
▼(Mature residue 1)
H1 -MKANLLVLLCALA--AAD--------ADTICIGYHANNSTDTVDTVLEKNVTVTHSVNL
H2 ---MAIIYLILLFT--AVR--------GDQICIGYHSNNSTEKVDTILERNVTVTHAQNI
H3 --MKTIIALSYIFCLALGQDLPGNDNSTATLCLGHHAVPNGTLVKTITDDQIEVTNATEL
H4 --MLSIVILFLLIAENS----SQNYTGNPVICMGHHAVANGTMVKTLADDQVEVVTAQEL
H5 --MERIVLLLAIVS--LVK--------SDQICIGYHANKSTKQVDTIMEKNVTVTHAQDI
H6 --MIAIIVVAILAT--AGR--------SDKICIGYHANNSTTQIDTILEKNVTVTHSVEL
H7 --MNTQILVFALVAVIPTN--------ADKICLGHHAVSNGTKVNTLTERGVEVVNATET
H8 --MEKFIAIAT-LASTNA---------YDRICIGYQSNNSTDTVNTLIEQNVPVTQTMEL
H9 --METKAIIAALLMVTAAN---------ADKICIGYQSTNSTETVDTLTESNVPVTHTKEL
H10 --MYKVVVIIALLGAVKG---------LDRICLGHHAVANGTIVKTLTNEQEEVTNATET
H11 --MEKTLLFAAIFL--CVK--------ADEICIGYLSNNSTDKVDTIIENNVTVTSSVEL
H12 --MEKFIILSTVLAASFA---------YDKICIGYQTNNSTETVNTLSEQNVPVTQVEEL
H13 -MALNVIATLTLIS-VCVH--------ADRICVGYLSTNSSERVDTLLENGVPVTSSIDL
H14 --MIALILVALALSHTAYSQITNGTTGNPIICLGHHAVENGTSVKTLTDNHVEVVSAKEL
H15 --MNTQIIVILVLGLSMVK--------SDKICLGHHAVANGTKVNTLTERGVEVVNATET
H16 -MMIKVLYFLIIVLGRYSK---------ADKICIGYLSNNSSDTVDTLTENGVPVTSSVDL
H17 MELIVLLILLNPYT--FVL---------GDRICIGYQANQNNQTVNTLLEQNVPVTGAQEI
▲(Mature residue 1)
▼(Residue Ap) (Residue Cp)▼
H1 LEDSHNGKLCRLKGIAPLQLGKCNIAGWLLGNPECDPLLPVRSWSYIVETPNSENGICYP
H2 LEKTHNGKLCKLNGIPPLELGDCSIAGWLLGNPECDRLLTVPEWSYIMEKENPRNGLCYP
H3 VQSSSTGKICNN-PHRILDGIDCTLIDALLGDPHCDVFQNET-WDLFVERSKAFS-NCYP
H4 VESQNLPELCPS-PLRLVDGQTCDIINGALGSPGCDHLNGAE-WDVFIERPNAVD-TCYP
H5 LERTHNGKLCSLNGVKPLILRDCSVAGWLLGNPMCDEFLNLPEWLYIVEKDNPINSLCYP
H6 LENQKEERFCKILKKAPLDLKGCTIEGWILGNPQCDLLLGDQSWSYIVERPTAQNGICYP
H7 VERTNIPKICSK-GKRTTDLGQCGLLGTITGPPQCDQFLEFS-ADLIIERREGND-VCYP
H8 VETEKHPAYCNTDLGAPLELRDCKIEAVIYGNPKCDIHLKDQGWSYIVERPSAPEGMCYP
H9 LHTEHNGMLCATDLGHPLILDTCTIEGLIYGNPSCDILLGGKEWSYIVERSSAVNGMCYP
H10 VESINLNKLCMK-GRSYKDLGNCHPVGMLIGTPVCDPHLTGT-WDTLIERENAIA-HCYP
H11 VETEHTGSFCSINGKQPISLGDCSFAGWILGNPMCDELIGKTSWSYIVEKPNPTNGICYP
H12 VHRGIDPILCGTELGSPLVLDDCSLEGLILGNPKCDLYLNGREWSYIVERPKEMEGVCYP
H13 IETNHTGTYCSLNGVSPVHLGDCSFEGWIVGNPACTSNPGIREWSYLIEDPAAPHGLCYP
H14 VETNHTDELCPS-PLKLVDGQDCHLINGALGSPGCDRLQDTT-WDVFIERPTAVD-TCYP
H15 VEITGIDKVCTK-GKKAVDLGSCGILGTIIGPPQCDLHLEFK-ADLIIERRNSSD-ICYP
H16 VETNHTGTYCSLNGISPIHLGDCSFEGWIVGNPSCATNINIREWSYLIEDPNAPNKPCYP
H17 LETNHNGKLCSLNGVPPLDLQSCTLAGWLLGNPNCDSLLEAEEWSYIKINESAPDDLCFP
▲(Residue Ap) (Residue Cp)▲
H1 GDFIDYEELREQLSSVSSFERFEIFPKESSWPNHNTNGVTAACSHE-GKSSFYRNLLWLT
H2 GSFNDYEELKHLLSSVTHFEKVKILPKDRWTQHTTTGG-SRACAVS-GNPSFFRNMVWLT
H3 YDVPDYASLRSLVASSGTLE--FITEGFTW-TGVTQNGGSNACKRG-PGNGFFSRLNWLT
H4 FDVPEYQSLRSILANNGKFE--FIAEEFQW-NTVKQNGKSGACKRA-NVDDFFNRLNWLV
H5 GDFNDYEELKYLLSSTNHFEKIRIIPRSSWSNHDASSGVSSACPYI-GRSSFLRNVVWLI
H6 GVLNEVEELKALIGSGERVERFEMFPKSTWTGVDTSSGVTRACPYN-SGSSFYRNLLWII
H7 GKFVNEEALRQILRGSGGID--KETMGFTY-SGIRTNGTTSACRRS-G-SSFYAEMEWLL
H8 GSVENLEELRFVFSSAASYKRIRLFDYSRWNVTRS--GTSKACNASTGGQSFYRSINWLT
H9 GNVENLEELRSLFSSAKSYKRIQIFPDKTWNVTYS--GTSRACSN-----SFYRSMRWLT
H10 GATINEEALRQKIMESGGIS--KMSTGFTYGSSITSAGTTKACMRN-GGDSFYAELKWLV
H11 GTLESEEELRLKFSGVLEFNKFEVFTSNGWGAVNSGVGVTAACKFG-GSNSFFRNMVWLI
H12 GSIENQEELRSLFSSIKKYERVKMFDFTKWNVTYT--GTSKACNNTSNQGSFYRSMRWLT
H13 GELNNNGELRHLFSGIRSFSRTELIPPTSWGEVLD--GTTSACRDNTGTNSFYRNLVWFI
H14 FDVPDYQSLRSILASSGSLE--FIAEQFTW-NGVKVDGSSSACLRG-GRNSFFSRLNWLT
H15 GRFTNEEALRQIIRESGGID--KESMGFRY-SGIRTDGATSACKRT-V-SSFYSEMKWLS
H16 GELDNNGELRHLFSGVNSFSRTELINPSKWGNVLD--GVTASCLDR-GASSFYRNLVWIV
H17 GNFENLQDLLLEMSGVQNFTKVKLFNPQSMTG-VTTNNVDQTCPFE-GKPSFYRNLNWIQ

