon on most of the days tested. Interestingly, there were no significant differences between the three doses of vaccination in H9HA Foldon immunized groups on all the days tested. However, with H9HA Foldon-scFv higher HI antibodies were produced with 20 μ g and 35 μ g doses compared to 2 μ g dose on most of the days tested. Furthermore, the HI antibody titre with 20 μ g and 35 μ g of H9HA Foldon-scFv was also higher than that of the inactivated virus vaccine group.

[0422] The HI assay only takes account of the antibodies that can block influenza hemagglutinin glycoprotein binding to sialic acid residues of receptor proteins and prevent hemagglutination of RBCs. However, it misses all other antibodies that could possibly neutralize the virus via different route and hence, does not give the total measure of anti-HA antibodies produced in the immunized serum. Thus, we used ELISA to measure the total amount of anti-HA IgY, IgM and IgA antibodies in the serum of the immunized chickens with 35 μ g dose of vaccines at 6, 14, 21 and 28 days ppv. As expected, the amount of IgY and IgM antibodies were higher than that of IgA antibodies in the immunized serum (FIG. 10). On day 6 ppv the amount of IgY and IgM

antibodies were significantly higher in H9HA Foldon-CD83 scFv and H9HA Foldon-Dec205 scFv groups compared to H9HA Foldon group. Furthermore, there was no difference in the amount of IgY antibodies between any groups on day 14 and day 21 ppv, but a significantly higher amount of IgM antibodies was seen with H9HA Foldon-CD83 scFv and H9HA Foldon-CD11c scFv compared to H9HA Foldon. On day 28 ppv a significantly higher amount of IgY and IgM antibodies were produced with H9HA Foldon-CD11c scFv, whereas with H9HA Foldon-CD83 scFv produced significantly higher anti-HA IgY compared to the H9HA Foldon group. Overall, differences were observed between H9HA Foldon and H9HA Foldon-scFv groups only with anti-HA IgM antibodies.

[0423] In addition, virus MNT assay was also performed with 35 μ g immunized sera at day 28 ppv. The virus neutralization titre with all H9HA Foldon-scFv groups were significantly higher than H9HA Foldon group, and H9HA Foldon-CD83 scFv gave the highest titre compared to all other vaccinated groups (FIG. 11, Table 3).

H9HA Foldon-CD83 scFv is Better at Reducing the Viral Load in H9N2 Virus Challenged Chickens Compared to H9HA Foldon

[0424] To determine the protective efficacy of H9HA Foldon-CD83 scFv against the H9N2 infection, different groups of chickens were vaccinated twice with H9HA Foldon, H9HA Foldon-CD83 scFv and inactivated H9N2 vaccines, and challenged with H9N2 virus after 7 days post boost vaccination. The control (PBS treated) and H9HA Foldon-CD83 scFv vaccinated groups also had un-infected chickens serving as contacts. The contact groups were there to provide evidence on whether the vaccinated chickens have a reduced chance of getting infection from the unvaccinated directly infected chickens while sharing the same air space, food and water.

[0425] The clinical signs observed in the virus infected chickens include diarrhoea, rapid breathing, weight loss, half eyes shut, ruffled feathers and isolated behaviour. All vaccinated groups (direct and contact) had 100% survival rate whereas only about 58% of the chickens in directly infected PBS control group survived the virus challenge. In addition, the survival rate of PBS treated contact group chickens was only about 87% (FIG. 12A). Furthermore, the average weight gain of all directly infected vaccinated chickens remained fairly consistent after the virus infection, whereas there was a significant decrease in the average weight gain in directly infected PBS control group on day 3 and day 4 post virus infection (FIG. 12B). We lost total of 3 chickens in directly infected PBS control group on day 3 and day 4 post virus infection, and the chickens that survived had a similar average weight gain pattern as the vaccinated groups after day 4 post virus infection.

[0426] The viral load in chickens was determined by performing plaque assay on the buccal swabs collected on day 1 to day 7 post infection. No virus shedding was observed via the cloacal route (data not shown). On day 1, 2 and 3 post infection significantly lower virus titre was observed in the vaccinated groups compared to the PBS control group (Figure). The average virus titre in directly infected PBS control groups ranged from 23,000 pfu/ml to 12,000 pfu/ml on the first three days post virus infection, whereas for all vaccinated groups the average virus titre ranged from 6200 pfu/ml to 390 pfu/ml. Furthermore, H9HA Foldon-CD83 scFv vaccinated chickens had signifi-

cantly lower average virus titre compared to H9HA Foldon vaccinated chickens on day 2 post infection (H9HA Foldon-CD83 scFv: 1220 pfu/ml, H9HA Foldon: 3052 pfu/ml) and day 3 post infection (H9HA Foldon-CD83 scFv: 390 pfu/ml, H9HA Foldon: 1257 pfu/ml). We did not see any significant difference between H9HA Foldon-CD83 scFv and inactivated virus vaccine groups in terms of virus load on all days post virus infection. By day 4 post infection, the virus was almost cleared from all the directly infected vaccinated groups whereas some virus was still left in the directly infected PBS control group. On the other hand, the H9HA Foldon-CD83 scFv contact group chickens had significantly lower virus titre compared to PBS contact group, and the chickens in vaccinated contact group showed virus one day later than PBS contact group (FIG. 13). By day 6 post infection the virus was cleared from all the groups (direct and contacts).

Summary

[0427] This study provides evidence that TADV or ATV containing antigens fused with antibodies specific to receptor molecules on the surface of APCs induce faster and stronger immunity in chickens. The prototype TADV consisted of hemagglutinin antigen of H9N2 AIV as a model antigen that was fused with scFv antibodies specific for chicken APC receptors CD83, CD11c and Dec205. The resultant modified hemagglutinin antigen fused with CD83 scFv, CD11c scFv or Dec205 scFv antibodies were produced as recombinant soluble trimeric glycoproteins in insect cells and characterized using Western blot and ELISA assays. The results suggested that the fusion of hemagglutinin antigen to scFv antibodies does not abrogate the functional activity of hemagglutinin or the scFv antibodies. Immunizations of chickens with these APC-targeted H9HA Foldon-scFv vaccines induced faster and stronger hemagglutinin antigen-specific antibody responses compared to the untargeted counterpart or the conventionally killed H9N2 virus vaccine.

[0428] For example, recombinant H9HA Foldon-CD83 scFv induced higher serum HI and virus neutralizing antibodies compared to the untargeted H9HA Foldon. Furthermore, chickens vaccinated with TADV (H9HA Foldon-CD83 scFv) also showed reduced clinical disease signs and reduced shedding of virus from buccal cavities when challenged with H9N2 virus. These studies demonstrated that targeting antigens via antibodies to chicken APCs enhanced the immunogenicity and protective efficacy of poultry vaccine against AIV. In addition, H9HA Foldon-scFv were also able to stimulate chicken splenocytes in vitro inducing the expression of pro-inflammatory cytokines (IFN γ , IL1 β and IL6) and chemokine (CXCLi2). Hence, scFv antibodies could be serving as built-in adjuvants along with delivering antigen cargo to the APCs, and increasing the immunostimulatory potential of the antigen.

Example 2—Antibody Targeted Vaccines Show Enhanced Immunogenicity Compared with Commercial Vaccines

Viruses and Vaccines

[0429] The hemagglutinin used for making the recombinant H9HA Foldon and recombinant H9HA Foldon-CD83 scFv was synthetically produced by incorporating consensus sequence of hemagglutinin of H9N2 viruses derived from

analysis of over 2000 H9HA sequences (from the public database) of G1-like H9 virus lineage. This synthetic hemagglutinin has about 98% amino acid sequence similarity to hemagglutinin ectodomain of A/Chicken/Pakistan/UDL 01/2008 (UDL 01/08) H9N2 virus (GenBank accession number: ACP50708.1, HA1: 19-349 and HA2: 1-174) virus.

[0430] The commercial vaccine tested was inactivated virus vaccine and had mixtures of A/Chicken/UAE/415/99 (UAE/415) H9N2 virus and Newcastle disease (ND) virus.

Chicken Vaccination and Blood Sample Collection

[0431] This animal study was carried out in order to compare the performance of the recombinant antibody targeted vaccines with the currently available commercial avian influenza vaccine. Chickens were divided into five groups (n=10 per group): commercial vaccine, H9HA Foldon (35 μ g per dose), H9HA Foldon-CD83 scFv (equivalent to 35 μ g of H9HA Foldon according to the molecular weight), Inactivated H9N2 (A/Chicken/Pakistan/UDL 01/2008, the sequence of which is publicly available at GenBank accession number: ACP50708.1) and unvaccinated control groups. H9HA Foldon, H9HA Foldon-CD83 scFv groups were further divided into single and double vaccination groups where the later received boost vaccination. All vaccines were formulated as per the industrial requirement. The volume of the recombinant vaccines and inactivated H9N2 vaccine were kept at 0.2 ml per dose to keep it consistent with the previous experiments. However, the volume of the commercial vaccine was kept at 0.25 ml per dose as per the industrial requirement. The chickens were vaccinated at 1 day old (SPF White Leghorn) subcutaneously, as this mimic the mass application route of 1-day old chickens in the hatchery. Only H9HA Foldon, H9HA Foldon-CD83 scFv and inactivated H9N2 vaccine groups were given boost vaccination at day 7 old. In all cases, blood samples were collected from the wing vein 6, 14, 21, 28 and 35 days post vaccination.

Hemagglutination Assay (HA) and Hemagglutination Inhibition (HI) Assay

[0432] World Health Organisation guidelines were followed for the HI assay and HA assay. Both assays utilized 1% chicken red blood cells (RBCs).

Results

Antigenic Relationship Between the Commercial and Recombinant Vaccine Virus Strains

[0433] The H9N2 virus strains used in the recombinant H9HA Foldon/H9HA Foldon-CD83 scFv vaccines the commercial vaccine were A/Chicken/Pakistan/UDL01/2008 and A/Chicken/UAE/415/99 respectively. These two viruses had 94% amino acid sequence similarity. We carried out the HI assay on the antisera using both the homologous and heterologous viruses, in order to determine the antigenic relationships between the two viruses. The 'r' value is used to determine the extent of the antigenic difference between the two virus strains as described in Archetti et al., J Exp Med, 1950 Nov. 1; 92(5): 441-62.

Vaccine (Antisera)	Virus	
	UAE/415	UDL January 2008
Commercial vaccine (UAE/415)	103.97	630.35
Inactivated H9N2 Pirbright (UDL January 2008)	18.38	207.94

[0434] Table 5: HI titre of the commercial vaccine and inactivated H9N2 Pirbright vaccine antisera with both homologous and heterologous viruses. The homologous titres are indicated in bold.

[0435] The 'r' value between the two H9N2 viruses was 0.73 meaning these two viruses were antigenically similar ('r' value=1 indicates no antigenic difference). Hence, some cross-reactivity between the antisera is expected.

Analysis of the Antisera

Hi Antibody Titre with A/Chicken/Pakistan/UDL 01/2008 H9N2 Virus

[0436] A standard HI assay was conducted to test the antisera from the immunized chickens. A/Chicken/Pakistan/UDL 01/2008 was used for this assay. This virus is homologous for H9HA Foldon, H9HA Foldon-CD83 scFv and Inactivated H9N2 Pirbright vaccines and heterologous for the commercial vaccine. HI antibody titres were measured at 6, 14, 21, 28 and 35 days post primary vaccination (ppv). The results show that the titre of HI antibodies produced by H9HA Foldon-CD83 scFv is significantly higher than H9HA Foldon on all the days tested. Interestingly, there were no significant differences between the single and double vaccinated H9HA Foldon/H9HA Foldon-CD83 scFv groups i.e. the HI antibody titres with and without the boost vaccination were similar. Furthermore, we also saw good cross-reactivity of the commercial vaccine antisera with heterologous UDL 01/08 virus. However, no significant differences were observed between the HI antibody titres between the commercial vaccine and H9HA Foldon-CD83 scFv groups (both single and double vaccinated groups, FIG. 14, Table 4). The HI antibody titre of H9HA Foldon-CD83 scFv group was higher (not significant) than the commercial vaccine group on most of the days tested (Table 5). Moreover, HI antibody production in the immunized chickens were observed after 14 days post primary vaccination.

Hi Antibody Titre with A/Chicken/UAE/415/99

[0437] A standard HI assay was conducted to test the antisera from the immunized chickens. A/Chicken/UAE/415/99 was used for this assay. This virus is heterologous for H9HA Foldon, H9HA Foldon-CD83 scFv and Inactivated H9N2 Pirbright vaccines and homologous for the commercial vaccine. HI antibody titres were measured at 6, 14, 21, 28 and 35 days post primary vaccination. As expected, we saw reduction in the HI antibody titre of H9HA Foldon, H9HA Foldon-CD83 scFv and Inactivated H9N2 Pirbright vaccine antisera with heterologous UAE/415 virus. Surprisingly, antisera from chickens immunized with H9HA Foldon-CD83 scFv had higher cross-reactivity with UAE/415 virus compared to H9HA Foldon and Inactivated H9N2 Pirbright vaccine. In addition, HI antibody titre of H9HA Foldon-CD83 scFv group was similar to that of the commercial vaccine on all the days tested (FIG. 14, Table 4). HI antibody production in the immunized chickens were observed after 14 days post primary vaccination.

Summary

[0438] In this study, we evaluated the immunogenicity of H9HA Foldon-CD83 scFv vaccine in comparison with a commercial avian influenza vaccine. There were differences in the vaccine virus strains between H9HA Foldon-CD83 scFv vaccine (A/Chicken/Pakistan/UDL01/2008) and commercial vaccine (A/Chicken/UAE/415/99). However, these viruses have 94% amino acid similarity and 'r' value of 0.73 which suggested that these two vaccine virus strains were antigenically similar. With homologous virus UDL 01/08, the HI antibody titre of H9HA Foldon-CD83 scFv group was higher (not significant) than the commercial vaccine group. Interestingly, the HI antibody titre induced by H9HA Foldon-CD83 scFv with the heterologous UAE/415/99 virus was similar to that induced by the commercial vaccine. This suggests that targeting H9HA antigen with CD83 scFv could improve the cross reactivity of the vaccine to other heterologous viruses hence, this could be a strategy to enhance broadly cross-reactive antibody titres. Furthermore, no significant differences were observed in the HI antibody titres between the single and double vaccinated H9HA Foldon/H9HA Foldon-CD83 scFv groups. This provides evidence that a single vaccination schedule without a boost can enhance the immunogenicity of H9HA Foldon-CD83 scFv vaccine. This is very advantageous because a single vaccination is preferred in the field to reduce cost and time.

[0439] Overall, these findings suggest that H9HA Foldon-CD83 scFv subunit vaccine may perform better than the commercial whole killed virus vaccine.

Example 3—Antibody Targeted Vaccine Comprising Bispecific or Multispecific Binding Domains (IG10)

[0440] A bispecific single chain fragment variable (scFv) antibody having binding specificity for two antigens was generated. One scFv binds to the virus antigen (in this case avian influenza virus surface protein hemagglutinin).

[0441] The virus specific scFv (FIG. 18) is capable of binding to virus antigen and is non-neutralising meaning that it will bind to the virus but will not neutralise it (in this example the first binding domain binds to hemagglutinin on an inactivated virus).

[0442] The APC-specific scFv (FIG. 18) is capable of binding to a cell surface protein on APCs in this example the binding domain is a CD83 scFv, which will target avian host APCs.

[0443] To demonstrate the approach of linking inactivated whole avian influenza virus antigen to the APCs cell surface receptors, a non-neutralising scFv antibody was generated (referred to herein as IG10) that specifically binds to hemagglutinin antigen of H9N2 avian influenza virus.

[0444] The scFv sequence of IG10 scFv was fused with CD83 scFv using a linker sequence. The resulting construct IG10 scFv-CD83 scFv was expressed as a bispecific scFv antibody in insect S2 cells and was secreted as soluble antibody (IG10 scFv-CD83 scFv) into the S2 cell culture medium. The culture medium containing secreted IG10 scFv-CD83 scFv bound specifically to inactivated H9N2 virus. The antibody construct showed specific binding to avian influenza virus and specific binding to CD83 receptor antigen in ELISA assays. The results for IG10 are shown in FIG. 19 and FIG. 20.

[0445] The results in FIG. 19 show that IG10-CD83 bispecific antibody can bind to the H9N2 virus with the affinity similar to that of IG10 scFv.

[0446] The results in FIG. 20 show that IG10-CD83 can bind and recognise chicken CD83 receptor protein. The signal for both IG10-CD83 may be considered relatively low because the proteins were not purified for this assay. It is expected that the signals will be higher with the purified bispecific antibodies.

[0447] Results from FIG. 19 and FIG. 20 show that the IG10scFv-CD83 scFv bispecific antibody has bispecific binding ability and can bind to both the H9N2 virus and chicken CD83 receptor protein.

Example 4—Antibody Targeted Vaccine Comprising Bispecific or Multispecific Binding Domains (HD8)

[0448] To demonstrate the approach of linking inactivated whole avian influenza virus antigen to the APCs cell surface receptors, a non-neutralising scFv antibody was generated (referred to herein as HD8) that specifically binds to hemagglutinin antigen of H9N2 avian influenza virus.