FIG. 1A

```
H1          E-K-EGSYPKLKNSYVNKKGKEVLVLWGIHHPPNSKEQQNLYQNENAYVSVVTSNYNRRF
H2          K-K-GSNYPIAKGSYNNTSGEQMLIIWGVHHPNDETEQRTLYQNVGTYVSIGTSTLNKRS
H3          KS--GSTYPVLNVTMPNNDNFDKLYIWGVHHPSTNQEQTSLYVQESGRVTVSTRRSQQSI
H4          KSD-GNAYPLQNLTKINNGDYARLYIWGVHHPSTSTEQTNLYKNNPGRVTVSTKTSQTSV
H5          K-K-NNTYPTIKRSYNNTNQEDLLILWGIHHPNDAAEQTKLYQNPTTYVSVGTSTLNQRS
H6          KTK-SAAYSVIKGAYNNTGNQPILYFWGVHHPPDTNEQNTLYGSGDRYVRMGTESMNFAK
H7          SNTDNASFPQMTKSYKNTRRESALIVWGIHHSGSTTEQTKLYGSGNKLITVGSSKYHQSF
H8          KKE-PDTYDFNEGAYVNNEDGDIIFLWGIHHPPDTKEQTTLYKNANTLSSVTTNTINRSF
H9          HK--SNSYPFQNAHYTNNERENILFMWGIHHPPTDTEQTDLYKNADTTTSVTTEDINRTF
H10         SKTKGQNFPQTTNTYRNTDTAEHLIIWGIHHPSSTQEKNDLYGTQSLSISVESSTYQNNF
H11         H-Q-SGTYPVIKRTFNNTKGRDVLIVWGIHHPATLTEHQDLYKKDSSYVAVGSETYNRRF
H12         LK--SGQFPVQTDEYKNTRDSDIVFTWAIHHPPTSDEQVKLYKNPDTLSSVTTVEINRSF
H13         K-K-NTRYPVISKTYNNTTGRDVLVLWGIHHPVSVDETKTLYVNSDPYTLVSTKSWSEKY
H14         KAT-NGNYGPINVTKENTGSYVRLYLWGVHHPSSDNEQTDLYKVATGRVTVSTRSDQISI
H15         SSMNNQVFPQLNQTYRNTRKEPALIVWGVHHSSSLDEQNKLYGTGNKLITVGSSKYQQSF
H16         K-K-DEKYPVIKGDYNNTTGRDVLVLWGIHHPDTETTATNLYVNKNPYTLVSTKEWSKRY
H17         G----NSGLPFNIEIKNPTSNPLLLLWGIHNTKDAAQQRNLYGNDYSYTIFNFGEKSEEF

                                                    ▼(Residue Cq)
H1          TPEIAERPKVRDQAGRMNYYWTLLKPGDTIIFEANGNLIAPMYAFALSRGFG--------
H2          IPVIATRPKVNGQGGRMEFSWTILDIWDTINFESTGNLIAPEYGFRISKRGS--------
H3          IPNIGSRPWVRGQSSRISIYWTIVKPGDVLVINSNGNLIAPRGYFKMRTG---------K
H4          VPDIGSRPLVRGQSGRVSFYWTIVEPGDLIVFNTIGNLIAPRGHYKLNNQK--------K
H5          IPEIATRPKVNGQSGRMEFFWTILKPNDAINFESNGNFIAPRYAYKIVKKGD--------
H6          SPEIAARPAVNGQRGRIDYYWSILKPGETLNVESNGNLIAPWYAFRFVSTSNK-------
H7          VPSPGTRPQINGQSGRIDFHWLILDPNDTVTFSFNGAFIAPNRASFLR----------GK
H8          QPNIGPRPLVRGQQGRMDYYWGILKRGETLKIRTNGNLIAPEFGYLLKGESY--------
H9          KPVIGPRPLVNGQQGRIDYYWSVLKPGQTLRIRSNGNLIAPWYGHVLTGESH--------
H10         VPVVGARPQVNGQSGRIDFHWTLVQPGDNITFSDNGGLIAPSRVSKLT----------GR
H11         TPEINTRPRVNGQAGRMTFYWKIVKPGESITFESNGAFLAPRYAFEIVSVGN--------
H12         KPNIGPRPLVRGQQGRMDYYWAVLKPGQTVKIQTNGNLIAPEYGHLITGKSH--------
H13         KLETGVRPGYNGQRSWMKIYWSLIHPGEMITFESNGGFLAPRYGYIIEEYGK--------
H14         VPNIGSRPRVRNQSGRISIYWTLVNPGDSIIFNSIGNLIAPRGHYKISKST--------K
H15         SPSPGARPKVNGQAGRIDFHWMLLDPGDTVTFTFNGAFIAPDRATFLRSNAPSGIEYNGK
H16         ELEIGTRIG-DGQRSWMKLYWHLMHPGERIMFESNGGLIAPRYGYIIEKYGT--------
H17         RPEIGQRDEVKAHQDRIDYYWGSLPAQSTLRIESTGNLIAPEYGFYYKRKEGK-------
                                                    ▲(Residue Cq)

                        ▼(Residue Aq)                   ▼(Residue Bq)
H1          SGIITS-NASMHECNTKCQTPLGAINSSLPYQNIHPVTIGECPKYVRSAKLRMVTGLRNN
H2          SGIMKT-EGTLENCETKCQTPLGAINTTLPFHNVHPLTIGECPKYVKSERLVLATGLRNV
H3          SSIMSSDAPIDT-CISECITPNGSIPNDKPFQNVNKITYGACPKYVKQNTLKLATGMRNV
H4          STILNTAIPIGS-CVSKCHTDKGSLSTTKPFQNISRIAVGDCPRYVKQGSLKLATGMRNI
H5          SAIMKS-GLAYGNCDTKCQTPVGEINSSMPFHNIHPHTIGECPKYVKSDRLVLATGLRNV
H6          GAVFKS-NLPIENCDATCQTVAGVLRTNKTFQNVSPLWIGECPKYVKSESLRLATGLRNV
H7          SMGIQSDVQVDANCEGECYHSGGTITSRLPFQNINSRAVGKCPRYVKQESLLLATGMKNV
H8          GRIIQNEDIPIGNCNTKCQTYAGAINSSKPFQNASRHYMGECPKYVKKASLRLAVGLRNT
H9          GRILKT-DLNNGNCVVQCQTEKGGLNTTLPFHNISKYAFGNCPKYVGVKSLKLPVGLRNV
H10         DLGIQSEALIDNSCESKCFWRGGSINTKLPFQNLSPRTVGQCPKYVNQRSLLLATGMRNV
H11         GKLFRS-ELNIESCSTKCQTEIGGINTNKSFHNVHRNTIGDCPKYVNVKSLKLATGPRNV
H12         GRILKN-NLPMGQCVTECQLNEGVMNTSKPFQNTSKHYIGKCPKYIPSGSLKLAIGLRNV
H13         GRIFQS-RIRMSRCNTKCQTSVGGINTNRTFQNIDKNALGDCPKYIKSGQLKLATGLRNV
H14         STVLKSDKRIGS-CTSPCLTDKGSIQSDKPFQNVSRIAIGNCPKYVKQGSLMLATGMRNI
H15         SLGIQSDAQIDESCEGECFYSGGTINSPLPFQNIDSRAVGKCPRYVKQSSLPLALGMKNV
H16         GRIFQS-GVRMARCNTKCQTSLGGINTNKTFQNIERNALGDCPKYIKSGQLKLATGLRNV
H17         GGLMKS-KLPISDCSTKCQTPLGALNSTLPFQNVHQQTIGNCPKYVKATSLMLATGLRNN
                        ▲(Residue Aq)                   ▲(Residue Bq)
```

FIG. 1B

```
                    ▼(HA2 domain starts)
H1          P----SIQSRGLFGAIAGFIEGGWTGMIDGWYGYHHQNEQGSGYAADQKSTQNAINGITN
H2          P----QIESRGLFGAIAGFIEGGWQGMIDGWYGYHHSNDQGSGYAADKESTQKAIDGITN
H3          P----EKQTRGLFGAIAGFIENGWEGMIDGWYGFRHQNSEGTGQAADLKSTQAAIDQING
H4          P----EKASRGLFGAIAGFIENGWQGLIDGWYGFRHQNAEGTGTAADLKSTQAAIDQING
H5          P----QRKKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQGSGYAADKESTQKAIDGITN
H6          P----QIETRGLFGAIAGFIEGGWTGMIDGWYGYHHENSQGSGYAADRESTQKAVDGITN
H7          PEPSKKRKKRGLFGAIAGFIENGWEGLVDGWYGFRHQNAQGEGTAADYKSTQSAIDQITG
H8          P----SVEPRGLFGAIAGFIEGGWSGMIDGWYGFHHSNSEGTGMAADQKSTQEAIDKITN
H9          P----AVSSRGLFGAIAGFIEGGWPGLVAGWYGFQHSNDQGVGMAADKGSTQKAIDKITS
H10         P---EVVQGRGLFGAIAGFIENGWEGMVDGWYGFRHQNAQGTGQAADYKSTQAAIDQITG
H11         P----AIASRGLFGAIAGFIEGGWPGLINGWYGFQHRDEEGTGIAADKESTQKAIDQITS
H12         P----QVQDRGLFGAIAGFIEGGWPGLVAGWYGFQHQNAEGTGIAADRDSTQRAIDNMQN
H13         P----AISNRGLFGAIAGFIEGGWPGLINGWYGFQHQNEQGTGIAADKESTQKAIDQITT
H14         P----GKQAKGLFGAIAGFIENGWQGLIDGWYGFRHQNAEGTGTAADLKSTQAAIDQING
H15         P---EKIRTRGLFGAIAGFIENGWEGLIDGWYGFRHQNAQGQGTAADYKSTQAAIDQITG
H16         P----SIGERGLFGAIAGFIEGGWPGLINGWYGFQHQNEQGTGIAADKASTQKAINEITT
H17         P----QMEGRGLFGAIAGFIEGGWQGMIDGWYGYHHENQEGSGYAADKEATQKAVDAITN
                    ▲(HA2 domain starts)

H1          KVNTVIEKMNIQFTAVGKEFNKLEKRMENLNKKVDDGFLDIWTYNAELLVLLENERTLDF
H2          RVNSVIEKMNTQFEAVGKEFSNLEKRLENLNKKMEDGFLDVWTYNAELLVLMENERTLDF
H3          KLNRVIEKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDL
H4          KLNRLIEKTNDKYHQIEKEFEQVEGRIQDLENYVEDTKIDLWSYNAELLVALENQHTIDV
H5          KVNSIIDKMNTRFEAVGKEFNNLERRVENLNKKMEDGFLDVWTYNVELLVLMENERTLDF
H6          KVNSIIDKMNTQFEAVDHEFSNLERRIDNLNKRMEDGFLDVWTYNAELLVLLENERTLDL
H7          KLNRLIEKTNQQFELIDNEFTEVEKQIGNLINWTKDSITEVWSYNAELIVAMENQHTIDL
H8          KVNNIVDKMNREFEVVNHEFSEVEKRINMINDKIDDQIEDLWAYNAELLVLLENQKTLDE
H9          KVNNIIDKMNKQYEVIDHEFNELEARLNMINNKIDDQIQDIWAYNAELLVLLENQKTLDE
H10         KLNRLIEKTNTEFESIESEFSETEHQIGNVINWTKDSITDIWTYNAELLVAMENQHTIDM
H11         KVNNIVDRMNTNFESVQHEFSEIEERINQLSKHVDDSVVDIWSYNAQLLVLLENEKTLDL
H12         KLNNVIDKMNKQFEVVNHEFSEVESRINMINSKIDDQITDIWAYNAELLVLLENQKTLDE
H13         KINNIIDKMNGNYDSIRGEFNQVEKRINMLADRIDDAVTDIWSYNAKLLVLLENDKTLDM
H14         KLNRLIEKTNEKYHQIEKEFEQVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDV
H15         KLNRLIEKTNKQFELIDNEFTEVEQQIGNVINWTRDSLTEIWSYNAELLVAMENQHTIDL
H16         KINNIIEKMNGNYDSIRGEFNQVEKRINMLADRVDDAVTDIWSYNAKLLVLLENDRTLDL
H17         KVNSIIDKMNSQFESNIKEFNRLELRIQHLSDRVDDALLDIWSYNTELLVLLENERTLDF

H1          HDSNVKNLYEKVKSQLKNNAKEIGNGCFEFYHKCDNECMESVRNGTYDYPKYSEESKLNR
H2          HDSNVKNLYDRVRMQLRDNAKELGNGCFEFYHKCDDECMNSVKNGTYDYPKYEEESKLNR
H3          TDSEMNKLFEKTRRQLRENAEDMGNGCFKIYHKCDNACIESIRNGTYDHDVYRDEALNNR
H4          TDSEMNKLFERVRRQLRENAEDKGNGCFEIFHKCDNNCIESIRNGTYDHDIYRDEAINNR
H5          HDSNVNNLYDKVRLQLKDNARELGNGCFEFYHKCDNECMESVRNGTYDYPQYSEEARLNR
H6          HDANVKNLYERVKSQLRDNAMILGNGCFEFWHKCDDECMESVKNGTYDYPKYQDESKLNR
H7          ADSEMNRLYERVRKQLRENAEEDGTGCFEIFHKCDDDCMASIRNNTYDHSKYREEAMQNR
H8          HDSNVKNLFDEVKRRLSANAIDAGNGCFDILHKCDNECMETIKNGTYDHKEYEEEAKLER
H9          HDANVNNLYNKVKRALGSNAVEDGNGCFELYHKCDDQCMETIRNGTYDRQKYQEESRLER
H10         ADSEMLNLYERVRKQLRQNAEEDGKGCFEIYHTCDDSCMESIRNNTYDHSQYREEALLNR
H11         HDSNVRNLHEKVRRMLKDNAKDEGNGCFTFYHKCDNKCIERVRNGTYDHKEFEEESKINR
H12         HDANVRNLHDRVRRVLRENAIDTGDGCFEILHKCDNNCMDTIRNGTYNHKEYEEESKIER
H13         HDANVKNLHEQVRRELKDNAIDEGNGCFELLHKCNDSCMETIRNGTYDHTEYAEESKLKR
H14         TDSEMNKLFERVRRQLRENAEDQGNGCFEIFHQCDNNCIESIRNGTYDHNIYRDEAINNR
H15         ADSEMNKLYERVRRQLRENAEEDGTGCFEIFHRCDDQCMESIRNNTYNHTEYRQEALQNR
H16         HDANVRNLHDQVKRALKSNAIDEGDGCFNLLHKCNDSCMETIRNGTYNHEDYREESQLKR
H17         HDANVKNLFEKVKAQLKDNAIDEGNGCFLLLHKCNNSCMDDIKNGTYKYMDYREESHIEK
```

FIG. 1C

```
H1              EKVDGVKLESMG-IYQILAIYSTVASSLVLLVSLGAISFWMCSNGSLQCRICI
H2              NEIKGVKLSNMG-VYQILAIYATVAGSLSLAIMIAGISLWMCSNGSLQCRICI
H3              FQIKGVELKSGY--KDWILWISFAISCFLLCVVLLGFIMWACQRGNIRCNICI
H4              FQIQGVKLTQGY--KDIILWISFSISCFLLVALLLAFILWACQNGNIRCQICI
H5              EEISGVKLESMG-VYQILSIYSTVASSLALAIMIAGLSFWMCSNGSLQCRICI
H6              QEIESVKLESLG-VYQILAIYSTVSSSLVLVGLIIAVGLWMCSNGSMQCRICI
H7              IQIDPVKLSSGY--KDVILWFSFGASCFLLLAIAMGLVFICVKNGNMRCTICI
H8              SKINGVKLEENT-TYKILSIYSTVAASLCLAILIAGGLILGMQNGSCRCMFCI
H9              QKIEGVKLESEG-TYKILTIYSTVASSLVLAMGFAAFLFWAMSNGSCRCNICI
H10             LNINPVKLSSGY--KDIILWFSFGESCFVLLAVVMGLVFFCLKNGNMRCTICI
H11             QEIEGVKLDSSGNVYKILSIYSCIASSLVLAALIMGFMFWACSNGSCRCTICI
H12             QKVNGVKLEENS-TYKILSIYSSVASSLVLLLMIIGGFIFGCQNGNVRCTFCI
H13             QEIDGIKLKSEDNVYKALSIYSCIASSVVLVGLILSFIMWACSSGNCRFNVCI
H14             IKINPVTLTMGY--KDIILWISFSMSCFVFVALILGFVLWACQNGNIRCQICI
H15             IMINPVKLSSGY--KDVILWFSFGASCVMLLAIAMGLIFMCVKNGNLRCTICI
H16             QEIEGIKLKTEDNVYKVLSIYSCIASSIVLVGLILAFIMWACSNGSCRFNVCI
H17             QKIDGVKLTDYS-RYYIMTLYSTIASSVVLGSLIIAAFLWGCQKGSIQCKICI
```

FIG. 1D

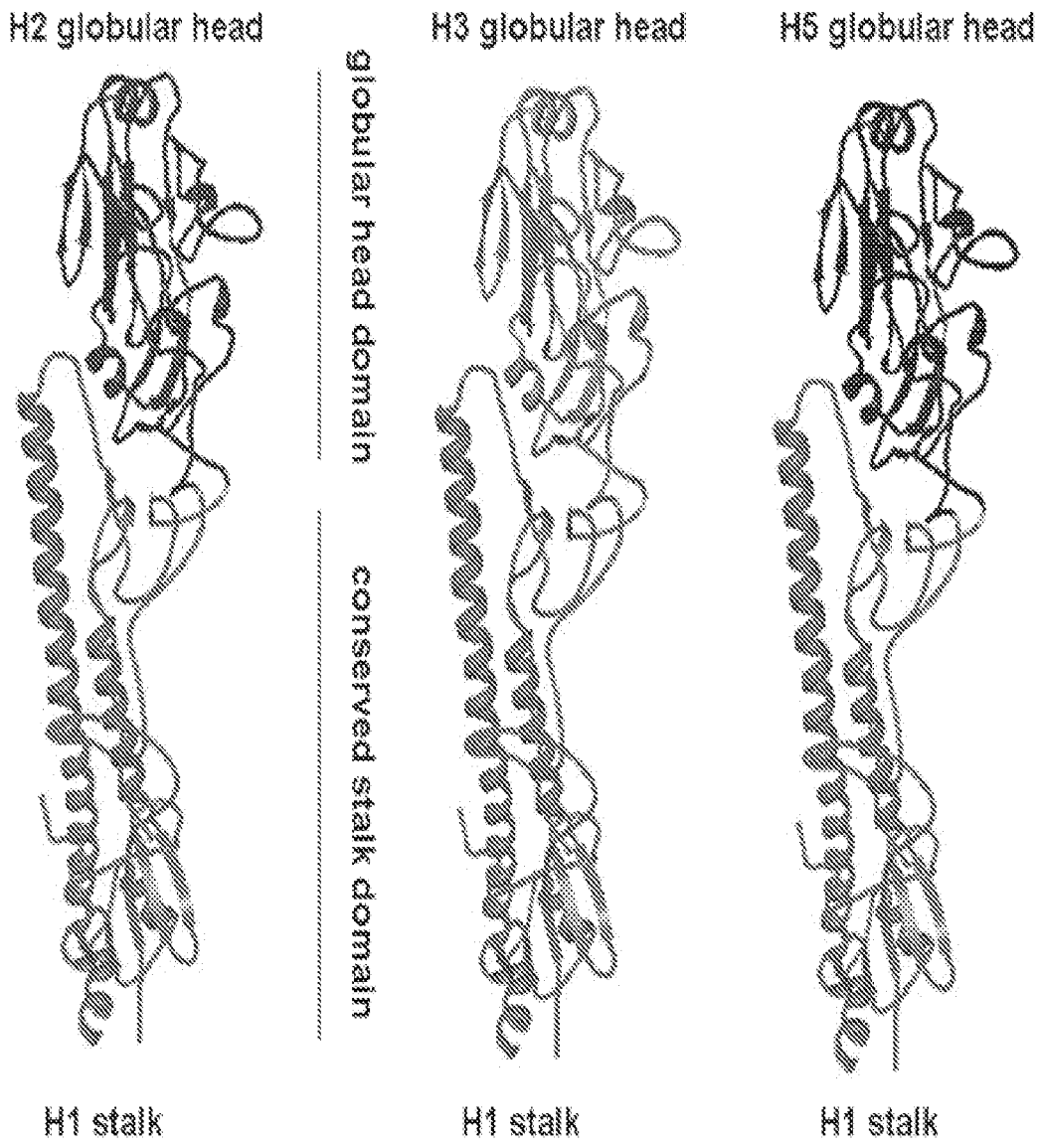

H2 globular head
H3 globular head
H5 globular head
globular head domain
conserved stalk domain
H1 stalk
H1 stalk
H1 stalk