[0449] The scFv sequence of HD8 scFv was fused with CD83 scFv using a linker sequence. The resulting construct HD8 scFv-CD83 scFv was expressed as a bispecific scFv antibody in insect S2 cells and was secreted as soluble antibody (HD8 scFv-CD83 scFv) into the S2 cell culture medium. The culture medium containing secreted IG10 scFv-D83 scFv bound specifically to inactivated H9N2 virus.

[0450] The binding activity of the HD8 scFv-CD83 scFv is tested by ELISA as described in Example 3.

Example 5—Vaccine Formulation Bispecific Antibodies

[0451] During vaccine formulation bispecific or multispecific antibodies are mixed with an inactivated virus (such as a commercial killed virus vaccine formulation). This will result in the formation of antibody conjugated with the inactivated virus. In this way the antigen of inactivated virus targets the APCs. This vaccine formulation enhances the efficacy of the inactivated vaccine by APCs without the need for any chemical conjugation.

Example 6—Immunization with H5HA Fused with CD83 scFv Protein Induces Faster and Higher Humoral Response

Construction of H5HA and H5HA-CD83scFv Expression Plasmids.

[0452] H5HA vaccine constructs (H5HA-Foldon-CD83scFv and H5HA-Foldon) were generated using the same method that describes the generation of H9HA expression cassettes H9HA-Foldon-CD83scFv (FIG. 1) and H9HA-Foldon (FIG. 2). The expression cassette (BIP-H5HA-Foldon-CD83scFv-Ctag, SEQ ID NO: 72) contained an ectodomain sequence (amino acid 17-527) of H5HA antigen of avian influenza H5N8 virus strain (A/duck/Egypt/SS19/2017, accession no. MH893738.1). The H5HA sequence was modified to change HA cleavage site from polybasic to monobasic, the hemagglutinin gene signal peptide was replaced by *Drosophila* BiP protein signal sequence and the TM domain, replaced with a 30 amino acid

trimerization foldon sequence of the trimeric protein fibrin from bacteriophage T4. Four amino acid “EPEA” sequence as tag (termed as EPEA tag or Ctag) was fused at the c-terminus of the expression cassette that serve for recombinant protein expression detection and purification. The second expression cassette (BIP-H5HA-Foldon-Ctag, SEQ ID NO: 73) lacks the CD83scFv sequence. Both expression cassettes were cloned into *Drosophila melanogaster* expression vector (pS2V1) using EcoR1 and SacII cloning sites. Expression and Purification of H5HA and H5HA Fused scFv Proteins

[0453] Recombinant proteins were produced and purified using the *Drosophila* Expression System (DES®, Life technologies). Briefly, pExpreS2-V1 plasmids containing expression cassettes (BIP-H5HA-Foldon-CD83scFv-Ctag or BIP-H5HA-Foldon-Ctag) were transfected into *Drosophila* S2 cells. Antibiotic selection was carried out for four weeks using Zeocine at a concentration of 1.5 mg/mL Zeocin. The Zeocine selected cells were cultured at 28° C. in serum free media (EX-CELL®, Merck). Recombinant proteins were secreted into culture supernatant and then purified using Ctag Affinity Matrix (CaptureSelect™, Thermo Fisher). Concentration of purified recombinant proteins was determined by Bradford assay and the purity was assessed by standard SDS-PAGE and Western Blot.

Chicken Vaccination

[0454] Groups (N=4 per groups) of one-day-old white leghorn SPF chickens were immunized with vaccine containing equimolar concentration of H5HA protein in H5HA-Foldon-CD83scFv and H5HA-Foldon vaccines. The proteins were formulated in Montanide™ ISA 71 VG (Seppic) adjuvant. The ratio of protein to adjuvant volume was 1:3. The vaccine dose (0.2 ml) was administered subcutaneous, delivered at the back of the neck. Blood samples were collected from the wing vein on 7, 14, 21 28, and 35 days of age and serum was analysed for the presence of H5HA specific antibodies using HI assays.

Results:

Production of H5HA-Foldon-CD83scFv and H5HA-Foldon Vaccines

[0455] The expression levels of recombinant H5HA-Foldon-CD83scFv and H5HA-Foldon proteins were in the range from 90-120 mg/litre of culture supernatants. The purity of proteins visualised using SDS-PAGE analysis that showed single band of monomeric proteins with molecular weight of 100 kDa of H5HA-Foldon-CD83scFv and 70 kDa of H5HA-Foldon. The purity of both proteins was estimated up to 99%.

Immunization with H5HA Foldon-CD83scFv Vaccine Induces Faster and Higher Humoral Response

[0456] One-day-old chicks were vaccinated with 0.2 mL per dose of vaccine containing equimolar concentration of purified H5HA-Foldon-CD83scFv (49 µg) and H5HA-Foldon (35 µg). Serum samples collected at 7, 14, 21 28, and 35 days of age were analysed using standard HI assay against the inactivated virus antigens (A/Duck/Egypt/SS19/2017). The data present in FIG. 22 show that the chickens vaccinated with H5HA-Foldon-CD83scFv contained markedly higher levels of HI titres compared with chickens vaccinated with H5HA-Foldon. Conclusion: The AIV

H5HA fused with CD83scFv antibody induced significantly faster and higher immune responses compared with the AIV H5HA that lacks CD83scFv antibody.

Example 7—Recombinant Herpesvirus of Turkey (rHVT) Expressing H9HA-Foldon-CD83scFv Proteins Induces Faster and Higher Humoral Response in Chickens

Results:

Generation of rHVT-H9HA-Foldon-CD83scFv and rHVT-H9HA-Foldon Using HDR CRISPR/Cas9 System.

[0457] The rHVT-H9HA-Foldon vaccine and rHVT-H9HA-Foldon-CD83scFv vaccine was generated using HDR-CRISPR/Cas9 is illustrated in FIG. 23. The expression cassette that produce H9HA-Foldon and H9HA-Foldon-CD83scFv proteins was integrated into the intergenic region of HVT genome between UL45/UL46 contained glycoprotein B (gB) promoter from pseudorabies virus (PRV) and polyA terminator of feline alpha herpesvirus 1. The HA protein sequence was derived from H9N2 virus strain A/chicken/Pakistan/SKP/2016 (Genbank accession number: AVX19091.1).

[0458] To rescue the rHVT, CEF cells were transfected with GFP gRNA plasmid and each of the donor plasmid containing expression cassettes (H9HA-Foldon vaccine and rHVT-H9HA-Foldon-CD83scFv) which were flanked by sequences homologous to the Cas9 cut sites. This was followed by infection with rHVT-GFP at a multiplicity of infection (MOI) of 0.01 at 12 hours post transfection. The rHVT virus plaques containing expression cassette of H9HA-Foldon or rHVT-H9HA-Foldon-CD83scFv were identified H9HA-Foldon vaccine and rHVT-H9HA-Foldon-CD83 scFv which were negative for green fluorescence. These GFP negative plaques are either correct rHVT-H9HA positive clones or false positive clones with silenced GFP. Viral DNA was extracted and subjected to PCR analysis using primers targeting the region within H9HA insert. In total, 11% and 22% of the clones were positive for H9HA-Foldon and H9HA-Foldon-CD83scFv insertions, respectively. One of the positive rHVT clone from each construct was taken forward for plaque purification, vaccine stock preparation, in vitro replication kinetics, insert stability and evaluation of immunogenicity in chickens.

The rHVT-H9HA-Foldon-CD83scFv and rHVT-H9HA-Foldon Show Similar in Vitro Replication Kinetics as that of the Wild-Type HVT.

[0459] The replication fitness of rHVT-H9HA-Foldon-CD83scfv was compared with the wild-type HVT to determine whether insertion of expression cassettes (H9HA-Foldon or H9HA-Foldon-CD83scFv) affected the infectivity and replication of rHVT constructs in cultured cells. For this, chicken embryo fibroblast (CEF) cells were infected with 100 pfu of either HVT wild-type, rHVT-H9HA or rHVT-H9HA-CD83scFv. Virus replication rates were measured by counting plaque (FIG. 24A) and qRT-PCR for genome copy numbers (FIG. 24B). The rate of virus replication measured by plaque assays or qRT-PCR showed no differences in virus replication fitness between the wild-type HVT and the rHVT-H9HA-Foldon and rHVT-H9HA-Foldon-CD83scFv vaccine constructs.

The rHVT-H9HA-Foldon-CD83scFv Induces Higher Antibody Responses Compared to rHVT-H9HA-Foldon in Vaccinated Chickens.

[0460] Groups of one-day-old White Leghorn SPF chickens (n=20 per group) were immunised with 4000 pfu of rHVT-H9HA-Foldon and rHVT-H9HA-Foldon-CD83scFv, subcutaneously. Blood samples were collected from the wing veins on day 6, 14, 21, 28 and 35 pv and serum samples were subjected to HI, anti-HA IgY ELISA and virus MNT assays to measure the HA antigen-specific antibody titres.

[0461] The chickens vaccinated with rHVT-H9HA-Foldon-CD83scFv vaccine showed detectable HI antibodies on day 21 pv whereas those group of chickens vaccinated with rHVT-H9HA-Foldon showed detectable levels of HI antibodies only from day 28 pv. (FIG. 25).

[0462] Comparison of HI antibody titres between rHVT-H9HA-Foldon-CD83scFv and rHVT-H9HA-Foldon demonstrated that the rHVT-H9HA-Foldon-CD83scFv was able to induce significantly higher HI antibody titres than the rHVT-H9HA-Foldon after day 21 pv (day 28 pv: p<0.05, day 35 pv: p<0.0001, day 42 pv: p<0.0001) (FIG. 25).

[0463] Analysis of IgY antibodies titres in serum samples collected from vaccinated chickens at different time points (day 6, 14, 21, 28 and 35 pv) also showed that rHVT-H9HA-Foldon-CD83scFv vaccine induced higher anti-H9HA IgY antibody titres compared to the rHVT-H9HA-Foldon vaccine (FIG. 26). The rHVT-H9HA-Foldon-CD83scFv group showed significantly higher anti-HA IgY antibodies compared to rHVT-H9HA-Foldon group on day 21 pv (p<0.05), day 28 pv (p<0.001) and day 35 pv (p<0.05).

[0464] The analysis of serum antibodies levels that specifically neutralise the H9N2 virus were determined using micro-neutralisation (MNT) assay. The serum samples collected on day 42 pv from chickens vaccinated with rHVT-H9HA-Foldon-CD83scFv showed significantly higher levels virus neutralising antibodies compared to rHVT-H9HA-Foldon (p<0.01) (FIG. 27).

Example 8—Generation of Recombinant Newcastle Disease Virus (rNDV) Expressing H9HA-Foldon-CD83scFv

[0465] A rNDV was generated carrying an expression cassette (H9HA-Foldon-CD83scFv) that encodes a soluble form of trimeric H9HA antigen fused with scFv antibody specific for chicken APCs receptor, CD83. The expression cassette (SEQ ID NO: 75) was chemically synthesised, and codon optimised for expression in chicken (*Gallus gallus*) cells (GenScript). The expression cassette was integrated in the NDV Intergenic region (between P/V and M genes) of LaSota strain (FIG. 30). The generated rNDV vaccine constructs produced secreted forms of H9HA-Foldon-CD83scFv antigens. Vaccination of 7-day-old chickens with the rNDV-H9HA-Foldon-CD83scFv vaccine elicited strong H9HA-antigen-specific HI antibody titres (FIG. 31). The results conclude that NDV can be used as a vector for the production and delivery of APCs targeting vaccines in chicken.

[0466] All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be under-

stood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the inven-

tion which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

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Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
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<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-CD11c antibody clone 8F2 heavy chain amino acid sequence

<400> SEQUENCE: 14

```
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1           5          10          15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20          25          30

Val Leu His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
35          40          45

Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys Phe Asn Glu Lys Phe
50          55          60

Lys Gly Lys Ala Thr Leu Thr Ser Asp Thr Ser Ser Ser Thr Ala Phe
65          70          75          80

Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85          90          95

Ala Arg Gly Asp Asn Leu Arg Pro Tyr Tyr Phe Asp Tyr Trp Gly Gln
100         105         110

Gly Thr Thr Leu Thr Val Ser Ser
115         120
```

<210> SEQ ID NO 15
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-CD11c antibody clone 8F2 heavy chain amino acid CDR-H1

<400> SEQUENCE: 15

```
Asn Tyr Val Leu His
1           5
```

<210> SEQ ID NO 16
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-CD11c antibody clone 8F2 heavy chain amino acid CDR-H2

<400> SEQUENCE: 16

```
Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys Phe Asn Glu Lys Phe Lys
1           5          10          15

Gly
```

<210> SEQ ID NO 17
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-CD11c antibody clone 8F2 heavy chain

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amino acid CDR-H3

<400> SEQUENCE: 17

Gly Asp Asn Leu Arg Pro Tyr Tyr Phe Asp Tyr
1 5 10

<210> SEQ ID NO 18
<211> LENGTH: 318
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc feature
<223> OTHER INFORMATION: anti-CD11c antibody clone 8F2 light chain nucleotide sequence

<400> SEQUENCE: 18

caaattgttc tcacccatc tccagcaatc atgtctgcat ctccaggaga gaaggtcacc	60
atgacctgca gtgccagctc aagtgttaat ttcatgtact ggtaccagca gaagccagga	120
tccctccccca gactcctgct ttatgacaca tccagcctgt cttctggagt ccctgttgc	180
ttcagtggca gtggctctgg gacctttac tctctcacaa tcagccgaat ggaggctgaa	240
gatgctgcca cttattactg ccagcagtgg agtcgttacc caccgacggtt cggtggaggc	300
accaagctgg aaatcaaa	318

<210> SEQ ID NO 19
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-CD11c antibody clone 8F2 heavy chain amino acid sequence

<400> SEQUENCE: 19

Gln Ile Val Leu Thr His Ser Pro Ala Ile Met Ser Ala Ser Pro Gly	
1 5 10 15	

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Phe Met	
20 25 30	

Tyr Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Arg Leu Leu Tyr	
35 40 45	

Asp Thr Ser Ser Leu Ser Ser Gly Val Pro Val Arg Phe Ser Gly Ser	
50 55 60	

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Met Glu Ala Glu	
65 70 75 80	

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Tyr Pro Pro Thr	
85 90 95	

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys	
100 105	

<210> SEQ ID NO 20
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:

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<221> NAME/KEY: MISC_FEATURE
 <223> OTHER INFORMATION: anti-CD11c antibody clone 8F2 heavy chain
 amino acid CDR-L1

<400> SEQUENCE: 20

Ser Ala Ser Ser Ser Val Ser Phe Met Tyr
 1 5 10

<210> SEQ ID NO 21
 <211> LENGTH: 7
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: synthetic peptide
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <223> OTHER INFORMATION: anti-CD11c antibody clone 8F2 heavy chain
 amino acid CDR-L2

<400> SEQUENCE: 21

Asp Thr Ser Ser Leu Ser Ser
 1 5

<210> SEQ ID NO 22
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: synthetic peptide
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <223> OTHER INFORMATION: anti-CD11c antibody clone 8F2 heavy chain
 amino acid CDR-L3

<400> SEQUENCE: 22

Gln Gln Trp Ser Arg Tyr Pro Pro Thr
 1 5

<210> SEQ ID NO 23
 <211> LENGTH: 357
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: synthetic construct
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <223> OTHER INFORMATION: anti-DEC205 antibody clone F887/AD6 heavy
 chain nucleotide sequence

<400> SEQUENCE: 23

gagggtgcaac tgggtggagtc tgggggagac tttagtgaagc ctggagggtc cctgaaactc	60
tcctgtgcag cctctggatt cactttcagt agctatggca tgtcttggt tcgccagact	120
ccagacaaga ggctggagtg ggtcgcaacc attagtagtg gtggtagtta cacctactat	180
ccagacagtg tgaaggggcg attcaccatt tccagagaca atgccaagaa catcctgtat	240
ctgcaaatga gcagtctgaa gtctgaagac acagccatgt attactgtgc aagactttca	300
acctgggact ggtacttcga tgtctgggc acagggacca cggtcacccgt ctcctca	357

<210> SEQ ID NO 24
 <211> LENGTH: 119
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: synthetic polypeptide

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<220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <223> OTHER INFORMATION: anti-DEC205 antibody clone F887/AD6 heavy chain amino acid sequence

<400> SEQUENCE: 24

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Asp	Leu	Val	Lys	Pro	Gly	Gly
1					5				10			15			

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Gly Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Trp Val
 35 40 45

Ala Thr Ile Ser Ser Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ile Leu Tyr
 65 70 75 80

Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95

Ala Arg Leu Ser Thr Trp Asp Trp Tyr Phe Asp Val Trp Gly Thr Gly
 100 105 110

Thr Thr Val Thr Val Ser Ser
 115

<210> SEQ ID NO 25
 <211> LENGTH: 5
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: synthetic peptide
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <223> OTHER INFORMATION: anti-DEC205 antibody clone F887/AD6 heavy chain amino acid CDR-H1

<400> SEQUENCE: 25

Ser	Tyr	Gly	Met	Ser
1			5	

<210> SEQ ID NO 26
 <211> LENGTH: 17
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: synthetic peptide
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <223> OTHER INFORMATION: anti-DEC205 antibody clone F887/AD6 heavy chain amino acid CDR-H2

<400> SEQUENCE: 26

Ser	Ser	Gly	Gly	Ser	Tyr	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys	Gly	Arg
1					5				10			15			

Phe

<210> SEQ ID NO 27
 <211> LENGTH: 10
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: synthetic peptide
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE

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<223> OTHER INFORMATION: anti-DEC205 antibody clone F887/AD6 heavy chain amino acid CDR-H3

<400> SEQUENCE: 27

Leu	Ser	Thr	Trp	Asp	Trp	Tyr	Phe	Asp	Val
1			5			10			

<210> SEQ ID NO 28

<211> LENGTH: 324

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: synthetic construct

<220> FEATURE:

<221> NAME/KEY: misc_feature

<223> OTHER INFORMATION: anti-DEC205 antibody clone F887/AD6 light chain nucleotide sequence

<400> SEQUENCE: 28

gaaatttgtc	tcacccagtc	tccagcactc	atggctgcat	ctccaggggga	gaaggtcacc	60
atcacctgca	gtgtcagctc	aagtataagt	tccggcaact	ttcaactggta	ccagcagaag	120
tcagggaaacct	cccccaaact	ctggatttat	ggcacatcca	acctggottc	tggagtccct	180
gttcgccttca	gtggcagtgg	atctgggacc	tcttattctc	tcacaatcag	cagcatggag	240
gctgaagatg	ctgccactta	ttactgtcaa	cagtggagta	gttaccatt	cacgttcggc	300
tcggggacaa	agttggaaat	aaaa				324

<210> SEQ ID NO 29

<211> LENGTH: 108

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: synthetic polypeptide

<220> FEATURE:

<221> NAME/KEY: MISC_FEATURE

<223> OTHER INFORMATION: anti-DEC205 antibody clone F887/AD6 light chain amino acid sequence

<400> SEQUENCE: 29

Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Leu	Met	Ala	Ala	Ser	Pro	Gly
1			5			10			15						

Glu	Lys	Val	Thr	Ile	Thr	Cys	Ser	Val	Ser	Ser	Ile	Ser	Ser	Gly
		20			25			30						

Asn	Phe	His	Trp	Tyr	Gln	Gln	Lys	Ser	Gly	Thr	Ser	Pro	Lys	Leu	Trp
			35		40			45							

Ile	Tyr	Gly	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Val	Arg	Phe	Ser
			50		55			60							

Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	Ser	Ser	Met	Glu
			65		70		75		80						

Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ser	Tyr	Pro
					85		90			95					

Phe	Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys
					100			105			

<210> SEQ ID NO 30

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: synthetic peptide

-continued

<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-DEC205 antibody clone F887/AD6 amino acid
CDR-L1

<400> SEQUENCE: 30

Ser Val Ser Ser Ser Ile Ser Ser Gly Asn Phe His
1 5 10

<210> SEQ ID NO 31
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-DEC205 antibody clone F887/AD6 amino acid
CDR-L2

<400> SEQUENCE: 31

Gly Thr Ser Asn Leu Ala Ser
1 5

<210> SEQ ID NO 32
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-DEC205 antibody clone F887/AD6 amino acid
CDR-L3

<400> SEQUENCE: 32

Gln Gln Trp Ser Ser Tyr Pro Phe Thr
1 5

<210> SEQ ID NO 33
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Cleavable linker

<400> SEQUENCE: 33

Leu Glu Ala Gly Cys Lys Asn Phe Phe Pro Arg Ser Phe Thr Ser Cys
1 5 10 15

Gly Ser Leu Glu
20

<210> SEQ ID NO 34
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 34

Gly Gly Ser Gly Gly Ser
1 5

-continued

<210> SEQ ID NO 35
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 35

Ser Gly Ser Gly Ser Gly Ser
1 5

<210> SEQ ID NO 36
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 36

Gly Gly Gly Gly Ser Gly Gly Gly Ser
1 5 10

<210> SEQ ID NO 37
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 37

Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser
1 5 10

<210> SEQ ID NO 38
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 38

Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Ser
1 5 10

<210> SEQ ID NO 39
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 39

Gly Gly Gly Ser Gly Gly Ser Gly Gly Gly Ser
1 5 10 15

<210> SEQ ID NO 40
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 40

Met Pro Leu Leu Leu Leu Pro Leu Leu Trp Ala Gly Ala Leu Ala

-continued

1	5	10	15
Met			
<210> SEQ_ID NO 41			
<211> LENGTH: 491			
<212> TYPE: PRT			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: synthetic polypeptide			
<220> FEATURE:			
<221> NAME/KEY: MISC_FEATURE			
<223> OTHER INFORMATION: H9HA ectodomain			
<400> SEQUENCE: 41			
Asp Lys Ile Cys Ile Gly His Gln Ser Thr Asn Ser Thr Glu Thr Val			
1 5 10 15			
Asp Thr Leu Thr Glu Thr Asn Val Pro Val Thr His Ala Lys Glu Leu			
20 25 30			
Leu His Thr Glu His Asn Gly Met Leu Cys Ala Thr Asn Leu Gly His			
35 40 45			
Pro Leu Ile Leu Asp Thr Cys Thr Ile Glu Gly Leu Ile Tyr Gly Asn			
50 55 60			
Pro Ser Cys Asp Leu Leu Gly Gly Arg Glu Trp Ser Tyr Ile Val			
65 70 75 80			
Glu Arg Pro Ser Ala Val Asn Gly Thr Cys Tyr Pro Gly Asn Val Glu			
85 90 95			
Asn Leu Glu Leu Arg Thr Leu Phe Ser Ser Ser Ser Tyr Gln			
100 105 110			
Arg Ile Gln Ile Phe Pro Asp Thr Ile Trp Asn Val Thr Tyr Thr Gly			
115 120 125			
Thr Ser Lys Ser Cys Ser Asp Ser Phe Tyr Arg Asn Met Arg Trp Leu			
130 135 140			
Thr Gln Lys Ser Gly Leu Tyr Pro Val Gln Asp Ala Gln Tyr Thr Asn			
145 150 155 160			
Asn Arg Gly Lys Asp Ile Leu Phe Val Trp Gly Ile His His Pro Pro			
165 170 175			
Thr Asp Thr Ala Gln Thr Asn Leu Tyr Thr Arg Thr Asp Thr Thr Thr			
180 185 190			
Ser Val Thr Thr Glu Asn Leu Asp Arg Thr Phe Lys Pro Val Ile Gly			
195 200 205			
Pro Arg Pro Leu Val Asn Gly Leu Ile Gly Arg Ile Asn Tyr Tyr Trp			
210 215 220			
Ser Val Leu Lys Pro Gly Gln Thr Leu Arg Val Arg Ser Asn Gly Asn			
225 230 235 240			
Leu Ile Ala Pro Trp Tyr Gly His Val Leu Ser Gly Glu Ser His Gly			
245 250 255			
Arg Ile Leu Lys Thr Asp Leu Asn Ser Gly Asn Cys Val Val Gln Cys			
260 265 270			
Gln Thr Glu Lys Gly Gly Leu Asn Ser Thr Leu Pro Phe His Asn Ile			
275 280 285			
Ser Lys Tyr Ala Phe Gly Asn Cys Pro Lys Tyr Ile Gly Val Lys Ser			
290 295 300			
Leu Lys Leu Ala Ile Gly Leu Arg Asn Val Pro Ala Arg Ser Ser Arg			
305 310 315 320			

-continued

Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Pro Gly
325 330 335

Leu Val Ala Gly Trp Tyr Gly Phe Gln His Ser Asn Asp Gln Gly Val
340 345 350

Gly Met Ala Ala Asp Arg Asp Ser Thr Gln Lys Ala Val Asp Lys Ile
355 360 365

Thr Ser Lys Val Asn Asn Ile Val Asp Lys Met Asn Lys Gln Tyr Glu
370 375 380

Ile Ile Asp His Glu Phe Ser Glu Val Glu Thr Arg Leu Asn Met Ile
385 390 395 400

Asn Asn Lys Ile Asp Asp Gln Ile Gln Asp Val Trp Ala Tyr Asn Ala
405 410 415

Glu Leu Leu Val Leu Leu Glu Asn Gln Lys Thr Leu Asp Glu His Asp
420 425 430

Ala Asn Val Asn Asn Leu Tyr Asn Lys Val Lys Arg Ala Leu Gly Ser
435 440 445

Asn Ala Met Glu Asp Gly Lys Gly Cys Phe Glu Leu Tyr His Lys Cys
450 455 460

Asp Asp Gln Cys Met Glu Thr Ile Arg Asn Gly Thr Tyr Asn Arg Arg
465 470 475 480

Lys Tyr Lys Glu Glu Ser Arg Leu Glu Arg Gln
485 490

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<210> SEQ ID NO 42
<211> LENGTH: 348
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/IG10
heavy chain nucleotide sequence
```

<400> SEQUENCE: 42

```
gagggttcagc tgcagcagtc tgtggcagag cttgtggcagg cagggggctc agtcaaggtg 60
tcctgcacag cttctggctt caacattaaa aacacctata tgcactgggt gaagcgagg 120
cctgaacagg gcctggagtg gattggaaagg attgatcctg cgaatggta tactaggat 180
gccccgaagt tccagggcaa ggccactata actgcagaca catcctccaa cacagcctac 240
ctgcagctca gcagcctgac atctgaggac actgccatct attactgtgc ccgttattac 300
ttcggtcctg actactgggg ccaaggcacc actctcacag tctcctca 348
```

```
<210> SEQ ID NO 43
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/IG10
heavy chain amino acid sequence
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<400> SEQUENCE: 43

Glu Val Gln Leu Gln Gln Ser Val Ala Glu Leu Val Arg Pro Gly Ala
1 5 10 15

-continued

Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asn Thr
20 25 30

Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Arg Tyr Ala Pro Lys Phe
50 55 60

Gln Gly Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
65 70 75 80

Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Arg Tyr Tyr Phe Gly Pro Asp Tyr Trp Gly Gln Gly Thr Thr Leu
100 105 110

Thr Val Ser Ser
115

<210> SEQ ID NO 44
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/IG10
heavy chain amino acid CDR-H1

<400> SEQUENCE: 44

Asn Thr Tyr Met His
1 5

<210> SEQ ID NO 45
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/IG10
heavy chain amino acid CDR-H2

<400> SEQUENCE: 45

Arg Ile Asp Pro Ala Asn Gly Asn Thr Arg Tyr Ala Pro Lys Phe Gln
1 5 10 15

Gly

<210> SEQ ID NO 46
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/IG10
heavy chain amino acid CDR-H3

<400> SEQUENCE: 46

Tyr Tyr Phe Gly Pro Asp Tyr
1 5

-continued

```

<210> SEQ ID NO 47
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/IG10
    light chain nucleotide sequence

```

```

<400> SEQUENCE: 47

gacatcctga tgacccaatc tccatcctcc atgtctgtat ctctggaga cacagtcatc      60
atcaacttgcc atgcaagtca gggcatttgc agtaataatag ggtgggttgc gcagaaacca      120
gggaaatcat ttaaggccct gatctatcat gcaaccaact tggaaagatgg agttccatca      180
aggttcagtg gcggtggatc tggagcagat tattctctca ccatcagcag cctggaatct      240
gaagattttgc cagactattt ctgtgtacag tatggtcagt ttccattcac gttcggctcg      300
gggacaaaatg tggaaataaa a                                         321

```

```

<210> SEQ ID NO 48
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/IG10
    light chain amino acid sequence

```

```

<400> SEQUENCE: 48

Asp Ile Leu Met Thr Gln Ser Pro Ser Ser Met Ser Val Ser Leu Gly
1           5          10          15
Asp Thr Val Ile Ile Thr Cys His Ala Ser Gln Gly Ile Ser Ser Asn
20          25          30
Ile Gly Trp Leu Gln Gln Lys Pro Gly Lys Ser Phe Lys Gly Leu Ile
35          40          45
Tyr His Ala Thr Asn Leu Glu Asp Gly Val Pro Ser Arg Phe Ser Gly
50          55          60
Gly Gly Ser Gly Ala Asp Tyr Ser Leu Thr Ile Ser Ser Leu Glu Ser
65          70          75          80
Glu Asp Phe Ala Asp Tyr Tyr Cys Val Gln Tyr Gly Gln Phe Pro Phe
85          90          95
Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100         105

```

```

<210> SEQ ID NO 49
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/IG10
    light chain amino acid CDR-L1

```

```

<400> SEQUENCE: 49

His Ala Ser Gln Gly Ile Ser Ser Asn Ile Gly
1           5          10

```

-continued

```

<210> SEQ ID NO 50
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/IG10
    light chain amino acid CDR-L2

<400> SEQUENCE: 50

```

```

His Ala Thr Asn Leu Glu Asp
1           5

```

```

<210> SEQ ID NO 51
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/IG10
    light chain amino acid CDR-L3

<400> SEQUENCE: 51

```

```

Val Gln Tyr Gly Gln Phe Pro Phe Thr
1           5

```

```

<210> SEQ ID NO 52
<211> LENGTH: 354
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/HD8
    heavy chain nucleotide sequence

<400> SEQUENCE: 52

```

```

gagggttcagc tgcagcagtc tgtggcagag cttgtgaggc cagggggctc agtcaagttg      60
tcctgcacag cttctggctt caacattaaa aacacctata tgcactgggt gaagcagagg      120
cctgaacagg gcctggagtg gatttggaaagg attgtatcctg cgaatggtaa tactagatat      180
gccccgaaat tccagggcaa ggccactata actgcagaca catcctccaa cacagcctac      240
ctgcagctca gcagcctgac atctgacgac actgcccattct attactgtgg taggacagag      300
ttcaggaatg ctatggacta ctggggtcaa ggaacctcag tcaccgtctc ctca      354

```

```

<210> SEQ ID NO 53
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/HD8
    heavy chain amino acid sequence

<400> SEQUENCE: 53

```

```

Glu Val Gln Leu Gln Gln Ser Val Ala Glu Leu Val Arg Pro Gly Ala

```

-continued

1	5	10	15												
Ser	Val	Lys	Leu	Ser	Cys	Thr	Ala	Ser	Gly	Phe	Asn	Ile	Lys	Asn	Thr
	20				25				30						

Tyr	Met	His	Trp	Val	Lys	Gln	Arg	Pro	Glu	Gln	Gly	Leu	Glu	Trp	Ile
	35				40				45						

Gly	Arg	Ile	Asp	Pro	Ala	Asn	Gly	Asn	Thr	Arg	Tyr	Ala	Pro	Lys	Phe
	50				55				60						

Gln	Gly	Lys	Ala	Thr	Ile	Thr	Ala	Asp	Thr	Ser	Ser	Asn	Thr	Ala	Tyr
	65				70			75				80			

Leu	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Asp	Asp	Thr	Ala	Ile	Tyr	Tyr	Cys
	85				90			95							

Gly	Arg	Thr	Glu	Phe	Arg	Asn	Ala	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr
	100				105				110						

Ser	Val	Thr	Val	Ser	Ser										
						115									

```

<210> SEQ ID NO 54
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/HD8
heavy chain amino acid CDR-H1

<400> SEQUENCE: 54

```

1	5			
---	---	--	--	--

```

<210> SEQ ID NO 55
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/HD8
heavy chain amino acid CDR-H2

<400> SEQUENCE: 55

```

1	5	10	15	
---	---	----	----	--

Gly

```

<210> SEQ ID NO 56
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/HD8
heavy chain amino acid CDR-H3

<400> SEQUENCE: 56

```

1	5			
---	---	--	--	--

-continued

```

<210> SEQ ID NO 57
<211> LENGTH: 321
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/HD8
light chain nucleotide sequence

```

```

<400> SEQUENCE: 57

gacatccaga tgactcagtc tccagcctcc ctatctccat ctgtgggaga aactgtcacc      60
atgacatgtc gagcaagtga gaatatttac agtaatttag catggtatca gcagaaacag      120
ggaaaaatctc ctcagctcct ggtctatgtc gcaacaaaact tagcagatgg tgtgccatca      180
aggttcagtg gcagttggatc aggccacacag ttttctctga agatcaacag cctgcagcct      240
gaagattttg ggaatttatta ctgtcaacat ttttataata ctccgtacac gttcggaggg      300
gggaccaagc tggaaataaa a                                         321

```

```

<210> SEQ ID NO 58
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/HD8
light chain amino acid sequence

```

```

<400> SEQUENCE: 58

Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Pro Ser Val Gly
1           5           10          15

Glu Thr Val Thr Met Thr Cys Arg Ala Ser Glu Asn Ile Tyr Ser Asn
20          25           30

Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Val
35          40           45

Tyr Ala Ala Thr Asn Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50          55           60

Ser Gly Ser Gly Thr Gln Phe Ser Leu Lys Ile Asn Ser Leu Gln Pro
65          70           75           80

Glu Asp Phe Gly Asn Tyr Tyr Cys Gln His Phe Tyr Asn Thr Pro Tyr
85          90           95

Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys
100         105

```

```

<210> SEQ ID NO 59
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/HD8
light chain amino acid CDR-L1

```

```
<400> SEQUENCE: 59
```

```
Arg Ala Ser Glu Asn Ile Tyr Ser Asn Leu Ala
```

-continued

1

5

10

```

<210> SEQ ID NO 60
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/HD8
light chain amino acid CDR-L2

<400> SEQUENCE: 60

```

Ala	Ala	Thr	Asn	Leu	Ala	Asp
1						5

```

<210> SEQ ID NO 61
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: anti-hemagglutinin antibody clone F955/HD8
light chain amino acid CDR-L3

<400> SEQUENCE: 61