FIG. 2

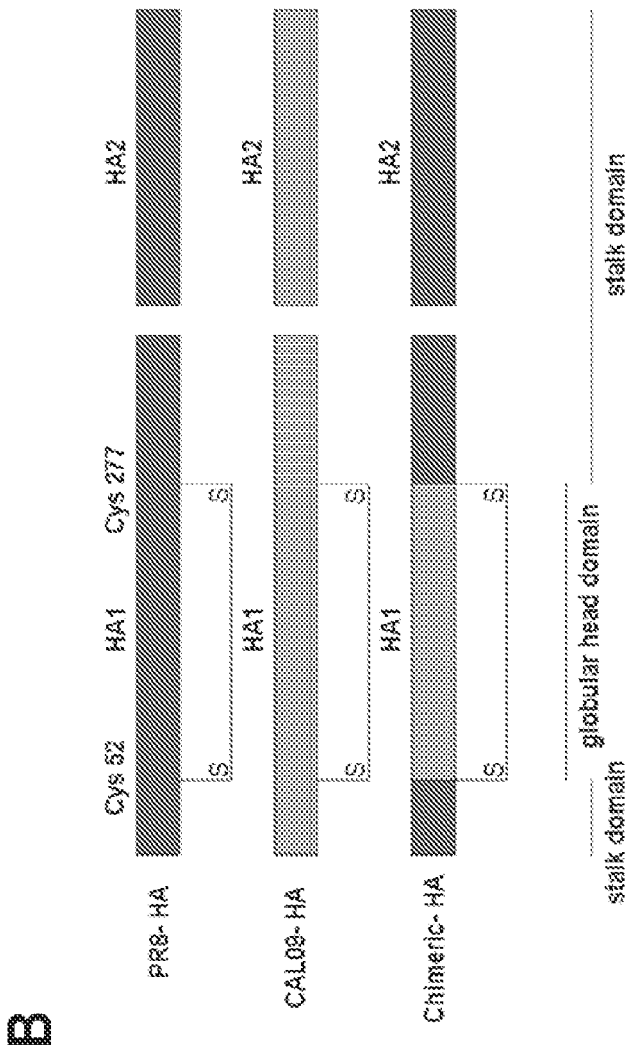

B
Cys 52
HA1
Cys 277
HA2
S
S
PR8- HA
CAL09- HA
Chimeric- HA
stalk domain
globular head domain
stalk domain
A
GFP
PR8 HA
CAL09 HA
Chimeric HA
Anti-PR8 HA (PY102)
Anti-CAL09 HA (35C2)
140
98
64
50
36
HAo
actin

FIG. 3

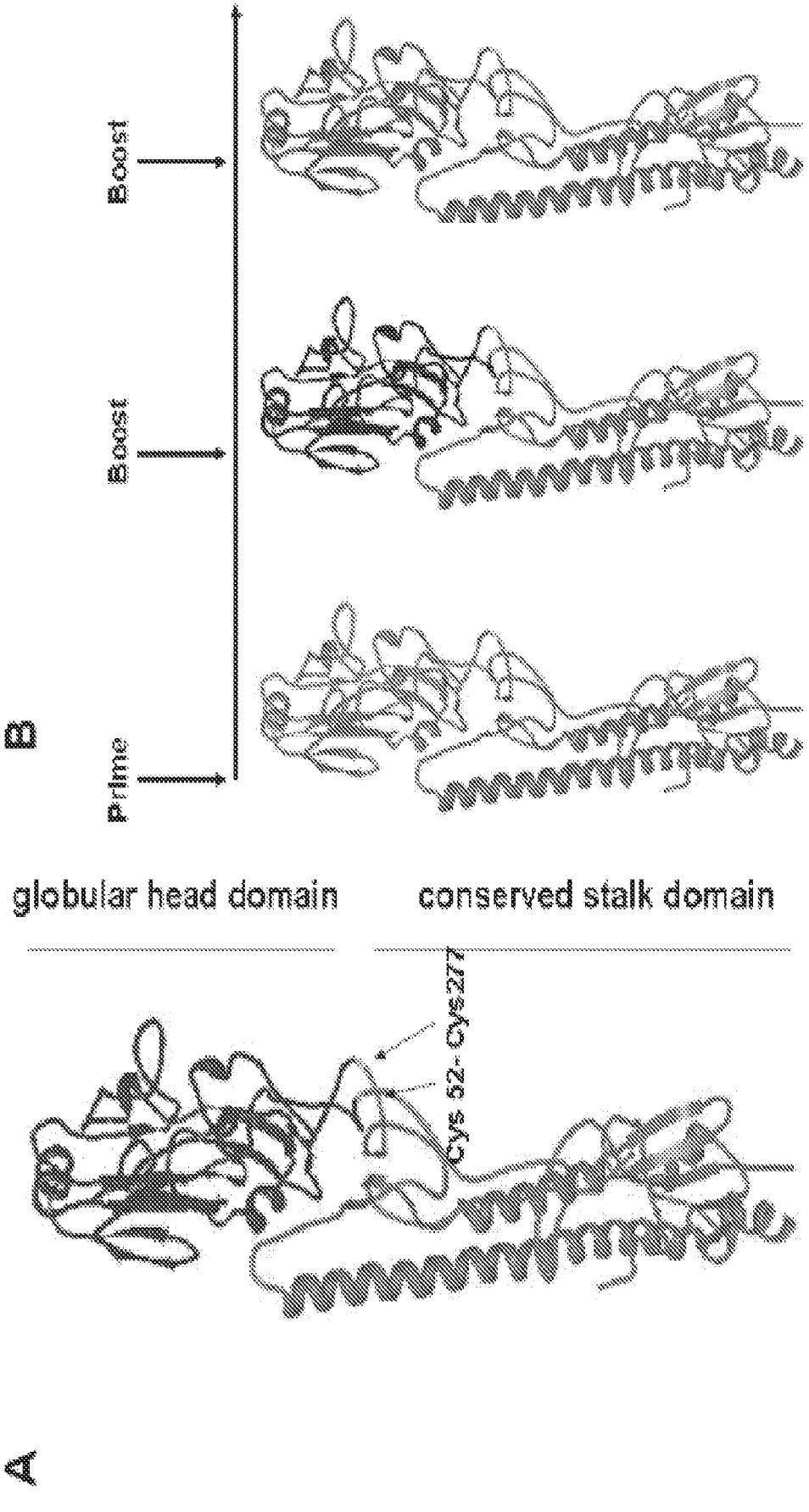

B
Prime
Boost
Boost
globular head domain
conserved stalk domain
Cys 52- Cys277
A

FIG. 4

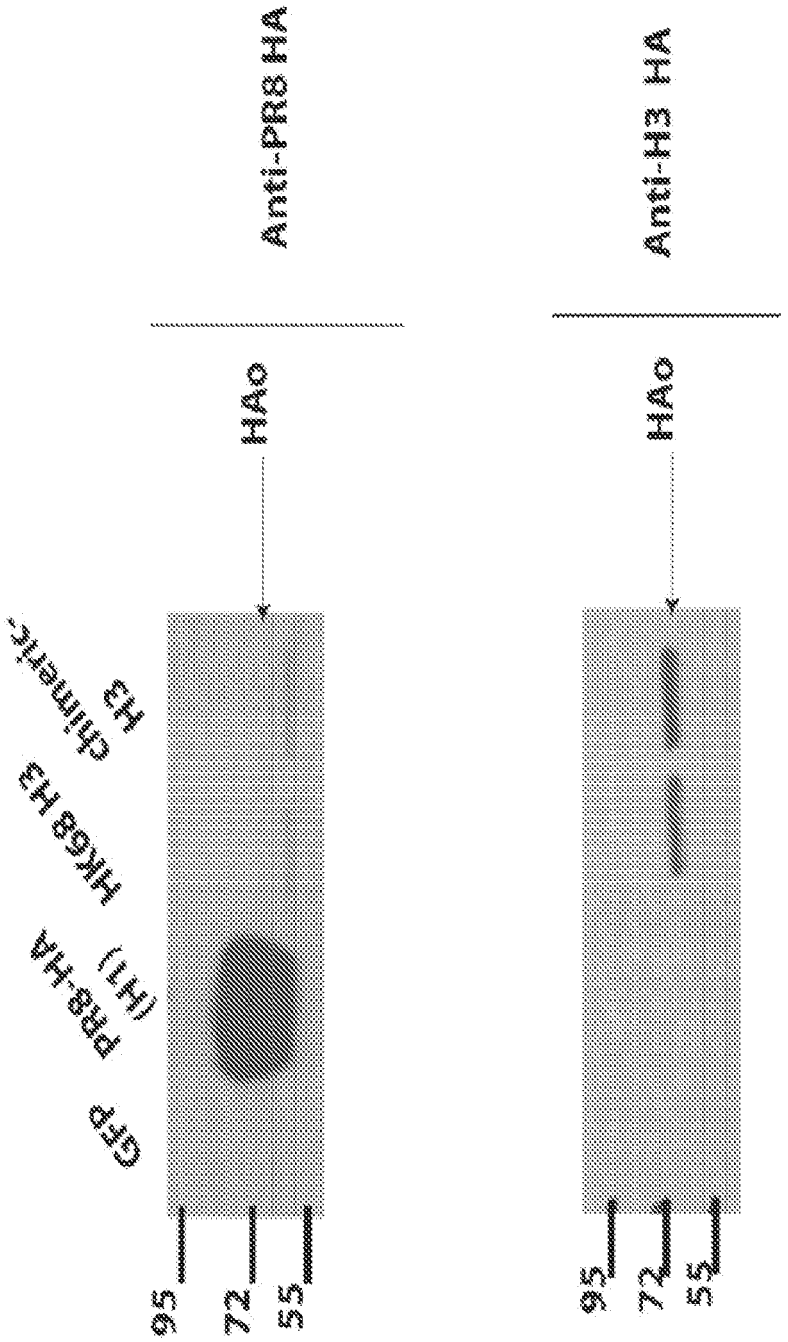

Anti-PR8 HA
Anti-H3 HA
HAo
HAo
GFP
PR8-HA (H1)
HK68 H3
chimeric H3
95
72
55
95
72
55