```

Gln	His	Phe	Tyr	Asn	Thr	Pro	Tyr	Thr
1								5

```

<210> SEQ ID NO 62
<211> LENGTH: 811
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: BIP signal-H9HA ectodomain-Foldon-LINKER-CD83
scFv-V5-His tag

<400> SEQUENCE: 62

```

Met	Lys	Leu	Cys	Ile	Leu	Leu	Ala	Val	Val	Ala	Phe	Val	Gly	Leu	Ser
1														10	15

Leu	Gly	Asp	Lys	Ile	Cys	Ile	Gly	His	Gln	Ser	Thr	Asn	Ser	Thr	Glu	
														20	25	30

Thr	Val	Asp	Thr	Leu	Thr	Glu	Thr	Asn	Val	Pro	Val	Thr	His	Ala	Lys
													35	40	45

Glu	Leu	Leu	His	Thr	Glu	His	Asn	Gly	Met	Leu	Cys	Ala	Thr	Asn	Leu
													50	55	60

Gly	His	Pro	Leu	Ile	Leu	Asp	Thr	Cys	Thr	Ile	Glu	Gly	Leu	Ile	Tyr	
													65	70	75	80

Gly	Asn	Pro	Ser	Cys	Asp	Leu	Leu	Gly	Gly	Arg	Glu	Trp	Ser	Tyr	
													85	90	95

Ile	Val	Glu	Arg	Pro	Ser	Ala	Val	Asn	Gly	Thr	Cys	Tyr	Pro	Gly	Asn
													100	105	110

Val	Glu	Asn	Leu	Glu	Leu	Arg	Thr	Leu	Phe	Ser	Ser	Ser	Ser	Ser	
													115	120	125

Tyr	Gln	Arg	Ile	Gln	Ile	Phe	Pro	Asp	Thr	Ile	Trp	Asn	Val	Thr	Tyr
													130	135	140

-continued

Thr Gly Thr Ser Lys Ser Cys Ser Asp Ser Phe Tyr Arg Asn Met Arg
 145 150 155 160
 Trp Leu Thr Gln Lys Ser Gly Leu Tyr Pro Val Gln Asp Ala Gln Tyr
 165 170 175
 Thr Asn Asn Arg Gly Lys Asp Ile Leu Phe Val Trp Gly Ile His His
 180 185 190
 Pro Pro Thr Asp Thr Ala Gln Thr Asn Leu Tyr Thr Arg Thr Asp Thr
 195 200 205
 Thr Thr Ser Val Thr Thr Glu Asn Leu Asp Arg Thr Phe Lys Pro Val
 210 215 220
 Ile Gly Pro Arg Pro Leu Val Asn Gly Leu Ile Gly Arg Ile Asn Tyr
 225 230 235 240
 Tyr Trp Ser Val Leu Lys Pro Gly Gln Thr Leu Arg Val Arg Ser Asn
 245 250 255
 Gly Asn Leu Ile Ala Pro Trp Tyr Gly His Val Leu Ser Gly Glu Ser
 260 265 270
 His Gly Arg Ile Leu Lys Thr Asp Leu Asn Ser Gly Asn Cys Val Val
 275 280 285
 Gln Cys Gln Thr Glu Lys Gly Gly Leu Asn Ser Thr Leu Pro Phe His
 290 295 300
 Asn Ile Ser Lys Tyr Ala Phe Gly Asn Cys Pro Lys Tyr Ile Gly Val
 305 310 315 320
 Lys Ser Leu Lys Leu Ala Ile Gly Leu Arg Asn Val Pro Ala Arg Ser
 325 330 335
 Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Trp
 340 345 350
 Pro Gly Leu Val Ala Gly Trp Tyr Gly Phe Gln His Ser Asn Asp Gln
 355 360 365
 Gly Val Gly Met Ala Ala Asp Arg Asp Ser Thr Gln Lys Ala Val Asp
 370 375 380
 Lys Ile Thr Ser Lys Val Asn Asn Ile Val Asp Lys Met Asn Lys Gln
 385 390 395 400
 Tyr Glu Ile Ile Asp His Glu Phe Ser Glu Val Glu Thr Arg Leu Asn
 405 410 415
 Met Ile Asn Asn Lys Ile Asp Asp Gln Ile Gln Asp Val Trp Ala Tyr
 420 425 430
 Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Gln Lys Thr Leu Asp Glu
 435 440 445
 His Asp Ala Asn Val Asn Asn Leu Tyr Asn Lys Val Lys Arg Ala Leu
 450 455 460
 Gly Ser Asn Ala Met Glu Asp Gly Lys Gly Cys Phe Glu Leu Tyr His
 465 470 475 480
 Lys Cys Asp Asp Gln Cys Met Glu Thr Ile Arg Asn Gly Thr Tyr Asn
 485 490 495
 Arg Arg Lys Tyr Lys Glu Glu Ser Arg Leu Glu Arg Gln Gly Ser Gly
 500 505 510
 Tyr Ile Pro Glu Ala Pro Arg Asp Gly Gln Ala Tyr Val Arg Lys Asp
 515 520 525
 Gly Glu Trp Val Leu Leu Ser Thr Phe Leu Gly Ser Gly Ser Gly Asp
 530 535 540

-continued

Ile	Val	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ala	Val	Ser	Val	Gly	Gln
545				550					555						560
Lys Val Thr Met Ser Cys Thr Ser Ser Gln Val Leu Leu His Ser Pro															
565					570					575					
Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser															
580					585					590					
Pro Lys Leu Leu Val Tyr Phe Ala Ser Thr Arg Glu Ser Gly Val Pro															
595				600					605						
Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile															
610				615					620						
Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His															
625				630					635				640		
Tyr Ser Thr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys															
645				650					655						
Gly Gly Gly Ser Gly Gly Ser Gly Gly Gly Ser Gly															
660				665					670						
Gly Gly Gly Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val															
675				680					685						
Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr															
690				695					700						
Phe Thr Asp Tyr Tyr Ile Asn Trp Val Lys Gln Ser His Gly Lys Ser															
705				710					715				720		
Leu Glu Trp Ile Gly Asp Ile Asn Pro Thr Asn Gly Asp Ser Thr Tyr															
725				730					735						
Ser Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser															
740				745					750						
Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Val Ser Ala															
755				760					765						
Val Tyr Tyr Cys Ala Arg Asp Tyr Ala Met Asp Tyr Trp Gly Gln Gly															
770				775					780						
Thr Ser Val Thr Val Ser Ser Gly Lys Pro Ile Pro Asn Pro Leu Leu															
785				790					795				800		
Gly Leu Asp Ser Thr His His His His His His															
805				810											

```

<210> SEQ ID NO 63
<211> LENGTH: 809
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: BIP signal-H9HA ectodomain-Foldon-LINKER-CD11c
scFv-V5-His tag

```

<400> SEQUENCE: 63

Met	Lys	Leu	Cys	Ile	Leu	Leu	Ala	Val	Val	Ala	Phe	Val	Gly	Leu	Ser
1				5				10							15
Leu Gly Asp Lys Ile Cys Ile Gly His Gln Ser Thr Asn Ser Thr Glu															
20				25					30						
Thr Val Asp Thr Leu Thr Glu Thr Asn Val Pro Val Thr His Ala Lys															
35				40					45						
Glu Leu Leu His Thr Glu His Asn Gly Met Leu Cys Ala Thr Asn Leu															
50				55					60						

-continued

Gly His Pro Leu Ile Leu Asp Thr Cys Thr Ile Glu Gly Leu Ile Tyr
65 70 75 80

Gly Asn Pro Ser Cys Asp Leu Leu Leu Gly Gly Arg Glu Trp Ser Tyr
85 90 95

Ile Val Glu Arg Pro Ser Ala Val Asn Gly Thr Cys Tyr Pro Gly Asn
100 105 110

Val Glu Asn Leu Glu Glu Leu Arg Thr Leu Phe Ser Ser Ser Ser
115 120 125

Tyr Gln Arg Ile Gln Ile Phe Pro Asp Thr Ile Trp Asn Val Thr Tyr
130 135 140

Thr Gly Thr Ser Lys Ser Cys Ser Asp Ser Phe Tyr Arg Asn Met Arg
145 150 155 160

Trp Leu Thr Gln Lys Ser Gly Leu Tyr Pro Val Gln Asp Ala Gln Tyr
165 170 175

Thr Asn Asn Arg Gly Lys Asp Ile Leu Phe Val Trp Gly Ile His His
180 185 190

Pro Pro Thr Asp Thr Ala Gln Thr Asn Leu Tyr Thr Arg Thr Asp Thr
195 200 205

Thr Thr Ser Val Thr Thr Glu Asn Leu Asp Arg Thr Phe Lys Pro Val
210 215 220

Ile Gly Pro Arg Pro Leu Val Asn Gly Leu Ile Gly Arg Ile Asn Tyr
225 230 235 240

Tyr Trp Ser Val Leu Lys Pro Gly Gln Thr Leu Arg Val Arg Ser Asn
245 250 255

Gly Asn Leu Ile Ala Pro Trp Tyr Gly His Val Leu Ser Gly Glu Ser
260 265 270

His Gly Arg Ile Leu Lys Thr Asp Leu Asn Ser Gly Asn Cys Val Val
275 280 285

Gln Cys Gln Thr Glu Lys Gly Gly Leu Asn Ser Thr Leu Pro Phe His
290 295 300

Asn Ile Ser Lys Tyr Ala Phe Gly Asn Cys Pro Lys Tyr Ile Gly Val
305 310 315 320

Lys Ser Leu Lys Leu Ala Ile Gly Leu Arg Asn Val Pro Ala Arg Ser
325 330 335

Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Trp
340 345 350

Pro Gly Leu Val Ala Gly Trp Tyr Gly Phe Gln His Ser Asn Asp Gln
355 360 365

Gly Val Gly Met Ala Ala Asp Arg Asp Ser Thr Gln Lys Ala Val Asp
370 375 380

Lys Ile Thr Ser Lys Val Asn Asn Ile Val Asp Lys Met Asn Lys Gln
385 390 395 400

Tyr Glu Ile Ile Asp His Glu Phe Ser Glu Val Glu Thr Arg Leu Asn
405 410 415

Met Ile Asn Asn Lys Ile Asp Asp Gln Ile Gln Asp Val Trp Ala Tyr
420 425 430

Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Gln Lys Thr Leu Asp Glu
435 440 445

His Asp Ala Asn Val Asn Asn Leu Tyr Asn Lys Val Lys Arg Ala Leu
450 455 460

-continued

Gly	Ser	Asn	Ala	Met	Glu	Asp	Gly	Lys	Gly	Cys	Phe	Glu	Leu	Tyr	His
465				470			475					480			
Lys	Cys	Asp	Asp	Gln	Cys	Met	Glu	Thr	Ile	Arg	Asn	Gly	Thr	Tyr	Asn
485				490				495							
Arg	Arg	Lys	Tyr	Lys	Glu	Glu	Ser	Arg	Leu	Glu	Arg	Gln	Gly	Ser	Gly
500				505				510							
Tyr	Ile	Pro	Glu	Ala	Pro	Arg	Asp	Gly	Gln	Ala	Tyr	Val	Arg	Lys	Asp
515				520			525								
Gly	Glu	Trp	Val	Leu	Leu	Ser	Thr	Phe	Leu	Gly	Ser	Gly	Ser	Gly	Glu
530				535			540								
Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly	Ala	Ser
545				550			555			560					
Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Asn	Tyr	Val
565				570			575								
Leu	His	Trp	Val	Lys	Gln	Lys	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	Gly
580				585			590								
Tyr	Ile	Asn	Pro	Tyr	Asn	Asp	Gly	Thr	Lys	Phe	Asn	Glu	Lys	Phe	Lys
595				600			605								
Gly	Lys	Ala	Thr	Leu	Thr	Ser	Asp	Thr	Ser	Ser	Thr	Ala	Phe	Met	
610				615			620								
Glu	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Ala
625				630			635			640					
Arg	Gly	Asp	Asn	Leu	Arg	Pro	Tyr	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	Gly
645				650			655								
Thr	Thr	Leu	Thr	Val	Ser	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly		
660				665			670								
Ser	Gly	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Ser	Gln	Ile	Val	Leu	Thr	
675				680			685								
His	Ser	Pro	Ala	Ile	Met	Ser	Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Met
690				695			700								
Thr	Cys	Ser	Ala	Ser	Ser	Val	Ser	Phe	Met	Tyr	Trp	Tyr	Gln	Gln	
705				710			715			720					
Lys	Pro	Gly	Ser	Ser	Pro	Arg	Leu	Leu	Leu	Tyr	Asp	Thr	Ser	Ser	Leu
725				730			735								
Ser	Ser	Gly	Val	Pro	Val	Arg	Phe	Ser	Gly	Ser	Gly	Thr	Ser	Ser	
740				745			750								
Tyr	Ser	Leu	Thr	Ile	Ser	Arg	Met	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr
755				760			765								
Tyr	Cys	Gln	Gln	Trp	Ser	Arg	Tyr	Pro	Pro	Thr	Phe	Gly	Gly	Thr	
770				775			780								
Lys	Leu	Glu	Ile	Lys	Gly	Lys	Pro	Ile	Pro	Asn	Pro	Leu	Leu	Gly	Leu
785				790			795			800					
Asp	Ser	Thr	His												
				805											

```

<210> SEQ ID NO 64
<211> LENGTH: 810
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: BIP signal-H9HA ectodomain-Foldon-LINKER-Dec205

```

-continued

scFv-V5-His tag

<400> SEQUENCE: 64

```

Met Lys Leu Cys Ile Leu Leu Ala Val Val Ala Phe Val Gly Leu Ser
1           5          10          15

Leu Gly Asp Lys Ile Cys Ile Gly His Gln Ser Thr Asn Ser Thr Glu
20          25          30

Thr Val Asp Thr Leu Thr Glu Thr Asn Val Pro Val Thr His Ala Lys
35          40          45

Glu Leu Leu His Thr Glu His Asn Gly Met Leu Cys Ala Thr Asn Leu
50          55          60

Gly His Pro Leu Ile Leu Asp Thr Cys Thr Ile Glu Gly Leu Ile Tyr
65          70          75          80

Gly Asn Pro Ser Cys Asp Leu Leu Leu Gly Gly Arg Glu Trp Ser Tyr
85          90          95

Ile Val Glu Arg Pro Ser Ala Val Asn Gly Thr Cys Tyr Pro Gly Asn
100         105         110

Val Glu Asn Leu Glu Glu Leu Arg Thr Leu Phe Ser Ser Ser Ser
115         120         125

Tyr Gln Arg Ile Gln Ile Phe Pro Asp Thr Ile Trp Asn Val Thr Tyr
130         135         140

Thr Gly Thr Ser Lys Ser Cys Ser Asp Ser Phe Tyr Arg Asn Met Arg
145         150         155         160

Trp Leu Thr Gln Lys Ser Gly Leu Tyr Pro Val Gln Asp Ala Gln Tyr
165         170         175

Thr Asn Asn Arg Gly Lys Asp Ile Leu Phe Val Trp Gly Ile His His
180         185         190

Pro Pro Thr Asp Thr Ala Gln Thr Asn Leu Tyr Thr Arg Thr Asp Thr
195         200         205

Thr Thr Ser Val Thr Thr Glu Asn Leu Asp Arg Thr Phe Lys Pro Val
210         215         220

Ile Gly Pro Arg Pro Leu Val Asn Gly Leu Ile Gly Arg Ile Asn Tyr
225         230         235         240

Tyr Trp Ser Val Leu Lys Pro Gly Gln Thr Leu Arg Val Arg Ser Asn
245         250         255

Gly Asn Leu Ile Ala Pro Trp Tyr Gly His Val Leu Ser Gly Glu Ser
260         265         270

His Gly Arg Ile Leu Lys Thr Asp Leu Asn Ser Gly Asn Cys Val Val
275         280         285

Gln Cys Gln Thr Glu Lys Gly Gly Leu Asn Ser Thr Leu Pro Phe His
290         295         300

Asn Ile Ser Lys Tyr Ala Phe Gly Asn Cys Pro Lys Tyr Ile Gly Val
305         310         315         320

Lys Ser Leu Lys Leu Ala Ile Gly Leu Arg Asn Val Pro Ala Arg Ser
325         330         335

Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Trp
340         345         350

Pro Gly Leu Val Ala Gly Trp Tyr Gly Phe Gln His Ser Asn Asp Gln
355         360         365

Gly Val Gly Met Ala Ala Asp Arg Asp Ser Thr Gln Lys Ala Val Asp
370         375         380

```

-continued

Lys	Ile	Thr	Ser	Lys	Val	Asn	Asn	Ile	Val	Asp	Lys	Met	Asn	Lys	Gln
385				390				395				400			
Tyr	Glu	Ile	Ile	Asp	His	Glu	Phe	Ser	Glu	Val	Glu	Thr	Arg	Leu	Asn
	405					410						415			
Met	Ile	Asn	Asn	Lys	Ile	Asp	Asp	Gln	Ile	Gln	Asp	Val	Trp	Ala	Tyr
	420					425						430			
Asn	Ala	Glu	Leu	Leu	Val	Leu	Leu	Glu	Asn	Gln	Lys	Thr	Leu	Asp	Glu
	435					440						445			
His	Asp	Ala	Asn	Val	Asn	Leu	Tyr	Asn	Lys	Val	Lys	Arg	Ala	Leu	
	450					455						460			
Gly	Ser	Asn	Ala	Met	Glu	Asp	Gly	Lys	Gly	Cys	Phe	Glu	Leu	Tyr	His
	465					470						475			480
Lys	Cys	Asp	Asp	Gln	Cys	Met	Glu	Thr	Ile	Arg	Asn	Gly	Thr	Tyr	Asn
	485					490						495			
Arg	Arg	Lys	Tyr	Lys	Glu	Glu	Ser	Arg	Leu	Glu	Arg	Gln	Gly	Ser	Gly
	500					505						510			
Tyr	Ile	Pro	Glu	Ala	Pro	Arg	Asp	Gly	Gln	Ala	Tyr	Val	Arg	Lys	Asp
	515					520						525			
Gly	Glu	Trp	Val	Leu	Leu	Ser	Thr	Phe	Leu	Gly	Ser	Gly	Ser	Gly	Glu
	530					535						540			
Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Leu	Met	Ala	Ala	Ser	Pro	Gly	Glu
	545					550						555			560
Lys	Val	Thr	Ile	Thr	Cys	Ser	Val	Ser	Ser	Ile	Ser	Ser	Gly	Asn	
	565					570						575			
Phe	His	Trp	Tyr	Gln	Gln	Lys	Ser	Gly	Thr	Ser	Pro	Lys	Leu	Trp	Ile
	580					585						590			
Tyr	Gly	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Val	Arg	Phe	Ser	Gly
	595					600						605			
Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	Ser	Ser	Met	Glu	Ala
	610					615						620			
Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ser	Tyr	Pro	Phe
	625					630						635			640
Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Gly	Gly	Gly	Ser	
	645					650						655			
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Gly	Ser	Glu	
	660					665						670			
Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Asp	Leu	Val	Lys	Pro	Gly	Gly	Ser
	675					680						685			
Leu	Lys	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr	Gly
	690					695						700			
Met	Ser	Trp	Val	Arg	Gln	Thr	Pro	Asp	Lys	Arg	Leu	Glu	Trp	Val	Ala
	705					710						715			720
Thr	Ile	Ser	Ser	Gly	Gly	Ser	Tyr	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys
	725					730						735			
Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Ile	Leu	Tyr	Leu
	740					745						750			
Gln	Met	Ser	Ser	Leu	Lys	Ser	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	Cys	Ala
	755					760						765			
Arg	Leu	Ser	Thr	Trp	Asp	Trp	Tyr	Phe	Asp	Val	Trp	Gly	Thr	Gly	Thr
	770					775						780			
Thr	Val	Thr	Val	Ser	Ser	Gly	Lys	Pro	Ile	Pro	Asn	Pro	Leu	Gly	

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785 790 795 800

Leu Asp Ser Thr His His His His His His
805 810

<210> SEQ ID NO 65
<211> LENGTH: 2444
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct

<400> SEQUENCE: 65

atgaaggattt gcataattact ggccgtcgtg gcctttgttgc gcctctcgct cgggataaga
tctgcacatgg ccaccagagc accaacacgca ccgagaccgt ggataccctg accgagacca
acgtggccagt gaccccacgccc aaggagctgc tgccacacccga gcacaacggaa atgctgtcg
ccaccaatct gggccaccccc ctgatccctgg atacctgcac catcgagggc ctgatctacg
gcaaccccccag ctgcgtatctg ctgctggag gaeccgagtg gtcctacatt gtggagcgcc
ccagcgcgcgt gaacggaaacc tgctatccag gcaacgtgga gAACCTGGAG gagctgcgc
ccctgttcag cagctcgagc agctaccaggc gcatccagat cttcccccgt accatctgg
acgtgaccta cacccggcacc agcaagagct gcagcgatag cttctaccgc aacatgcgc
ggctgaccca gaagttccggc ctgtaccctgg tgcaggatgc ccagtagatcc aacaatcg
gcaaggacat cctgttcgtg tggggcatcc accacccccc aaccgatacc gcccagacca
atctgtacac cccgaccatgat accaccacca gcgtgaccac cgagaatctg gatcgac
tcaagcccgat gatcgccca cggccactcg tgaatggact gatcgccgc atcaactact
atggagcgt gctgaagccc ggccagaccc tgegcgtgatc cagcaatggaa aatctgtac
ccccgttgta cggccacgtg ctgagccggag agagccacgg cccgattctg aagaccc
tgaacagcgcc caactgcgtg gtgcgtgc agaccgagaa gggccgcctg aatagcaccc
tgccttcac caacatctcg aagtacgcct tcggaaactg ccccaagtac atcgccgt
agtcctgaa gtcggccatc ggccgtcgca atgtgcgcac ccgcacgtatc cgccgactgt
tcggagccat tgcggcttc attgaggcg gctggccagg actggggcc ggtatggtac
gattccagca cagcaacgtatc cggccgtgg gaatggccgc cagatcgatc agtaccc
aggccgtgaa taagatcacc tccaaagtga acaacatctg ggacaagatg aacaagc
acgagatcat cggccacgtt ttcagccggag tggagaccccg cctgaacatg atcaacaaca
agatcgacca ccaagatccag gatgtgtggg cctacaacgc cggactgtc gtgcgt
agaaccagaaa gaccctggac gagcacgtatc ccaacgtgaa caatctgtat aacaaagt
agcgcgcgcctt gggcagcaac gccatggagg atggaaagggg atgcttcgag ctgtacc
agtcgcacga tcagtgcacatc gagaccatcc gcaacggcac ctacaacccgc cggca
aggaggagag cccgctggag cggccaggc gccgctacat cccagaggcc ccac
gacaggccata tgcgtcgac gatggcgatc ggggtgtctg gagcacccctt ctgg
gctctgggtga gatcgatctg acacagaccc cggccatgtat ggtgtctgc ccagg
aagtggccat tacctgcacgc tgcgtccacca gcatcagcgg cggcaacttc
agcagaagtc cggccacccgt ccgaagctgt ggtatctacgg aacaagcaat ctgg
gagtgccatc ggcgttttagt ggaagtggaa gcccggccaccc ctacagccctg accat
aactgtatc

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gcatggaagc cgaggatgcc gccacctact attgccagca gtggtegagc tacccctca	1920
ccttcggcag tggcaccaag ctggaaatca aaggcgagg cggaaagtggt ggccggaggtt	1980
caggtggtgg tggatcaggc ggagggtggta gtgaagtgca gctgggtgaa agcggcgag	2040
acctggtaa gccaggcgga agcctgaago tgagttgegc tgccagcgga ttcaectca	2100
gctcctacgg catgagctgg gtccgacaga caccgataa gcgcttggag tgggttgc当地	2160
ccatttagcag cggaggcagc tacacgtact accccgatag tgtgaaggga cgcttcacca	2220
tcaagccgcga taacgccaag aacatcctgt acctgcagat gagcagcctg aagtccgagg	2280
acaccgcccgt gtattactgc gccccgtctga gcacctggaa ttggacttc gatgtgtggg	2340
gcaccggAAC caccgtgaca gtttagtagtg gttctggctc tgggtgtaag cctatcccta	2400
accctctccgt cggtctcgat tctacgcata atcaccatca ccat	2444

<210> SEQ ID NO 66
<211> LENGTH: 2432
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct

<400> SEQUENCE: 66	
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tctgcacatcgcc accaacagca ccgagaccgt ggataccctg accgagacca	120
acgtgccagt gacccacgccc aaggagctgc tgcacaccga gcacaacgga atgctgtgc当地	180
ccaccaatctt gggccaaaaaccctt ctgatcctgg atacctgcac catcgaggcc ctgatctacg	240
gcaaccccaag ctgcgatctg ctgctggag gacgcgagtg gtcctacattt gtggagcgcc	300
ccagcgcgtt gaacggaaacc ttgatccag gcaacgtggaa gaacctggag gagctgc当地	360
ccctgttccat cagctcgagc agctaccaggc gcatccagat ttcccccgtt accatcttga	420
acgtgaccta caccggcacc agcaagagct gcagcgatag ttcttaccgc aacatgc当地	480
ggctgaccca gaagtccggc ctgtatccat tgcaggatgc ccagtacacc aacaatcgcc	540
gcaaggacat cctgttcgtt tggtttccatcc accacccccc aaccgtatcc gcccagacca	600
atctgtacac ccgcacccat accaccatca gctgtaccac cgagaatctg gatcgacat	660
tcaagcccgat gatcgccccca cggccactcg tgaatggact gatcgccgc atcaactact	720
attggagcgat gctgaagcccc ggccagaccc tgcgcgtgc当地 cagcaatggaa aatctgtatcg	780
ccccgtggta cggccacgtt ctgagcgagg agagccaccc cccgattctg aagaccgtt	840
tgaacagcgg caactgcgtt gtgcagtgc当地 agaccgagaa gggcgccctt aatagcaccc	900
tgccttccaa caacatctcg aagtacgcct tccggaaactt ccccaagtac atcggcgtt当地	960
agtccctgaa gctggccatc ggcctgc当地 atgtgccagc ccgcgttgc当地 cgccggactgt	1020
tccggccatc tgcggccatc attggggcg gctggccagg actgggtggcc ggtatggatcg	1080
gattccagca cagcaacgtt caggccgtgg gaatggccgc cgatcgatc agtaccaga	1140
aggccgtggta taagatcacc tccaaagtta acaacatcgat ggacaagatg aacaagc当地	1200
acgagatcat cggccacgtt ttcagcgagg tggagaccccg cctgaatcg atcaacaaca	1260
agatcgacga ccagatccag gatgtgtggg cctacaacgc cgagctgc当地 gtgc当地	1320
agaaccagaa gaccctggac gggccacgtt ccaacgtggaa caatctgtat aacaaagtta	1380

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agcgcgcctt	gggcagcaac	gccatggagg	atggaaaggg	atgcttcgag	ctgtaccaca	1440
agtgcgcacga	tcatgtcatg	gagaccatecc	gcaacggcac	ctacaaccgc	cgcaagtaca	1500
aggaggagag	ccgcctggag	cgcaggggca	gcggctacat	cccagaggcc	ccacgcgacg	1560
gacaggecta	tgtgcgcagaag	gatggcgagt	gggtgctgct	gagcaccttc	ctgggttctg	1620
gctctggta	tatctgtatg	acccagtcgc	caagcagtct	ggctgtgtcc	gtgggacaga	1680
aagtgcacat	gagctgcacc	agcagccagg	tgctgctgca	cagccccaaac	cagaagaatt	1740
acctggctg	gtatcagcag	aagcccgccc	aaagtccaa	gctgctggtc	tactttgcca	1800
gcacacgcga	gagcggagtg	ccagatcgtt	ttaccggaa	cggcagcggc	accgattca	1860
ccctgacaat	tagtagcgtg	caggccgagg	atctggccgt	gtattactgc	cagcagcact	1920
acagcacccc	gctgacattt	ggcgcggaa	cgaagctgaa	actgaaaggc	ggaggtgta	1980
gtgggtggcg	aggatcaggt	gggtgggttt	ctggcggtgg	tggaagtgaa	gtgcaactgc	2040
agcagagcgg	cccagagctg	gtcaaaccag	gtgccagcgt	gaagatcagc	tgcaaggcca	2100
gcggatacac	cttcaccgat	tactacatca	actgggtcaa	gcagagccac	ggcaagagcc	2160
tggaatggat	cgcgatatac	aacccacca	acggcgatag	cacctacagc	cagaagttca	2220
agggcaaaagc	cacgctgacc	gtggataaga	gtacgagcac	cgcctacatg	gaactgcgcga	2280
gcctgacaag	cgaagtgtcc	gccgtgtact	attgcgcccc	tgattacgcc	atggattact	2340
ggggacaggg	caccagtgt	accgttagta	gtggtaagcc	tatccctaacc	cctctccctcg	2400
gtctcgattc	tacgcatcat	caccatcacc	at			2432

<210> SEQ ID NO 67
<211> LENGTH: 2426
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct

<400> SEQUENCE: 67

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tctgcacatgg	ccaccagagc	accaacagca	ccgagaccgt	ggataccctg	accgagacca	120
acgtgcctgt	gaccacgc	aaggagctgc	tgcacacccg	gcacaacgg	atgctgtgc	180
ccaccaatct	gggcacccc	ctgatcctgg	atacctgcac	catcgaggcc	ctgatctacg	240
gcaacccca	ctgcgtatctg	ctgctggag	gacgcgagtg	gtcctacatt	gtggagcgcc	300
ccagcgcgt	gaacggaaacc	tgctatccag	gcaacgtgaa	gaacctggag	gagctgcgc	360
ccctgttcag	cagctcgagc	agctaccagc	gcatccagat	cttcccgat	accatctgga	420
acgtgaccta	cacccggcacc	agcaagagct	gcagcgatag	cttctaccgc	aacatgcgc	480
ggctgaccca	gaagtccggc	ctgtacccag	tgcaggatgc	ccagtcacacc	aacaatcg	540
gcaaggacat	cctgttcgt	tggggatcc	accacccccc	aaccgtatcc	gcccagacca	600
atctgtacac	ccgcaccgat	accaccacca	gcgtgaccac	cgagaatctg	gatcgacac	660
tcaagccgt	gatcgcccc	cgcccactcg	tgaatggact	gatcgccgc	atcaactact	720
atggagcg	gctgaagccc	ggccagaccc	tgcgcgtgc	cagcaatgg	aatctgatcg	780
ccccgtggta	cggccacgt	ctgagcggag	agagccacgg	ccgcattctg	aagaccgatc	840
tgaacagcgg	caactgcgt	gtgcagtgc	agaccgagaa	ggcgccctg	aatagcaccc	900

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tgccttcca	caacatctcg	aagtacgcct	tcggaaactg	ccccaaagtac	atcggcgtga	960
agtcacctgaa	gctggccatc	ggcctgcgca	atgtgccagg	ccgcagtagt	cgccggactgt	1020
tcggagccat	tgccggcttc	attgagggcg	gctggccagg	actggtgcc	ggatggtagc	1080
gattccagca	cagcaacat	cagggcgtgg	aatggccgc	cgatcgcgt	agtaccaga	1140
aggccgtgga	taagatcacc	tccaaagtga	acaacatcg	ggacaagatg	aacaagcagt	1200
acgagatcat	cgaccacgag	ttcagcggagg	tggagacccc	cctgaacatg	atcaacaaca	1260
agatcgcacg	ccagatccag	gatgtgtggg	cctacaacgc	cgagctgctg	gtgctgctgg	1320
agaaccagaa	gaccctggac	gagcacgtat	ccaacgtgaa	caatctgtat	aacaaagtga	1380
agcgcgcct	gggcagcaac	gccatggagg	atggaaagggg	atgcttcgag	ctgtaccaca	1440
agtgcgcacg	tcaagtgcatg	gagaccatcc	gcaacggcac	ctacaaccgc	cgcaagtaca	1500
aggaggagag	ccgcctggag	cgccaggcga	gcccgtacat	cccagaggcc	ccacgcgacg	1560
gacaggccta	tgtgcgcaag	gatggcgagt	gggtgctgt	gagcaccttc	ctgggttctg	1620
gctctggta	agtgcactg	caacaaagcg	gcccagagct	ggttaagcca	ggtgccagtg	1680
tgaagatgag	ctgcaaggcc	agcggctaca	ccttcaccaa	ctacgtgctg	cactgggtca	1740
agcagaagcc	cgcccaaggc	ctggaatgga	tcggctacat	caacccctac	aacgatggca	1800
c当地aaatcaa	cgagaagttc	aaggcCAAAG	ccacgctgac	cagcgatacc	agttagcagca	1860
ccgccttcat	ggaactgagc	agcctgacct	ccgaagatag	cgccgtgtac	tattgcgccc	1920
gtggcgataa	tctgcgcccc	tactacttcg	attactgggg	ccagggaaacg	accctgacag	1980
ttatgttcagg	tggcgagggt	agcggagggt	gtggatcagg	tgggtgggta	agtgggtggc	2040
gtggatccca	gattgtgctg	acacacagcc	ccgcatcat	gagtgttagc	ccaggcgaga	2100
aagtgaccat	gacatgcagt	gccagcagca	gcgtgtcctt	catgttatgg	tatcagcaaa	2160
agccggcag	cagccgcgt	ctgctgctgt	atgatacaag	ctccctgagc	agcggagtc	2220
ccgtgcgtt	tagtggaaac	ggatccggaa	ccagctactc	cctgaccatc	agtcgcatgg	2280
aagccgaaga	tgccgcccac	tactactgca	agcagtggtc	ccgttatccg	ccaacattcg	2340
gcggaggcac	caagctggaa	atcaaggta	agcttatccc	taacccttc	ctcggtctcg	2400
attctacgca	tcatcaccat	caccat				2426

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<210> SEQ ID NO 68
<211> LENGTH: 522
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic polypeptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: CD33 SIGNAL-IG10 scFv-(Glycine4Serine)4
linker-CD83 scFv-Ctag