FIG. 5

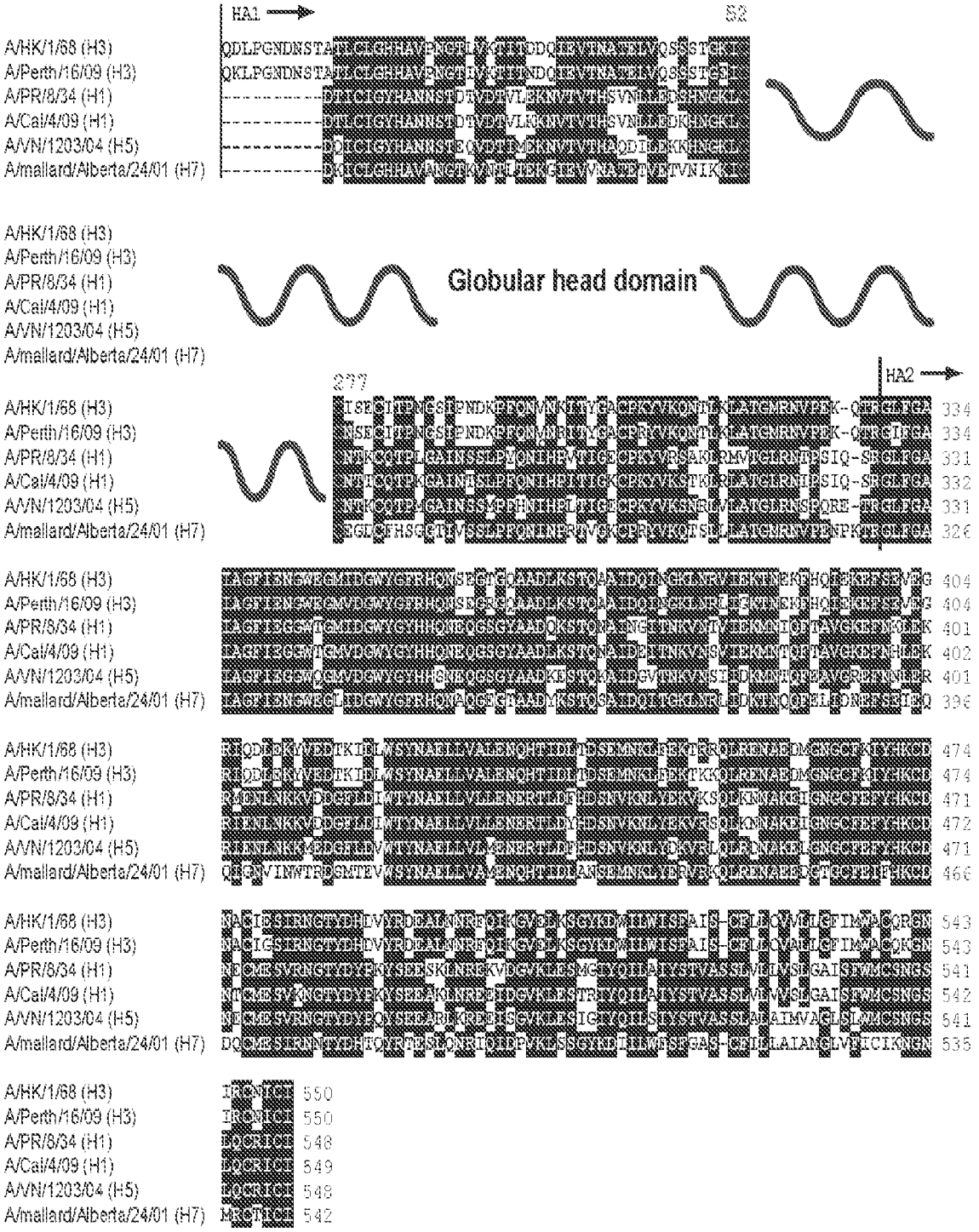

HA1
S2
A/HK/1/68 (H3)
A/Perth/16/09 (H3)
A/PR/8/34 (H1)
A/Cal/4/09 (H1)
A/VN/1203/04 (H5)
A/mallard/Alberta/24/01 (H7)
Globular head domain
HA2
277
334
404
474
543
550

FIG. 6

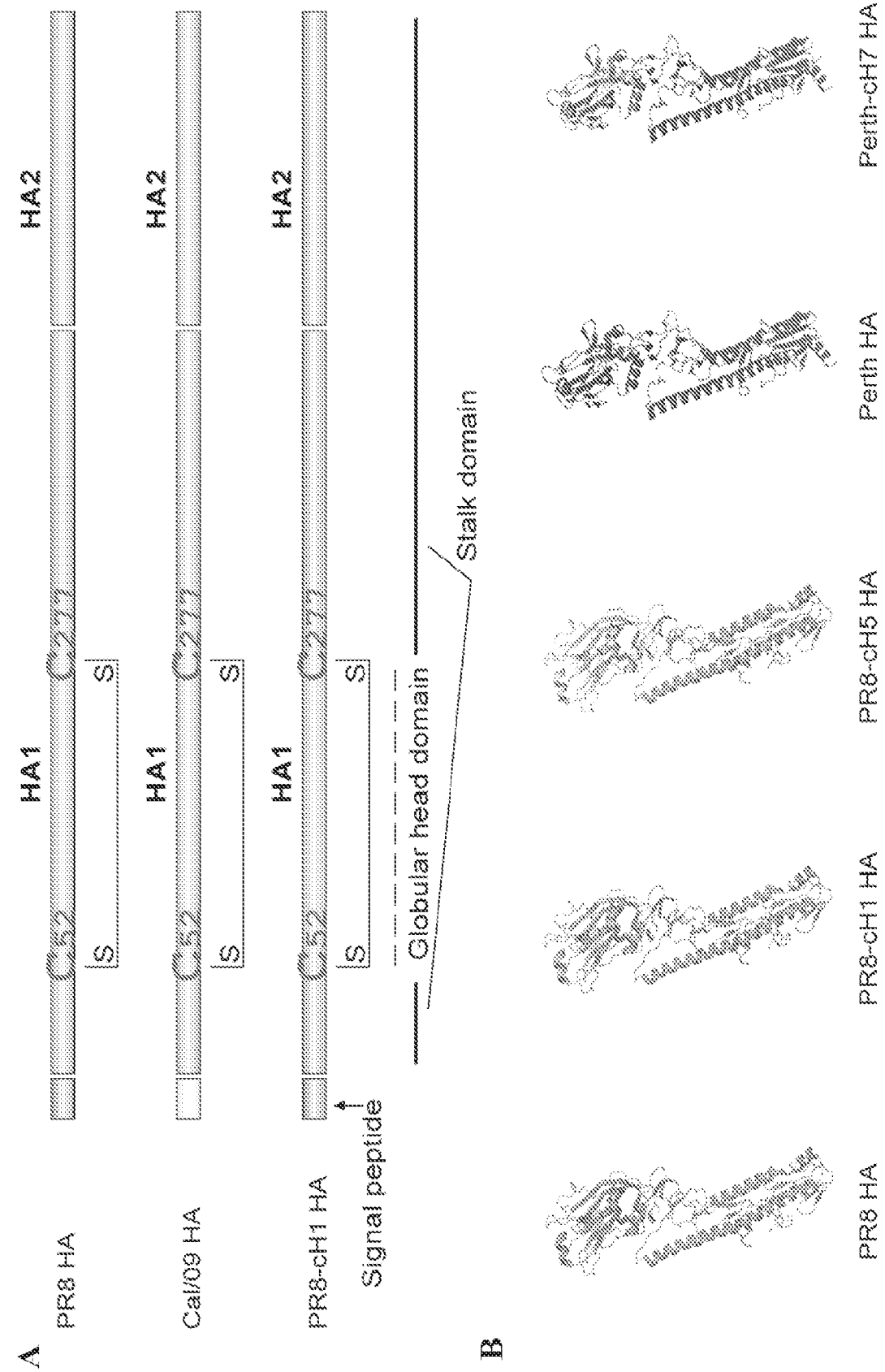

A
HA1
HA2
PR8 HA
Cal/09 HA
PR8-cH1 HA
Signal peptide
Globular head domain
Stalk domain
B
PR8 HA
PR8-cH1 HA
PR8-cH5 HA
Perth HA
Perth-cH7 HA