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<400> SEQUENCE: 68

Met	Pro	Leu	Leu	Leu	Leu	Pro	Leu	Leu	Trp	Ala	Gly	Ala	Leu	Ala
1														

1 5 10 15

Met	Asp	Ile	Leu	Met	Thr	Gln	Ser	Pro	Ser	Ser	Met	Ser	Val	Ser	Leu
20															

20 25 30

Gly	Asp	Thr	Val	Ile	Ile	Thr	Cys	His	Ala	Ser	Gln	Gly	Ile	Ser	Ser
35															

35 40 45

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Asn	Ile	Gly	Trp	Leu	Gln	Gln	Lys	Pro	Gly	Lys	Ser	Phe	Lys	Gly	Leu
50				55			60								
Ile	Tyr	His	Ala	Thr	Asn	Leu	Glu	Asp	Gly	Val	Pro	Ser	Arg	Phe	Ser
65				70			75			80					
Gly	Gly	Gly	Ser	Gly	Ala	Asp	Tyr	Ser	Leu	Thr	Ile	Ser	Ser	Leu	Glu
			85			90			95						
Ser	Glu	Asp	Phe	Ala	Asp	Tyr	Tyr	Cys	Val	Gln	Tyr	Gly	Gln	Phe	Pro
	100				105				110						
Phe	Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Gly	Gly	Gly	
115				120			125								
Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Glu	Val	Gln	Leu	Gln		
	130			135			140								
Gln	Ser	Val	Ala	Glu	Leu	Val	Arg	Pro	Gly	Ala	Ser	Val	Lys	Leu	Ser
145				150			155			160					
Cys	Thr	Ala	Ser	Gly	Phe	Asn	Ile	Lys	Asn	Thr	Tyr	Met	His	Trp	Val
	165				170			175							
Lys	Gln	Arg	Pro	Glu	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Arg	Ile	Asp	Pro
	180				185			190							
Ala	Asn	Gly	Asn	Thr	Arg	Tyr	Ala	Pro	Lys	Phe	Gln	Gly	Lys	Ala	Thr
	195				200			205							
Ile	Thr	Ala	Asp	Thr	Ser	Ser	Asn	Thr	Ala	Tyr	Leu	Gln	Leu	Ser	Ser
210				215			220								
Leu	Thr	Ser	Glu	Asp	Thr	Ala	Ile	Tyr	Tyr	Cys	Ala	Arg	Tyr	Tyr	Phe
225				230			235			240					
Gly	Pro	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Thr	Val	Ser	Ser	Gly	
	245				250			255							
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly		
	260				265			270							
Gly	Gly	Ser	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ala	Val
	275				280			285							
Ser	Val	Gly	Gln	Lys	Val	Thr	Met	Ser	Cys	Thr	Ser	Ser	Gln	Val	Leu
	290				295			300							
Leu	His	Ser	Pro	Asn	Gln	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys
305				310			315			320					
Pro	Gly	Gln	Ser	Pro	Lys	Leu	Leu	Val	Tyr	Phe	Ala	Ser	Thr	Arg	Glu
	325				330			335							
Ser	Gly	Val	Pro	Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe
	340				345			350							
Thr	Leu	Thr	Ile	Ser	Ser	Val	Gln	Ala	Glu	Asp	Leu	Ala	Val	Tyr	Tyr
	355				360			365							
Cys	Gln	Gln	His	Tyr	Ser	Thr	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys
	370				375			380							
Leu	Glu	Leu	Lys	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly		
	385				390			395			400				
Gly	Gly	Ser	Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys
	405				410			415							
Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe
	420				425			430							
Thr	Asp	Tyr	Tyr	Ile	Asn	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Ser	Leu
	435				440			445							
Glu	Trp	Ile	Gly	Asp	Ile	Asn	Pro	Thr	Asn	Gly	Asp	Ser	Thr	Tyr	Ser

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450	455	460
Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser		
465	470	475
		480
Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Val Ser Ala Val		
485	490	495
Tyr Tyr Cys Ala Arg Asp Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr		
500	505	510
Ser Val Thr Val Ser Ser Glu Pro Glu Ala		
515	520	

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<210> SEQ ID NO 69
<211> LENGTH: 1569
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct

<400> SEQUENCE: 69

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ctgatgactc aatcgccaag ctccatgagt gtctcgctcg ggcacacggg aataataact      120
tgtcacgcta gtcaggcat tagcagtaac ataggatggc tccagcaaaa accccggcaag      180
agctttaaagg gtttgattta ccacgcaacg aacctggaag atggagtgcc gagccgattt      240
agtggccgg agcgcgggtgc cgattacago ttgacaatat cgagtcgttgc atccggaggac      300
tttgcgcatt attattgtt acagtaggtt cagttccat ttacgttccg aagcggtacg      360
aatgtggaga taaaaggccg aggccggaaatggccggag gaagccggagg cggaggatcc      420
gaggttcaac tgcaacaatc gggtgcagaa ttggtaacggc caggagcaag tgtgaagctg      480
agctgcacgg cgagcggttt caacataag aacacttaca tgcactgggt gaagcaacgg      540
ccagagcagg gcttggagtg gattggacgc attgatccc ccaacggcaa cactaggatc      600
ggccggaaat tccaaggtaa agctacgatc actgcagata catcgatcaa caccgcctac      660
ctccaaactct cgagtctcac tagegaggat acggccatct attactgcgc acggatttat      720
ttcggacactg attactgggg tcagggaaacg actctgactg tatccagttt cggaggccga      780
agtggccggc gggaaagccg aggccggagg tccggccggag gggcagtttgc tatcgatgt      840
acccagtcgc caagcgttgc ggctgtgtcc gtgggacaga aagtggccat gagctgcacc      900
agcagccagg tgctgtgca cagccccaaac cagaagaatt acctggccctg gtatcagcag      960
aagccccggcc aaagtccgaa gctgtggtc tactttgcca gcacacgcga gagccggatg      1020
ccagatcggtt ttacccggaa cggcggccgc accgatttca ccctgacaaat tagtagcgat      1080
caggccgagg atctggccgt gtattactgc cagcagcact acagcaccctt gctgacattt      1140
ggccggccggaa cgaagcttggaa actgaaaggc ggaggtggta gtggggccgg aggatcagg      1200
ggtgtgggtt ctgaagtgc actgcagcag agcggccctg agctggccaa accaggtgcc      1260
agcgtgaaga tcaagctgaa ggccggccga tacaccttca ccgattacta catcaactgg      1320
gtcaagcaga gccacggcaa gagcctggaa tggatcgccg atatcaaccc caccacggc      1380
gatagcacct acagccagaa gttcaaggcc aaagccacgc tgaccgttgc taagatgtc      1440
agcaccgcct acatggaaact ggcggccctg acaagcgaag tgcgtccgt gtactattgc      1500
ggccgtgatt acggccatggaa ttactggggc caggccacca gtgtgaccgt tagtagtgag      1560

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ccagaggct	1569
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<210> SEQ ID NO 70
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 70

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Gly Phe Leu Gly
1

```

<210> SEQ ID NO 71
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic peptide

<400> SEQUENCE: 71

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Ala Leu Ala Leu
1

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<210> SEQ ID NO 72
<211> LENGTH: 2574
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct

<400> SEQUENCE: 72

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atgaagctgt gcatacctgct ggccgtgggt gcctttgtgg gatttagtgg tggacgttgt	60
ccatggcccg gggatcagat ctgttattggc tatcacgcaca ataacagcac cgaacaagtt	120
gacaccatta tggaaaagaa cgttaacagtc acgcattgtccc aggacattct cgagaaaacc	180
cacaatggca aactctgtga cttgaacggc gtcaaacctc tcatactgaa agactgttcg	240
gtcgccggct ggctcctcggt taatcctatg tgccgttgtt ttatccgtt accttgagtgg	300
tctgtatattg ttgagggggc aaacccggca aatgatctct gctatctgg aagcctcaac	360
gactatgaag aactcaaaca cctccctctcg cgcataaaatc acttcgaaaa aataactcatc	420
ataccaaaga gcgttggcc taaccacgg acatcgctcg cggttccgc agcctgtccc	480
tatcaggggaa ctccatcggtt tttccgtaat gtagtatggc tcataaaaaaa gaatgacgcc	540
tatcctacaa ttaaaattag ctataataac accaatcgcc aagacccctt gattatgtgg	600
ggatttccatc actccaataa cggccaaagaa cagaccaact tgtacaaaaa ccccaactacc	660
tatataagtgtgggtaccag tacactgaac caaaggctgg ttccaaagat agtactcgat	720
agccaggtta atggtaacgc gggacgtatg gacttctttt ggacaattttt gaaaccaaacc	780
gacgcataac attttggaaag caatggtaac tttatcgccc cagagtacgc ctataagatc	840
gtcaagaagg gagactcgac gataatgaag agtgaagtag aatacggcca ctgcaatacg	900
aaatgtcaaa cggccagtcgg tgccatcaac agtctcgatgc cggtccataa catccatccg	960
ctgacgatttggaggtgcccc gaaatacgtaa aagagcaata aattgggtttt ggccactggc	1020
ctccgtataa gttcccgaggc cgagacacgg ggtctgttcg gtgccatcgcc tggcttcatt	1080
gaggggaggtt ggcagggttat ggtggacgga tggtaacggct atcatcatag taatgaacag	1140

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ggtagtgtt acgcagcgga taaggagtcc acccagaagg ctatcgatgg agtgacaaac	1200
aaggtaaca gtattattga taaaatgaac acccaatttg aagcagtggg acgagagttc	1260
aataatctcg aacggcgcat cgaaaacctg aataaaaaga tggaaagacgg attcctcgac	1320
gtctggacct acaacgctga actgttggtg ctcatggaaa acgagcggac gctcgattc	1380
cacgatagca atgttaagaa tctgtatgac aaagtccggc tccaattgcg agacaacgcc	1440
aaagaactgg gaaacggttt ttttgagttc tatcataaat gtgataacga gtgcattgaa	1500
agtgtccgaa atggAACATA cgattaccctt cagtattcg aggaaagctcg tttgaaaagg	1560
gaagaaatat ccggcgtaaa actcgaaagt atcgAACCTT atcaagcggc cgcaggcagc	1620
ggctacatcc cagaggcccc acgacgacgga caggcctatg tgcgcaaggaa tggcgagtgg	1680
gtgctgctga gcaccccttctt gttaattaag aattcgcggc cgctcgaggg aagtggaaagc	1740
ggagatatcg tcatgttccca gtcgcggcaggc agtctggctg tgcggctggg acagaaatgt	1800
accatgagct gcaccaggcag ccaggtgctg ctgcacagcc ccaaccagaa gaattacctg	1860
gcctggatcc acgagaagcc cggccaaagt ccgaagctgc tggctactt tgccagcaca	1920
cgcgagagcg gactgtccaga tcgttttacc ggaagcggca gggcaccgaa ttccaccctg	1980
acaattatgttgcgtgcaggc cgaggatctg gccgtgtatt actgtccagca gcactacagc	2040
accccgctga catttggcgc cggAACGAAG ctggAACTGA aaggcggagg tggtagtgtt	2100
ggcggaggat cagggtgggg tggttctggc ggtggggaa gtgaagtgc actgcaggcag	2160
agcggcccag agctggtcaa accaggtgcc acgegtgaaga tcaagctgca ggcgcggaa	2220
tacaccccttca ccgattacta catcaactgg gtcaaggcaga gccacggca ggcctggaa	2280
tggatcgccgc atatcaaccc caccaacggc gatagcacct acagccagaa gttcaagggc	2340
aaagccacgc tgaccgttgc taagagtgc acgaccgcgt acatggact ggcgcgcctg	2400
acaaggcggcgt gtactattgc gcccgtgatt acgcccatttgc ttactgggg	2460
caggcgcacca gtgtgaccgt tagtagtttctt agagggccct tcgaaggtaa gcctatccct	2520
aaccctctcc tcggtctcgat ttctacgcgtt gaaaggcggcgc agccagaggc ttaa	2574

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<210> SEQ ID NO 73
<211> LENGTH: 1788
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: BIP-H5HA-Foldon

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<400> SEQUENCE: 73

atgaagctgt gcacccgtgtt ggccgtgggg gcctttgtgg gattgagttt gggacgtgtt
60
ccatggcccg gggatcagat ctgttattggc tatcacgcctt ataacacgcac cgaacaaggtt
120
gacaccattt tggaaaagaa cgtaacagtc acgcacgtccc aggacattctt cgagaaaacc
180
cacaatggca aactctgtga cttgtacggc gtcaaaccttc tcataactgaa agactgttgc
240
gtcgccggct ggctcctcgtaatcctatgt tgcgacgaaat ttatccgcgtt acctgagttgg
300
tcgtatatttggatggggc aaacccggca aatgtatctt ctgtatctgg aagcctcaac
360
gactatgttgcgtt aactcaaaaca cctccctctcg cgcataaaatc acttcgaaaa aataactctt
420
ataccaaaaga gcaatggcc taaccacgcgtt acatcgctcg ggcgttccgc agcctgtccc
480

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tatcagggaa ctccatcggtt tttccgtaat gtagtatggc tcataaaaaaa gaatgacgcc	540
tatcctacaa ttaaaattag ctataataac accaatcgcg aagacccctt gattatgtgg	600
ggtattcatc actccaataa cgccgaagaa cagaccaact tgtacaaaaa ccccactacc	660
tatataagtg tgggtaccag tacactgaac caaaggctgg ttccaaagat agctactcgt	720
agccaggta atggtaaacg gggacgtatg gacttcttt ggacaatttt gaaaccaaac	780
gacgcaatac attttgcggaa caatggtaac ttatcgcccc cagagtacgc ctataagatc	840
gtcaagaagg gagactcgac gataatgaag agtgaagtag aatacggcca ctgcaatacg	900
aatatgtcaaa cgccagtcgg tgccatcaac agctcgatgc cgttccataa catccatccg	960
ctgacgattg gagagtgcgg gaaatacgtaa aagagaata aattggctt ggccactggc	1020
ctccgtataa gtccccaggg cgagacacgg ggtctgttcg gtgcctatcgc tggcttcatt	1080
gagggaggtt ggcagggtat ggtggacggg tggtacggct atcatcatag taatgaacag	1140
ggtagtggtt acgcagcgga taaggagttt acccagaagg ctatcgatgg agtgcacaaac	1200
aaggtaaca gtattattgtaa taaaatgaac acccaatttg aagcagtggg acgagagttc	1260
aataatctcg aacggcgcat cgaaaacctg aataaaaaga tggaagacgg attcctcgac	1320
gtctggacct acaacgctga actgttgggtc ctcatggaaa acgagcgac gctcgatttc	1380
cacgatagca atgttaagaa tctgtatgac aaagtccggc tccaaattgcg agacaacgcc	1440
aaagaactgg gaaacggttt tttttagttt tatcataat gtgataacga tggcatggaa	1500
agtgtccgaa atggaacata cgattaccca cagtattcgg aggaagctcg tttgaaaagg	1560
gaagaaatat ccggcgtaaa actcgaaagt atcggaaacct atcaagcgcc cgccaggcagc	1620
ggctacatcc cagaggcccc acgcgacggc caggcctatg tgcgcagga tggcgagtgg	1680
gtgctgtcga gcacccctt gtcttagaggg cccttcgaag gtaaggctat ccctaaccct	1740
ctccctcggtc tcgattctac gcgtgaaggc ggccggccag aggcttaa	1788

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<210> SEQ ID NO 74
<211> LENGTH: 1632
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: H9HA-Foldon

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<400> SEQUENCE: 74

atggaggcta ttagtctgtat gattattctg ctgggtggta caacctcaaa tgccgataaa 60
atttgtattt gacatcagtc tactaacagc acagagactg tggacaccct gacagaatcc 120
aacatccccg tgacccaggc aaaagagctg ctgcacacag aacacaacgg gatgtgtgc 180
gccaccaatc tggccaggcc cctgatcctg gacacttgca ccgtggaggg cctgatctac 240
ggaaaccctta gctgcgtatct gctgtgggc ggaagagagttt gtagctacat cgtggaaagg 300
ccctccgcag tgaatggaaatgttaccct gggAACGTGGAAAATCTGAGGAACGTGCCGG 360
atgtgttca gtcggccag ctccattaccat agaatccaga ttttcctgatgttctatctgg 420
aacgtgcacct acgcggaaac aagcaatcc tgcagcaact ccttctacag aaatatgagg 480
tggctgaccc agaagaacgg gaattaccca attcaggatg ctcagtgacac aaacaatccg 540

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ggaaaggaca	tcctgttat	ttggggatac	caccacccc	ctactgatac	cacacagacc	600
aacctgtaca	caagaactga	cactaccaca	agcgtgacta	ccgagaatct	ggatagaact	660
ttcaaacctc	tgatcgccc	aaggcccctg	gtgaacggcc	tgattggacg	catcaattac	720
tactggagcg	tgctgaagcc	tggacagact	ctgcgcgtgc	ggtccaacgg	caatctgatc	780
gccccatggt	acggacacgt	gctgagcgg	gagtcccacg	gaaggattct	gaagaccgac	840
ctgaaatccg	ggaactgcgt	ggtgcagtgc	cagactgaaa	aagggggct	gaacagcacc	900
ctgccattcc	acaatatctc	caaatacgc	tccggcacat	gccccaaagta	catcgagtg	960
aagagcctga	aactggctat	tggcctgcgc	aacgtgccag	caaagtccaa	tcggggctg	1020
ttcggcgccaa	ttgcggggtt	tatcgaggg	ggatggccag	gcctggtggc	tggatggtag	1080
gggttccagc	acagcaacga	tcagggagtg	ggaatggcag	ctgacagggg	aagcacacag	1140
aaggcagtgg	ataaaaatcac	ttccaagggt	aacaacatca	tgcataagat	gaacaggcag	1200
tacgagatca	ttgaccacga	atttccgag	attgaaacac	gcctgaatat	gattaacaac	1260
aagatcgatg	accagattca	ggacgtgtgg	gcttacaacg	cagagctgct	ggtgtgtctg	1320
aaaaatcaga	agactctgga	tgagoacgac	gccaacgtga	acaatctgt	caataaggtg	1380
aaaaagagccc	ttggggagcaa	cgctatggag	gatgggaaag	gctgcttga	actgtaccac	1440
aagtgcgatg	accagtgcat	ggaaacaato	aggaacggca	cttacaatag	aaggaagttac	1500
acagaggaaa	gccgcctgga	gaggcaggga	tccggataca	tccctgaagc	accacgcgt	1560
ggacaggcct	acgtgaggaa	ggatggggag	tgggtgtcgc	tgagtacatt	tctggggaa	1620
cctgaggcat	aa					1632

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<210> SEQ ID NO 75
<211> LENGTH: 2382
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: H9HA-Poldon-CD83scFv

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<400> SEQUENCE: 75						
atggaggcta	ttagtctgat	gattattctg	ctgggtgtga	caacctcaaa	tgccgataaa	60
atttgtattg	gacatcagtc	tactaacgc	acagagactg	tggacaccc	gacagaatcc	120
aacatccccg	tgacccaggc	aaaagagctg	ctgcacacag	aacacaacgg	gatgctgtgc	180
gccaccaatc	tgggcaggcc	cctgatcctg	gacacttgca	ccgtggaggg	cctgatctac	240
ggaaacccta	gctgcgatct	gctgctgggc	ggaagagagt	ggagctacat	cgtggaaagg	300
ccctccgcag	tgaatggAAC	atgcgtaccct	gggaacgtgg	aaaatctgga	ggaactgcgg	360
atgctgttca	gctccgcag	ctcctaccag	agaatccaga	ttttcctga	tgctatctgg	420
aacgtgacct	acgacggAAC	aagcaaattcc	tgcagcaact	ccttctacag	aaatatgagg	480
tggctgaccc	agaagaacgg	gaattaccca	attcaggatg	ctcagttacac	aaacaatcg	540
ggaaaggaca	tcctgttat	ttggggatac	caccacccc	ctactgatac	cacacagacc	600
aacctgtaca	caagaactga	cactaccaca	agcgtgacta	ccgagaatct	ggatagaact	660
ttcaaacctc	tgatcgccc	aaggcccctg	gtgaacggcc	tgattggacg	catcaattac	720
tactggagcg	tgctgaagcc	tggacagact	ctgcgcgtgc	ggtccaacgg	caatctgatc	780

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gccccatgg	acggacacgt	gctgagcgga	gagtcccacg	gaaggattct	gaagaccgac	840
ctgaaatccg	ggaactgcgt	ggtgcagtgc	cagactgaaa	aagggggcct	gaacagcacc	900
ctgccattcc	acaatatctc	caaatacgcc	tccggcacat	gccccaaagta	catcgagtg	960
aagagcctga	aactggctat	tggectgcgc	aacgtgccag	caaagtccaa	tcggggctg	1020
ttcggcgaa	ttgccccgtt	tatcgaggga	ggatggccag	gcctggtggc	tggatggtac	1080
gggttccagc	acagcaacga	tcaggagtg	ggaatggcag	ctgacagggg	aagcacacag	1140
aaggcagtgg	ataaaaatcac	ttccaagggt	aacaacatca	tcgataagat	gaacaggcag	1200
tacgagatca	ttgaccacga	attttccag	attgaaacac	gcctgaatat	gattaacaac	1260
aagatcgatg	accagattca	ggacgtgtgg	gcttacaacg	cagagctgct	ggtgcgtctg	1320
aaaaatcaga	agactctgga	tgagcacgac	gccaacgtga	acaatctgta	caataagtg	1380
aaaaagagccc	tggggagcaa	cgctatggag	gatgggaaag	gctgccttga	actgtaccac	1440
aagtgcgatg	accagtgcac	ggaaaacaatc	aggaacggca	cttacaatag	aaggaagtag	1500
acagaggaaa	gcccgcctgga	gagggcaggga	tccggataca	tccctgaagc	accacgcgat	1560
ggacaggccct	acgtgaggaa	ggatggggag	tgggtgctgc	ttagtacatt	tctggggccc	1620
ggggatattg	tgatgaccca	gtctcctagt	agcctggccg	tgtccgtgg	gcagaaagtg	1680
accatgtctt	gtacccctc	tcaggtgctg	ctgcaactccc	ctaaccagaa	aaattacctg	1740
gcctggtacc	agcagaaacc	tggccagago	cctaagctgc	tggtgtactt	cgccagcact	1800
agagagtcgg	gcgtgccaga	tagttcaca	ggatccggga	gcggcactga	ctttaccctg	1860
acaatcgatc	ccgtgcaggc	cgaggatctg	gccgtgtact	actgccagca	gcactacagc	1920
accccccgt	catttggagc	agggacaaaa	ctggaaactga	agggcggagg	aggatccgga	1980
ggaggaggaa	gcggaggagg	agggtccggc	ggaggaggaa	gcgagggtca	gctgcagcag	2040
agcggaccag	aactggtgaa	acccggagca	agcgtgaaaa	tctcctgca	ggccagcgg	2100
tacacttca	ccgattacta	cattaactgg	gtgaaacagt	cccacggaa	gagcctggaa	2160
tggatcgccg	atattaaccc	tactaatgg	gactccacct	acagccagaa	gtttaaaggc	2220
aaggctacac	tgactgtgga	caagagctcc	agcacccgcat	acatggagct	gagatccctg	2280
acaagcgaag	tgtccgcctg	gtactactgt	gccagagatt	atgctatgg	ctattgggc	2340
caggggacaa	gcgtgactgt	gagttccgaa	cctgaggcat	aa		2382

<210> SEQ ID NO 76

<211> LENGTH: 857

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: synthetic polypeptide

<400> SEQUENCE: 76

Met	Lys	Leu	Cys	Ile	Leu	Leu	Ala	Val	Val	Ala	Phe	Val	Gly	Ley	Ser	
1								5						10		15

Leu	Gly	Arg	Ser	Pro	Trp	Pro	Gly	Asp	Gln	Ile	Cys	Ile	Gly	Tyr	His
														25	30

Ala	Asn	Asn	Ser	Thr	Glu	Gln	Val	Asp	Thr	Ile	Met	Glu	Lys	Asn	Val	
														35	40	45

Thr	Val	Thr	His	Ala	Gln	Asp	Ile	Leu	Glu	Lys	Thr	His	Asn	Gly	Lys	
														50	55	60

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Leu Cys Asp Leu Asn Gly Val Lys Pro Leu Ile Leu Lys Asp Cys Ser
 65 70 75 80
 Val Ala Gly Trp Leu Leu Gly Asn Pro Met Cys Asp Glu Phe Ile Arg
 85 90 95
 Val Pro Glu Trp Ser Tyr Ile Val Glu Arg Ala Asn Pro Ala Asn Asp
 100 105 110
 Leu Cys Tyr Pro Gly Ser Leu Asn Asp Tyr Glu Glu Leu Lys His Leu
 115 120 125
 Leu Ser Arg Ile Asn His Phe Glu Lys Ile Leu Ile Ile Pro Lys Ser
 130 135 140
 Ser Trp Pro Asn His Glu Thr Ser Leu Gly Val Ser Ala Ala Cys Pro
 145 150 155 160
 Tyr Gln Gly Thr Pro Ser Phe Phe Arg Asn Val Val Trp Leu Ile Lys
 165 170 175
 Lys Asn Asp Ala Tyr Pro Thr Ile Lys Ile Ser Tyr Asn Asn Thr Asn
 180 185 190
 Arg Glu Asp Leu Leu Ile Met Trp Gly Ile His His Ser Asn Asn Ala
 195 200 205
 Glu Glu Gln Thr Asn Leu Tyr Lys Asn Pro Thr Thr Tyr Ile Ser Val
 210 215 220
 Gly Thr Ser Thr Leu Asn Gln Arg Leu Val Pro Lys Ile Ala Thr Arg
 225 230 235 240
 Ser Gln Val Asn Gly Gln Arg Gly Arg Met Asp Phe Phe Trp Thr Ile
 245 250 255
 Leu Lys Pro Asn Asp Ala Ile His Phe Glu Ser Asn Gly Asn Phe Ile
 260 265 270
 Ala Pro Glu Tyr Ala Tyr Lys Ile Val Lys Lys Gly Asp Ser Thr Ile
 275 280 285
 Met Lys Ser Glu Val Glu Tyr Gly His Cys Asn Thr Lys Cys Gln Thr
 290 295 300
 Pro Val Gly Ala Ile Asn Ser Ser Met Pro Phe His Asn Ile His Pro
 305 310 315 320
 Leu Thr Ile Gly Glu Cys Pro Lys Tyr Val Lys Ser Asn Lys Leu Val
 325 330 335
 Leu Ala Thr Gly Leu Arg Asn Ser Pro Gln Gly Glu Thr Arg Gly Leu
 340 345 350
 Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Gln Gly Met Val
 355 360 365
 Asp Gly Trp Tyr Gly Tyr His His Ser Asn Glu Gln Gly Ser Gly Tyr
 370 375 380
 Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala Ile Asp Gly Val Thr Asn
 385 390 395 400
 Lys Val Asn Ser Ile Ile Asp Lys Met Asn Thr Gln Phe Glu Ala Val
 405 410 415
 Gly Arg Glu Phe Asn Asn Leu Glu Arg Arg Ile Glu Asn Leu Asn Lys
 420 425 430
 Lys Met Glu Asp Gly Phe Leu Asp Val Trp Thr Tyr Asn Ala Glu Leu
 435 440 445
 Leu Val Leu Met Glu Asn Glu Arg Thr Leu Asp Phe His Asp Ser Asn
 450 455 460

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Val Lys Asn Leu Tyr Asp Lys Val Arg Leu Gln Leu Arg Asp Asn Ala
 465 470 475 480
 Lys Glu Leu Gly Asn Gly Cys Phe Glu Phe Tyr His Lys Cys Asp Asn
 485 490 495
 Glu Cys Met Glu Ser Val Arg Asn Gly Thr Tyr Asp Tyr Pro Gln Tyr
 500 505 510
 Ser Glu Glu Ala Arg Leu Lys Arg Glu Glu Ile Ser Gly Val Lys Leu
 515 520 525
 Glu Ser Ile Gly Thr Tyr Gln Ala Ala Ala Gly Ser Gly Tyr Ile Pro
 530 535 540
 Glu Ala Pro Arg Asp Gly Gln Ala Tyr Val Arg Lys Asp Gly Glu Trp
 545 550 555 560
 Val Leu Leu Ser Thr Phe Leu Leu Ile Lys Asn Ser Arg Pro Leu Glu
 565 570 575
 Gly Ser Gly Ser Gly Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu
 580 585 590
 Ala Val Ser Val Gly Gln Lys Val Thr Met Ser Cys Thr Ser Ser Gln
 595 600 605
 Val Leu Leu His Ser Pro Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln
 610 615 620
 Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Val Tyr Phe Ala Ser Thr
 625 630 635 640
 Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
 645 650 655
 Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val
 660 665 670
 Tyr Tyr Cys Gln Gln His Tyr Ser Thr Pro Leu Thr Phe Gly Ala Gly
 675 680 685
 Thr Lys Leu Glu Leu Lys Gly Gly Gly Ser Gly Gly Gly Ser Gly
 690 695 700
 Gly Gly Gly Ser Gly Gly Gly Ser Gly Val Gln Leu Gln Gln
 705 710 715 720
 Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys
 725 730 735
 Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Ile Asn Trp Val Lys
 740 745 750
 Gln Ser His Gly Lys Ser Leu Glu Trp Ile Gly Asp Ile Asn Pro Thr
 755 760 765
 Asn Gly Asp Ser Thr Tyr Ser Gln Lys Phe Lys Gly Lys Ala Thr Leu
 770 775 780
 Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu
 785 790 795 800
 Thr Ser Glu Val Ser Ala Val Tyr Tyr Cys Ala Arg Asp Tyr Ala Met
 805 810 815
 Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ser Arg Gly
 820 825 830
 Pro Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser
 835 840 845
 Thr Arg Glu Gly Glu Pro Glu Ala
 850 855

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<210> SEQ_ID NO 77
<211> LENGTH: 581
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic polypeptide

<400> SEQUENCE: 77

Met Lys Leu Cys Ile Leu Leu Ala Val Val Ala Phe Val Gly Leu Ser
1 5 10 15

Leu Gly Arg Ser Pro Trp Pro Gly Asp Gln Ile Cys Ile Gly Tyr His
20 25 30

Ala Asn Asn Ser Thr Glu Gln Val Asp Thr Ile Met Glu Lys Asn Val
35 40 45

Thr Val Thr His Ala Gln Asp Ile Leu Glu Lys Thr His Asn Gly Lys
50 55 60