FIG. 7A - 7B

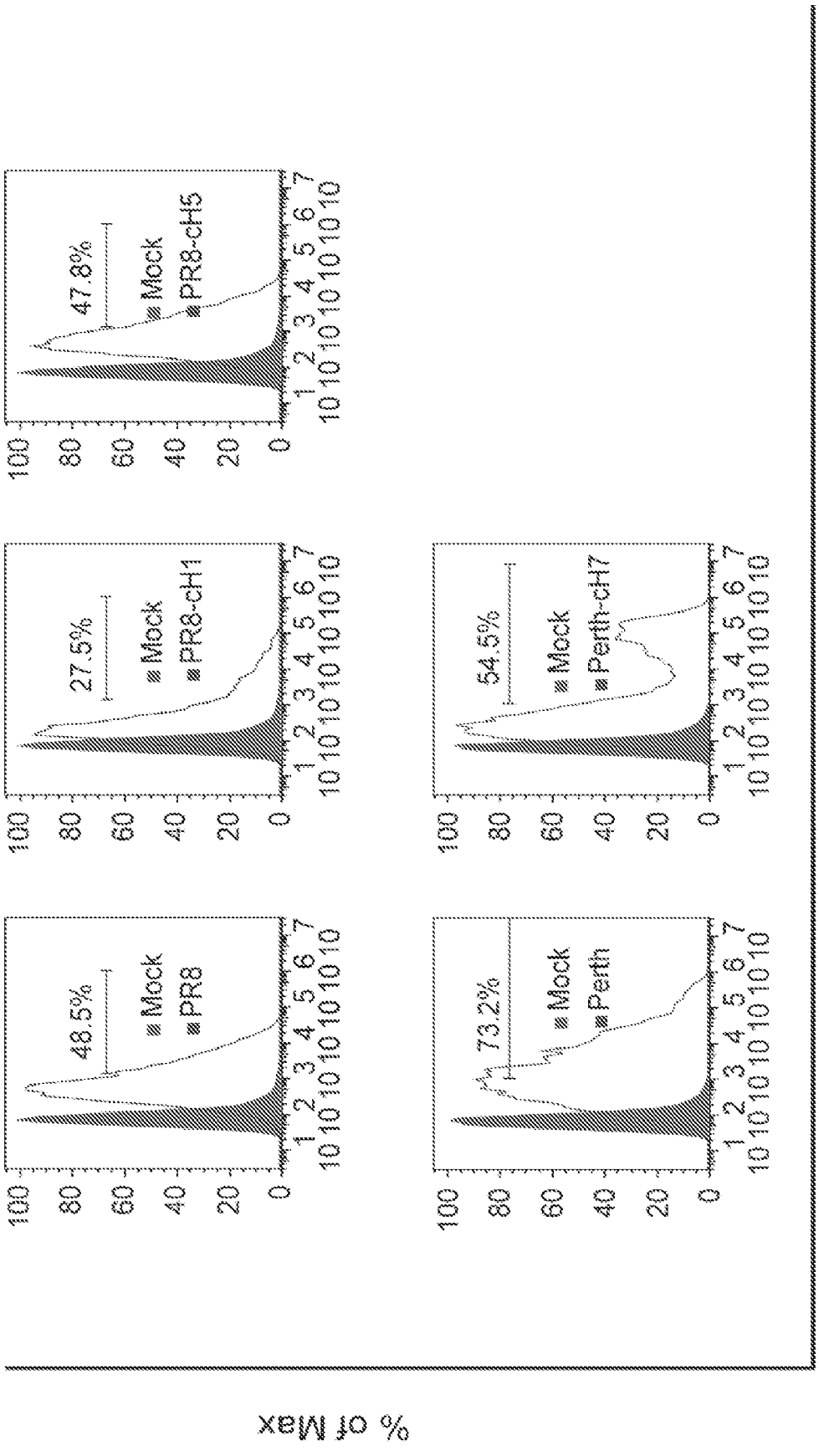

% of Max
Hemagglutinin
48.5%
Mock
PR8
27.5%
Mock
PR8-cH1
47.8%
Mock
PR8-cH5
73.2%
Mock
Perth
54.5%
Mock
Perth-cH7