Leu Cys Asp Leu Asn Gly Val Lys Pro Leu Ile Leu Lys Asp Cys Ser
65 70 75 80

Val Ala Gly Trp Leu Leu Gly Asn Pro Met Cys Asp Glu Phe Ile Arg
85 90 95

Val Pro Glu Trp Ser Tyr Ile Val Glu Arg Ala Asn Pro Ala Asn Asp
100 105 110

Leu Cys Tyr Pro Gly Ser Leu Asn Asp Tyr Glu Glu Leu Lys His Leu
115 120 125

Leu Ser Arg Ile Asn His Phe Glu Lys Ile Leu Ile Ile Pro Lys Ser
130 135 140

Ser Trp Pro Asn His Glu Thr Ser Leu Gly Val Ser Ala Ala Cys Pro
145 150 155 160

Tyr Gln Gly Thr Pro Ser Phe Phe Arg Asn Val Val Trp Leu Ile Lys
165 170 175

Lys Asn Asp Ala Tyr Pro Thr Ile Lys Ile Ser Tyr Asn Asn Thr Asn
180 185 190

Arg Glu Asp Leu Leu Ile Met Trp Gly Ile His His Ser Asn Asn Ala
195 200 205

Glu Glu Gln Thr Asn Leu Tyr Lys Asn Pro Thr Thr Tyr Ile Ser Val
210 215 220

Gly Thr Ser Thr Leu Asn Gln Arg Leu Val Pro Lys Ile Ala Thr Arg
225 230 235 240

Ser Gln Val Asn Gly Gln Arg Gly Arg Met Asp Phe Phe Trp Thr Ile
245 250 255

Leu Lys Pro Asn Asp Ala Ile His Phe Glu Ser Asn Gly Asn Phe Ile
260 265 270

Ala Pro Glu Tyr Ala Tyr Lys Ile Val Lys Lys Gly Asp Ser Thr Ile
275 280 285

Met Lys Ser Glu Val Glu Tyr Gly His Cys Asn Thr Lys Cys Gln Thr
290 295 300

Pro Val Gly Ala Ile Asn Ser Ser Met Pro Phe His Asn Ile His Pro
305 310 315 320

Leu Thr Ile Gly Glu Cys Pro Lys Tyr Val Lys Ser Asn Lys Leu Val
325 330 335

Leu Ala Thr Gly Leu Arg Asn Ser Pro Gln Gly Glu Thr Arg Gly Leu
340 345 350

Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Gln Gly Met Val

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355	360	365	
Asp Gly Trp Tyr Gly Tyr His His Ser Asn Glu Gln Gly Ser Gly Tyr			
370	375	380	
Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala Ile Asp Gly Val Thr Asn			
385	390	395	400
Lys Val Asn Ser Ile Ile Asp Lys Met Asn Thr Gln Phe Glu Ala Val			
405	410	415	
Gly Arg Glu Phe Asn Asn Leu Glu Arg Arg Ile Glu Asn Leu Asn Lys			
420	425	430	
Lys Met Glu Asp Gly Phe Leu Asp Val Trp Thr Tyr Asn Ala Glu Leu			
435	440	445	
Leu Val Leu Met Glu Asn Glu Arg Thr Leu Asp Phe His Asp Ser Asn			
450	455	460	
Val Lys Asn Leu Tyr Asp Lys Val Arg Leu Gln Leu Arg Asp Asn Ala			
465	470	475	480
Lys Glu Leu Gly Asn Gly Cys Phe Glu Phe Tyr His Lys Cys Asp Asn			
485	490	495	
Glu Cys Met Glu Ser Val Arg Asn Gly Thr Tyr Asp Tyr Pro Gln Tyr			
500	505	510	
Ser Glu Glu Ala Arg Leu Lys Arg Glu Glu Ile Ser Gly Val Lys Leu			
515	520	525	
Glu Ser Ile Gly Thr Tyr Gln Ala Ala Ala Gly Ser Gly Tyr Ile Pro			
530	535	540	
Glu Ala Pro Arg Asp Gly Gln Ala Tyr Val Arg Lys Asp Gly Glu Trp			
545	550	555	560
Val Leu Leu Ser Thr Phe Leu Leu Ile Lys Ile Lys Thr Arg Glu Gly			
565	570	575	
Gly Glu Pro Glu Ala			
580			

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<210> SEQ ID NO 78
<211> LENGTH: 793
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic polypeptide

<400> SEQUENCE: 78

Met Glu Ala Ile Ser Leu Met Ile Ile Leu Leu Val Val Thr Thr Ser
1 5 10 15

Asn Ala Asp Lys Ile Cys Ile Gly His Gln Ser Thr Asn Ser Thr Glu
20 25 30

Thr Val Asp Thr Leu Thr Glu Ser Asn Ile Pro Val Thr Gln Ala Lys
35 40 45

Glu Leu Leu His Thr Glu His Asn Gly Met Leu Cys Ala Thr Asn Leu
50 55 60

Gly Arg Pro Leu Ile Leu Asp Thr Cys Thr Val Glu Gly Leu Ile Tyr
65 70 75 80

Gly Asn Pro Ser Cys Asp Leu Leu Gly Gly Arg Glu Trp Ser Tyr
85 90 95

Ile Val Glu Arg Pro Ser Ala Val Asn Gly Thr Cys Tyr Pro Gly Asn
100 105 110

Val Glu Asn Leu Glu Glu Leu Arg Met Leu Phe Ser Ser Ala Ser Ser

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115	120	125
Tyr Gln Arg Ile Gln Ile Phe Pro Asp Ala Ile Trp Asn Val Thr Tyr		
130	135	140
Asp Gly Thr Ser Lys Ser Cys Ser Asn Ser Phe Tyr Arg Asn Met Arg		
145	150	155
160		
Trp Leu Thr Gln Lys Asn Gly Asn Tyr Pro Ile Gln Asp Ala Gln Tyr		
165	170	175
Thr Asn Asn Arg Gly Lys Asp Ile Leu Phe Ile Trp Gly Ile His His		
180	185	190
Pro Pro Thr Asp Thr Thr Gln Thr Asn Leu Tyr Thr Arg Thr Asp Thr		
195	200	205
Thr Thr Ser Val Thr Thr Glu Asn Leu Asp Arg Thr Phe Lys Pro Leu		
210	215	220
Ile Gly Pro Arg Pro Leu Val Asn Gly Leu Ile Gly Arg Ile Asn Tyr		
225	230	235
240		
Tyr Trp Ser Val Leu Lys Pro Gly Gln Thr Leu Arg Val Arg Ser Asn		
245	250	255
Gly Asn Leu Ile Ala Pro Trp Tyr Gly His Val Leu Ser Gly Glu Ser		
260	265	270
His Gly Arg Ile Leu Lys Thr Asp Leu Lys Ser Gly Asn Cys Val Val		
275	280	285
Gln Cys Gln Thr Glu Lys Gly Gly Leu Asn Ser Thr Leu Pro Phe His		
290	295	300
Asn Ile Ser Lys Tyr Ala Phe Gly Thr Cys Pro Lys Tyr Ile Gly Val		
305	310	315
320		
Lys Ser Leu Lys Leu Ala Ile Gly Leu Arg Asn Val Pro Ala Lys Ser		
325	330	335
Asn Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Trp		
340	345	350
Pro Gly Leu Val Ala Gly Trp Tyr Gly Phe Gln His Ser Asn Asp Gln		
355	360	365
Gly Val Gly Met Ala Ala Asp Arg Gly Ser Thr Gln Lys Ala Val Asp		
370	375	380
Lys Ile Thr Ser Lys Val Asn Asn Ile Ile Asp Lys Met Asn Arg Gln		
385	390	395
400		
Tyr Glu Ile Ile Asp His Glu Phe Ser Glu Ile Glu Thr Arg Leu Asn		
405	410	415
Met Ile Asn Asn Lys Ile Asp Asp Gln Ile Gln Asp Val Trp Ala Tyr		
420	425	430
Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Gln Lys Thr Leu Asp Glu		
435	440	445
His Asp Ala Asn Val Asn Asn Leu Tyr Asn Lys Val Lys Arg Ala Leu		
450	455	460
Gly Ser Asn Ala Met Glu Asp Gly Lys Gly Cys Phe Glu Leu Tyr His		
465	470	475
480		
Lys Cys Asp Asp Gln Cys Met Glu Thr Ile Arg Asn Gly Thr Tyr Asn		
485	490	495
Arg Arg Lys Tyr Thr Glu Glu Ser Arg Leu Glu Arg Gln Gly Ser Gly		
500	505	510
Tyr Ile Pro Glu Ala Pro Arg Asp Gly Gln Ala Tyr Val Arg Lys Asp		
515	520	525

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Gly Glu Trp Val Leu Leu Ser Thr Phe Leu Gly Pro Gly Asp Ile Val
530 535 540

Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Val Gly Gln Lys Val
545 550 555 560

Thr Met Ser Cys Thr Ser Ser Gln Val Leu Leu His Ser Pro Asn Gln
565 570 575

Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys
580 585 590

Leu Leu Val Tyr Phe Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg
595 600 605

Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser
610 615 620

Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His Tyr Ser
625 630 635 640

Thr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Gly Gly
645 650 655

Gly Gly Ser Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly
660 665 670

Gly Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro
675 680 685

Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
690 695 700

Asp Tyr Tyr Ile Asn Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu
705 710 715 720

Trp Ile Gly Asp Ile Asn Pro Thr Asn Gly Asp Ser Thr Tyr Ser Gln
725 730 735

Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr
740 745 750

Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Val Ser Ala Val Tyr
755 760 765

Tyr Cys Ala Arg Asp Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser
770 775 780

Val Thr Val Ser Ser Glu Pro Glu Ala
785 790

<210> SEQ ID NO 79
<211> LENGTH: 543
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic polypeptide

<400> SEQUENCE: 79

Met Glu Ala Ile Ser Leu Met Ile Ile Leu Leu Val Val Thr Thr Ser
1 5 10 15

Asn Ala Asp Lys Ile Cys Ile Gly His Gln Ser Thr Asn Ser Thr Glu
20 25 30

Thr Val Asp Thr Leu Thr Glu Ser Asn Ile Pro Val Thr Gln Ala Lys
35 40 45

Glu Leu Leu His Thr Glu His Asn Gly Met Leu Cys Ala Thr Asn Leu
50 55 60

Gly Arg Pro Leu Ile Leu Asp Thr Cys Thr Val Glu Gly Leu Ile Tyr
65 70 75 80

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Gly Asn Pro Ser Cys Asp Leu Leu Leu	Gly Gly Arg Glu Trp Ser Tyr		
85	90	95	
Ile Val Glu Arg Pro Ser Ala Val Asn Gly Thr Cys Tyr Pro Gly Asn			
100	105	110	
Val Glu Asn Leu Glu Glu Leu Arg Met Leu Phe Ser Ser Ala Ser Ser			
115	120	125	
Tyr Gln Arg Ile Gln Ile Phe Pro Asp Ala Ile Trp Asn Val Thr Tyr			
130	135	140	
Asp Gly Thr Ser Lys Ser Cys Ser Asn Ser Phe Tyr Arg Asn Met Arg			
145	150	155	160
Trp Leu Thr Gln Lys Asn Gly Asn Tyr Pro Ile Gln Asp Ala Gln Tyr			
165	170	175	
Thr Asn Asn Arg Gly Lys Asp Ile Leu Phe Ile Trp Gly Ile His His			
180	185	190	
Pro Pro Thr Asp Thr Thr Gln Thr Asn Leu Tyr Thr Arg Thr Asp Thr			
195	200	205	
Thr Thr Ser Val Thr Thr Glu Asn Leu Asp Arg Thr Phe Lys Pro Leu			
210	215	220	
Ile Gly Pro Arg Pro Leu Val Asn Gly Leu Ile Gly Arg Ile Asn Tyr			
225	230	235	240
Tyr Trp Ser Val Leu Lys Pro Gly Gln Thr Leu Arg Val Arg Ser Asn			
245	250	255	
Gly Asn Leu Ile Ala Pro Trp Tyr Gly His Val Leu Ser Gly Glu Ser			
260	265	270	
His Gly Arg Ile Leu Lys Thr Asp Leu Lys Ser Gly Asn Cys Val Val			
275	280	285	
Gln Cys Gln Thr Glu Lys Gly Gly Leu Asn Ser Thr Leu Pro Phe His			
290	295	300	
Asn Ile Ser Lys Tyr Ala Phe Gly Thr Cys Pro Lys Tyr Ile Gly Val			
305	310	315	320
Lys Ser Leu Lys Leu Ala Ile Gly Leu Arg Asn Val Pro Ala Lys Ser			
325	330	335	
Asn Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp			
340	345	350	
Pro Gly Leu Val Ala Gly Trp Tyr Gly Phe Gln His Ser Asn Asp Gln			
355	360	365	
Gly Val Gly Met Ala Ala Asp Arg Gly Ser Thr Gln Lys Ala Val Asp			
370	375	380	
Lys Ile Thr Ser Lys Val Asn Asn Ile Ile Asp Lys Met Asn Arg Gln			
385	390	395	400
Tyr Glu Ile Ile Asp His Glu Phe Ser Glu Ile Glu Thr Arg Leu Asn			
405	410	415	
Met Ile Asn Asn Lys Ile Asp Asp Gln Ile Gln Asp Val Trp Ala Tyr			
420	425	430	
Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Gln Lys Thr Leu Asp Glu			
435	440	445	
His Asp Ala Asn Val Asn Asn Leu Tyr Asn Lys Val Lys Arg Ala Leu			
450	455	460	
Gly Ser Asn Ala Met Glu Asp Gly Lys Gly Cys Phe Glu Leu Tyr His			
465	470	475	480

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Lys Cys Asp Asp Gln Cys Met Glu Thr Ile Arg Asn Gly Thr Tyr Asn
485 490 495

Arg Arg Lys Tyr Thr Glu Glu Ser Arg Leu Glu Arg Gln Gly Ser Gly
500 505 510

Tyr Ile Pro Glu Ala Pro Arg Asp Gly Gln Ala Tyr Val Arg Lys Asp
515 520 525

Gly Glu Trp Val Leu Leu Ser Thr Phe Leu Gly Glu Pro Glu Ala
530 535 540

<210> SEQ ID NO 80

<211> LENGTH: 511

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: synthetic polypeptide

<220> FEATURE:

<221> NAME/KEY: MISC_FEATURE

<223> OTHER INFORMATION: H5N8 ectodomain

<400> SEQUENCE: 80

Asp Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Gln Val
1 5 10 15

Asp Thr Ile Met Glu Lys Asn Val Thr Val Thr His Ala Gln Asp Ile
20 25 30

Leu Glu Lys Thr His Asn Gly Lys Leu Cys Asp Leu Asn Gly Val Lys
35 40 45

Pro Leu Ile Leu Lys Asp Cys Ser Val Ala Gly Trp Leu Leu Gly Asn
50 55 60

Pro Met Cys Asp Glu Phe Ile Arg Val Pro Glu Trp Ser Tyr Ile Val
65 70 75 80

Glu Arg Ala Asn Pro Ala Asn Asp Leu Cys Tyr Pro Gly Ser Leu Asn
85 90 95

Asp Tyr Glu Glu Leu Lys His Leu Leu Ser Arg Ile Asn His Phe Glu
100 105 110

Lys Ile Leu Ile Ile Pro Lys Ser Ser Trp Pro Asn His Glu Thr Ser
115 120 125

Leu Gly Val Ser Ala Ala Cys Pro Tyr Gln Gly Thr Pro Ser Phe Phe
130 135 140

Arg Asn Val Val Trp Leu Ile Lys Lys Asn Asp Ala Tyr Pro Thr Ile
145 150 155 160

Lys Ile Ser Tyr Asn Asn Thr Asn Arg Glu Asp Leu Leu Ile Met Trp
165 170 175

Gly Ile His His Ser Asn Asn Ala Glu Glu Gln Thr Asn Leu Tyr Lys
180 185 190

Asn Pro Thr Thr Tyr Ile Ser Val Gly Thr Ser Thr Leu Asn Gln Arg
195 200 205

Leu Val Pro Lys Ile Ala Thr Arg Ser Gln Val Asn Gly Gln Arg Gly
210 215 220

Arg Met Asp Phe Phe Trp Thr Ile Leu Lys Pro Asn Asp Ala Ile His
225 230 235 240

Phe Glu Ser Asn Gly Asn Phe Ile Ala Pro Glu Tyr Ala Tyr Lys Ile
245 250 255

Val Lys Lys Gly Asp Ser Thr Ile Met Lys Ser Glu Val Glu Tyr Gly
260 265 270

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His	Cys	Asn	Thr	Lys	Cys	Gln	Thr	Pro	Val	Gly	Ala	Ile	Asn	Ser	Ser
275															285
Met	Pro	Phe	His	Asn	Ile	His	Pro	Leu	Thr	Ile	Gly	Glu	Cys	Pro	Lys
290															300
Tyr	Val	Lys	Ser	Asn	Lys	Leu	Val	Leu	Ala	Thr	Gly	Leu	Arg	Asn	Ser
305															320
Pro	Gln	Gly	Glu	Thr	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile
325															335
Glu	Gly	Gly	Trp	Gln	Gly	Met	Val	Asp	Gly	Trp	Tyr	Gly	Tyr	His	His
340															350
Ser	Asn	Glu	Gln	Gly	Ser	Gly	Tyr	Ala	Ala	Asp	Lys	Glu	Ser	Thr	Gln
355															365
Lys	Ala	Ile	Asp	Gly	Val	Thr	Asn	Lys	Val	Asn	Ser	Ile	Ile	Asp	Lys
370															380
Met	Asn	Thr	Gln	Phe	Glu	Ala	Val	Gly	Arg	Glu	Phe	Asn	Asn	Leu	Glu
385															400
Arg	Arg	Ile	Glu	Asn	Leu	Asn	Lys	Lys	Met	Glu	Asp	Gly	Phe	Leu	Asp
405															415
Val	Trp	Thr	Tyr	Asn	Ala	Glu	Leu	Leu	Val	Leu	Met	Glu	Asn	Glu	Arg
420															430
Thr	Leu	Asp	Phe	His	Asp	Ser	Asn	Val	Lys	Asn	Leu	Tyr	Asp	Lys	Val
435															445
Arg	Leu	Gln	Leu	Arg	Asp	Asn	Ala	Lys	Glu	Leu	Gly	Asn	Gly	Cys	Phe
450															460
Glu	Phe	Tyr	His	Lys	Cys	Asp	Asn	Glu	Cys	Met	Glu	Ser	Val	Arg	Asn
465															480
Gly	Thr	Tyr	Asp	Tyr	Pro	Gln	Tyr	Ser	Glu	Glu	Ala	Arg	Leu	Lys	Arg
485															495
Glu	Glu	Ile	Ser	Gly	Val	Lys	Leu	Glu	Ser	Ile	Gly	Thr	Tyr	Gln	
500															510

1. An engineered protein comprising: at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell; and
 - a) at least one antigenic polypeptide; or
 - b) at least one binding domain which is capable of binding to at least one antigenic polypeptide.
2. An engineered protein according to claim 1, wherein said engineered protein is a genetically engineered protein.
3. An engineered protein according to claim 2, wherein the at least one binding domain capable of binding to a cell surface protein and the at least one antigenic polypeptide are comprised in a single recombinant protein; or the at least one binding domain capable of binding to a cell surface protein and the at least one binding domain which is capable of binding to at least one antigenic polypeptide are comprised in a single recombinant protein.
4. An engineered protein according to claim 1, wherein the at least one binding domain capable of binding to a cell surface protein is operably linked to the at least one antigenic polypeptide or the at least one binding domain which is capable of binding to an antigenic polypeptide.
5. An engineered protein according to claim 1, wherein the antigen presenting cell is at least one of a dendritic cell, macrophage, B cell or natural killer cell.
6. An engineered protein according to claim 1, wherein the cell surface protein is selected from an immunoglobulin family protein, an integrin family receptor or a C-type lectin.
7. An engineered protein according to claim 1, wherein the cell surface protein is selected from CD83, CD11c or Dec205.
8. An engineered protein according to claim 1, wherein the cell surface protein is CD83.
9. An engineered protein according to claim 1, wherein the at least one antigenic polypeptide is an avian influenza virus antigenic polypeptide, such as hemagglutinin.
10. An engineered protein according to claim 1, wherein the binding domain(s) is (are) based on the antigen binding site of an antibody or an antibody fragment such as a single-chain variable fragment (scFv), Fv, F(ab') or F(ab')2.
11. A nucleic acid construct which comprises a first polynucleotide which encodes at least one binding domain which is capable of binding to a cell surface protein on an avian antigen presenting cell as defined in claim 1; and a second polynucleotide which encodes at least one antigenic polypeptide or at least one binding domain which is capable of binding to at least one antigenic polypeptide as defined in claim 1.
12. A vector which comprises a nucleic acid construct according to claim 11.

- 13.** An engineered cell comprising a nucleic acid construct according to claim **11**.
- 14.** An avian vaccine comprising an engineered protein according to claim **1**.
- 15.** A vaccine according to claim **14** for use in treating and/or preventing disease in an avian subject.
- 16.** (canceled)
- 17.** A method for treating and/or preventing a disease in an avian subject which comprises the step of administering to a subject an effective amount of a vaccine according to claim **14**.
- 18.** A method according to claim **17**, wherein administration of said vaccine elicits a humoral and/or cellular immune response in the subject.
- 19.** A method according to claim **17**, wherein administration of said vaccine decreases the challenge pathogen load in the subject.
- 20.** A method according to claim **17**, wherein the subject is poultry, optionally a chicken, turkey, duck, quail, pigeon or goose.
- 21.** A method for the preparation of the vaccine according to claim **14**, the method comprising the step of admixing a genetically engineered protein according to claim **1**, and a pharmaceutically acceptable carrier.

* * * *