FIG. 8A

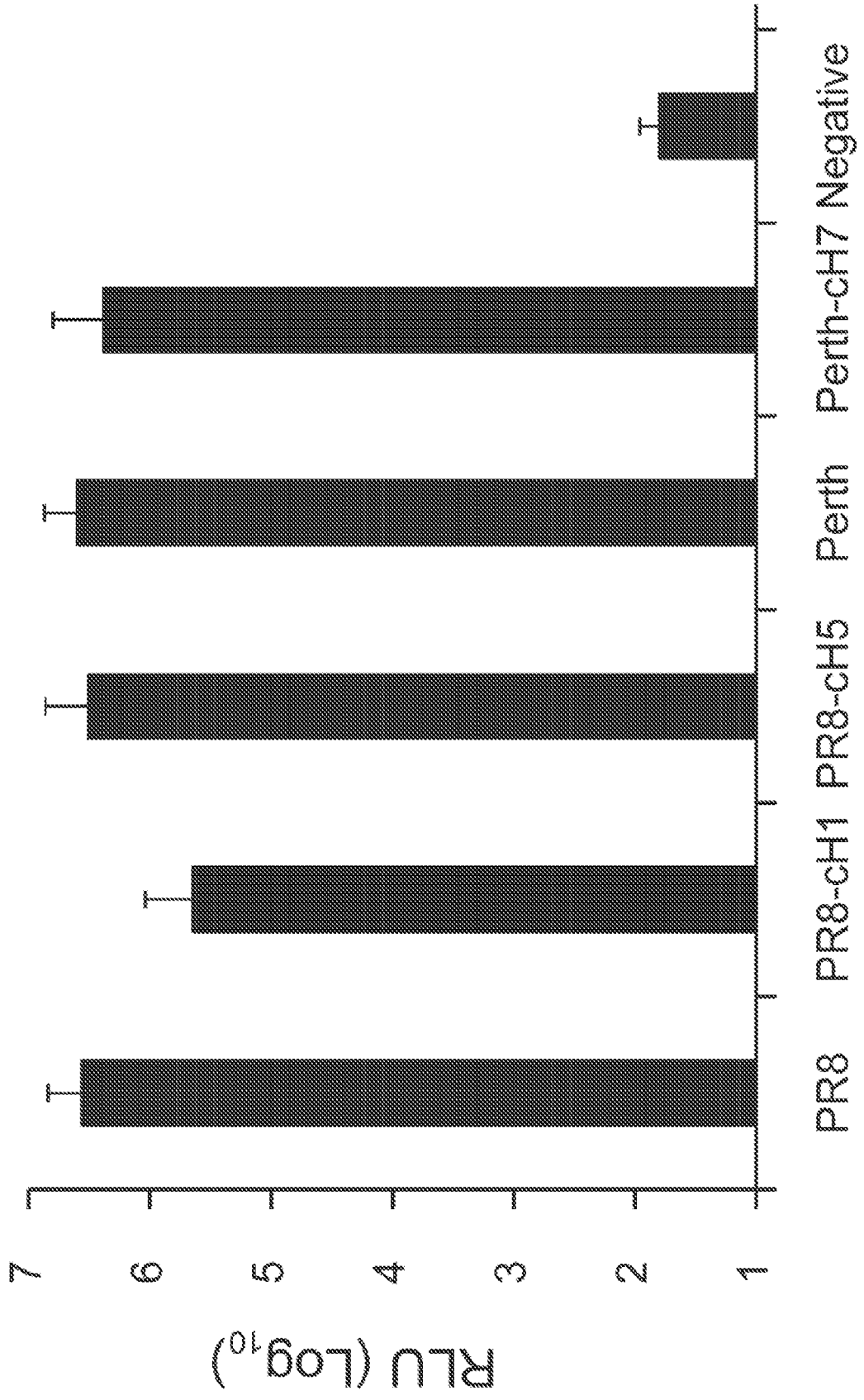

RLU (Log10)
7
6
5
4
3
2
1
PR8
PR8-cH1
PR8-cH5
Perth
Perth-cH7
Negative

FIG. 8B

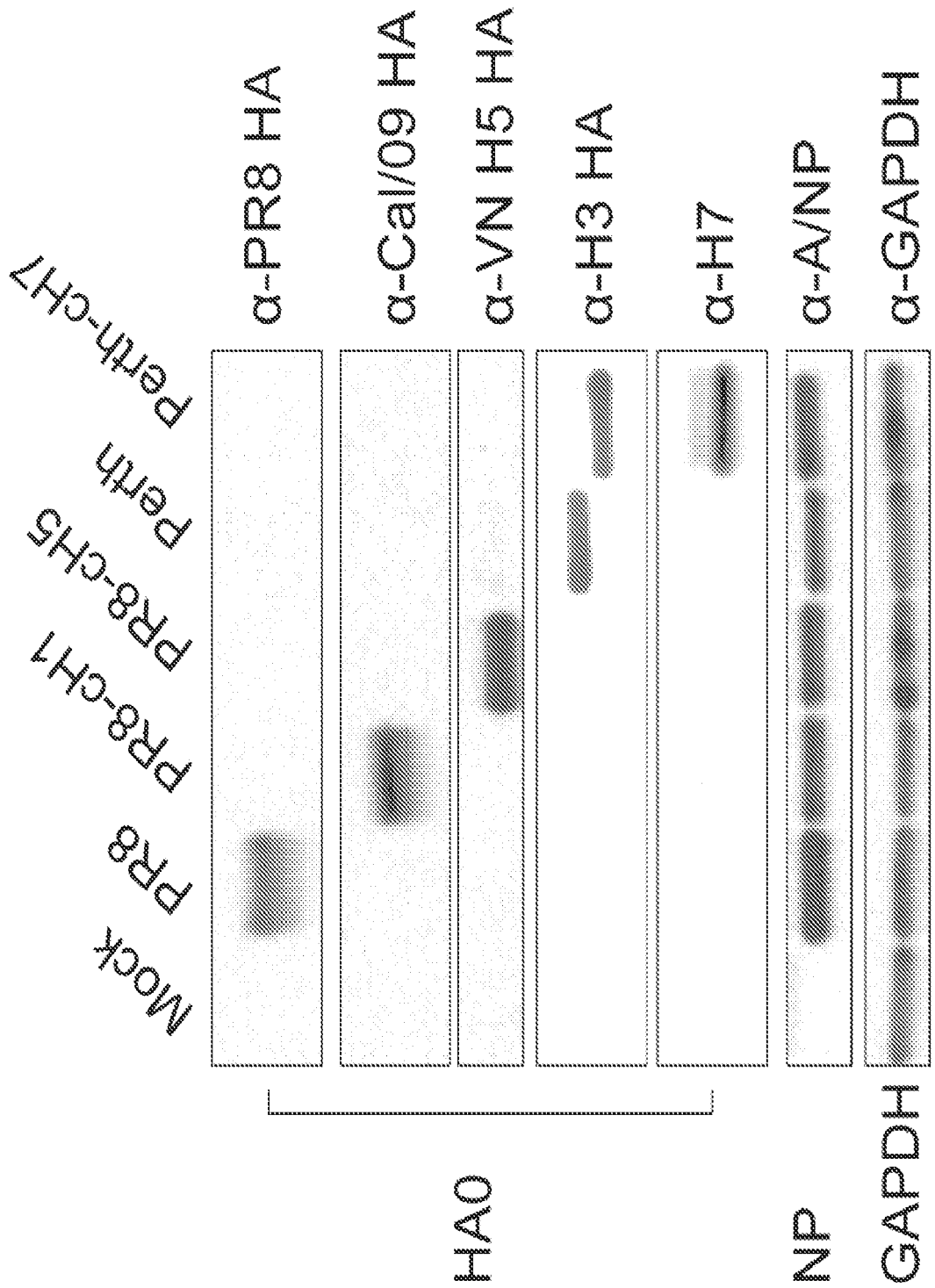

α-PR8 HA
α-Cal/09 HA
α-VN H5 HA
α-H3 HA
α-H7
α-A/NP
α-GAPDH
Mock
PR8
PR8-CH1
PR8-CH5
Perth
Perth-CH7
HA0
NP
GAPDH

FIG. 9A

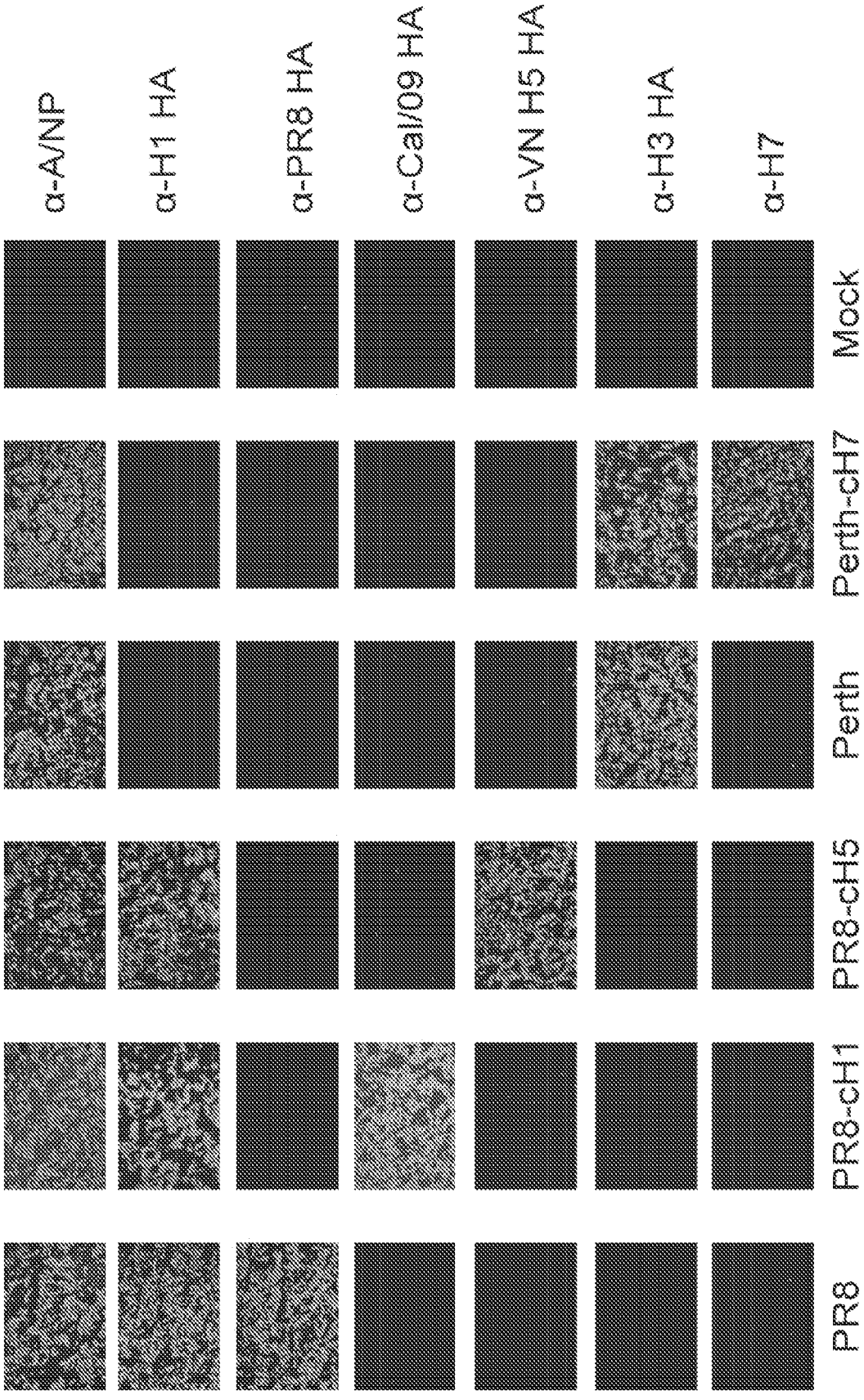

α-A/NP
α-H1 HA
α-PR8 HA
α-Cal/09 HA
α-VN H5 HA
α-H3 HA
α-H7
Mock
Perth-cH7
Perth
PR8-cH5
PR8-cH1
PR8

FIG. 9B

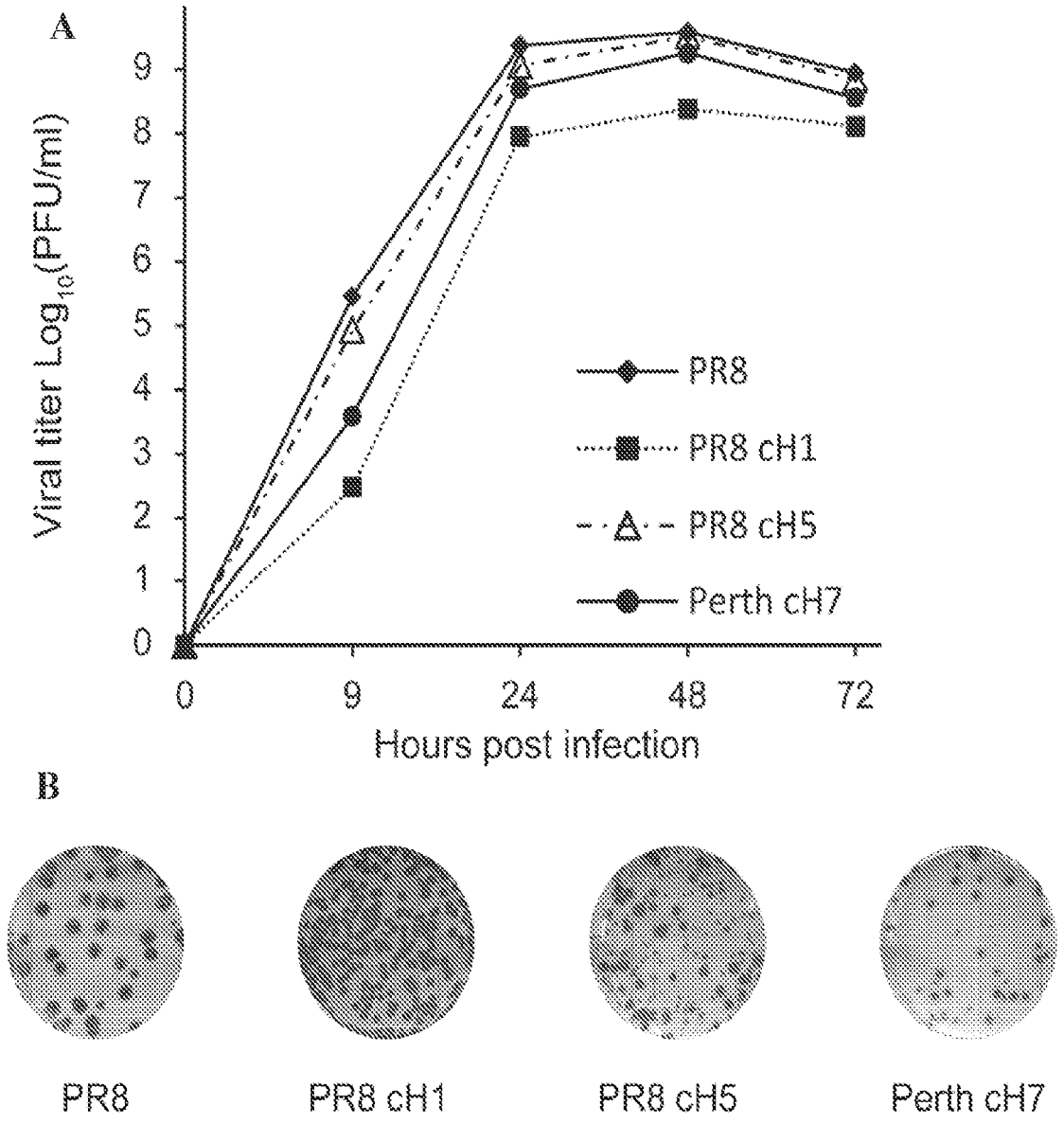

A
Viral titer Log10(PFU/ml)
0
1
2
3
4
5
6
7
8
9
0
9
24
48
72
Hours post infection
PR8
PR8 cH1
PR8 cH5
Perth cH7
B
PR8
PR8 cH1
PR8 cH5
Perth cH7

FIG. 10A - 10B

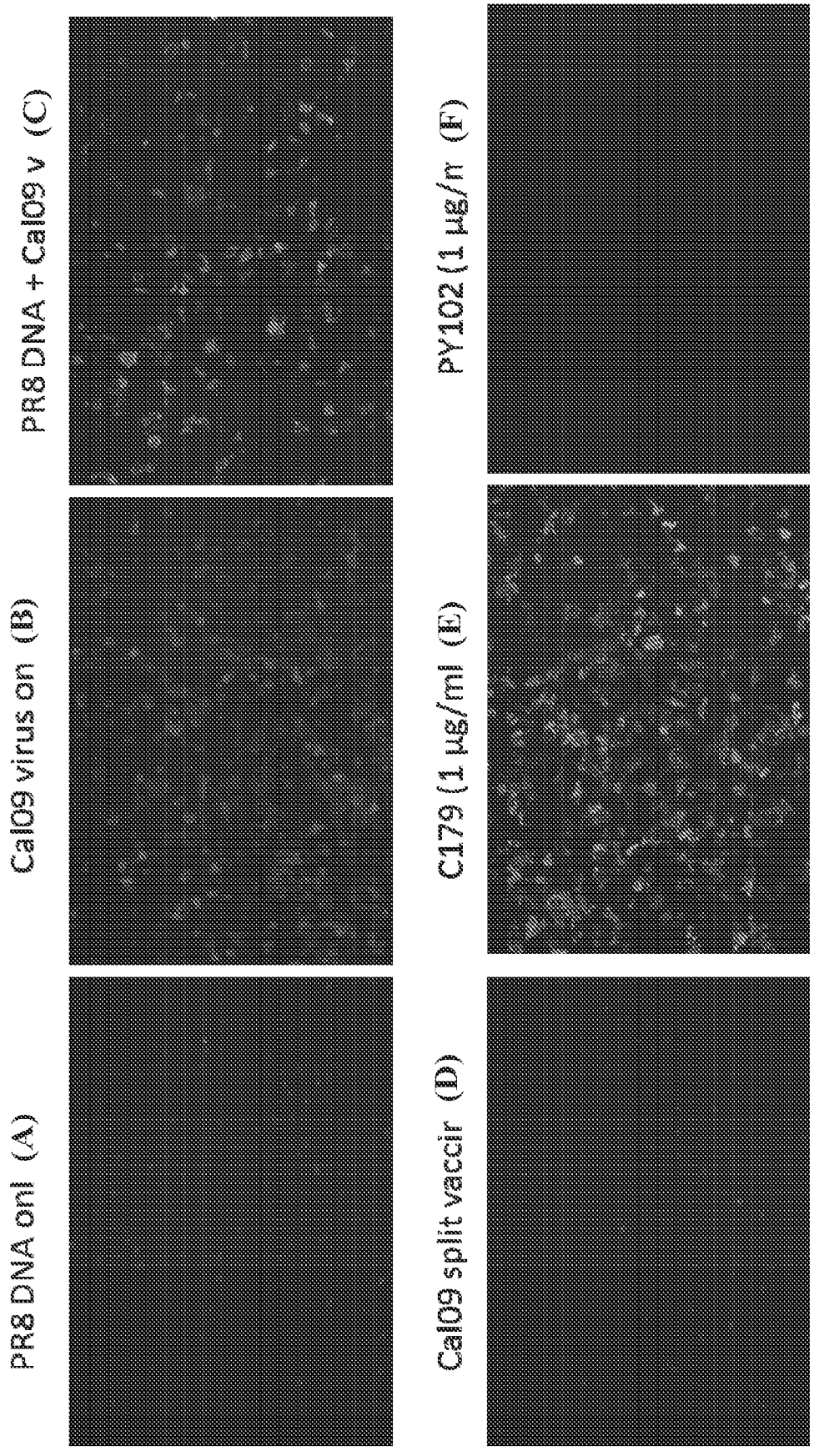

PR8 DNA onl (A)
Cal09 virus on (B)
PR8 DNA + Cal09 v (C)
Cal09 split vaccir (D)
C179 (1 µg/ml (E)
PY102 (1 µg/m (F)

FIG. 11A - 11B

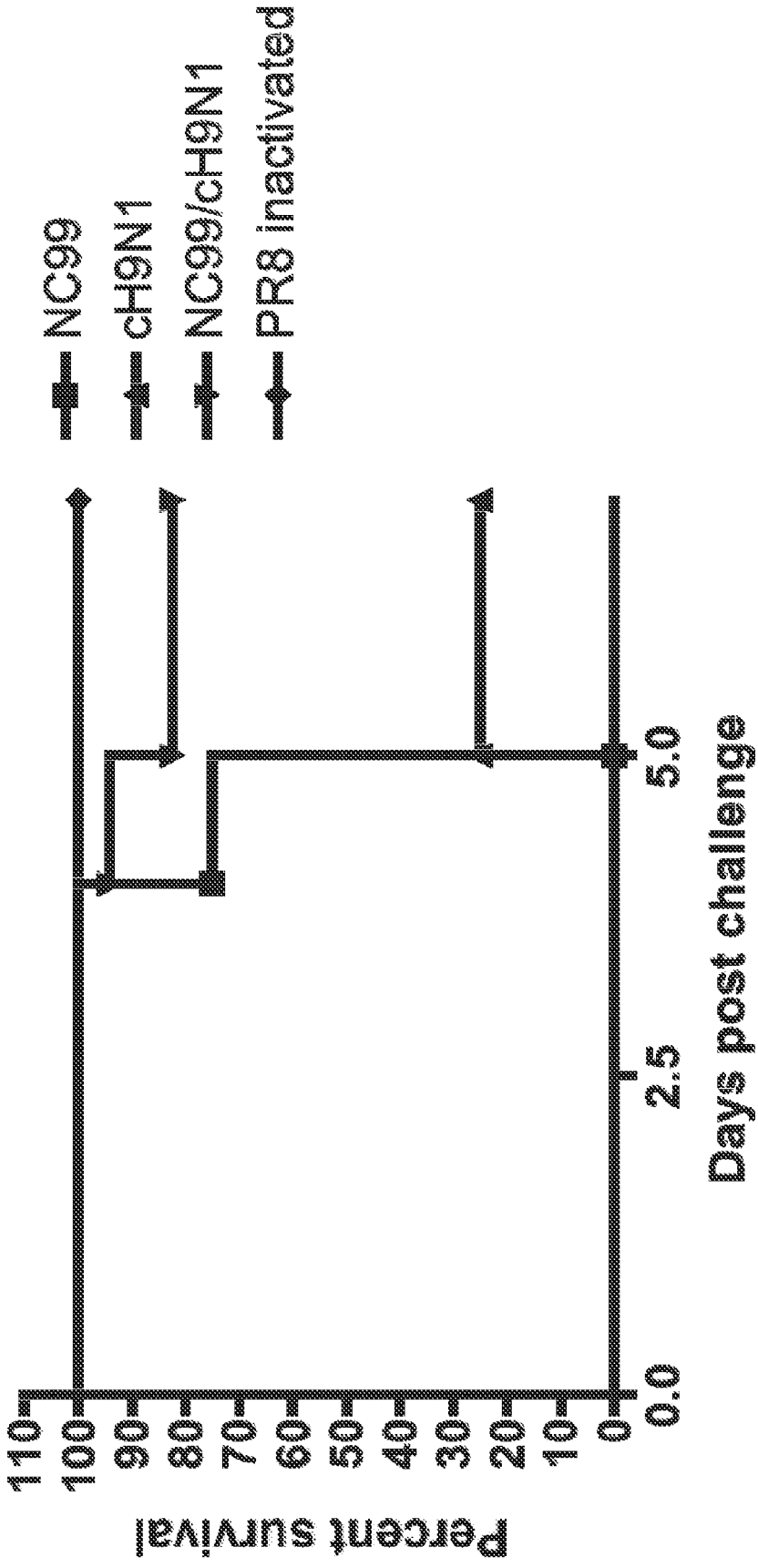

NC99
cH9N1
NC99/cH9N1
PR8 inactivated
Percent survival
110
100
90
80
70
60
50
40
30
20
10
0
0.0
2.5
5.0
Days post challenge

FIG. 12

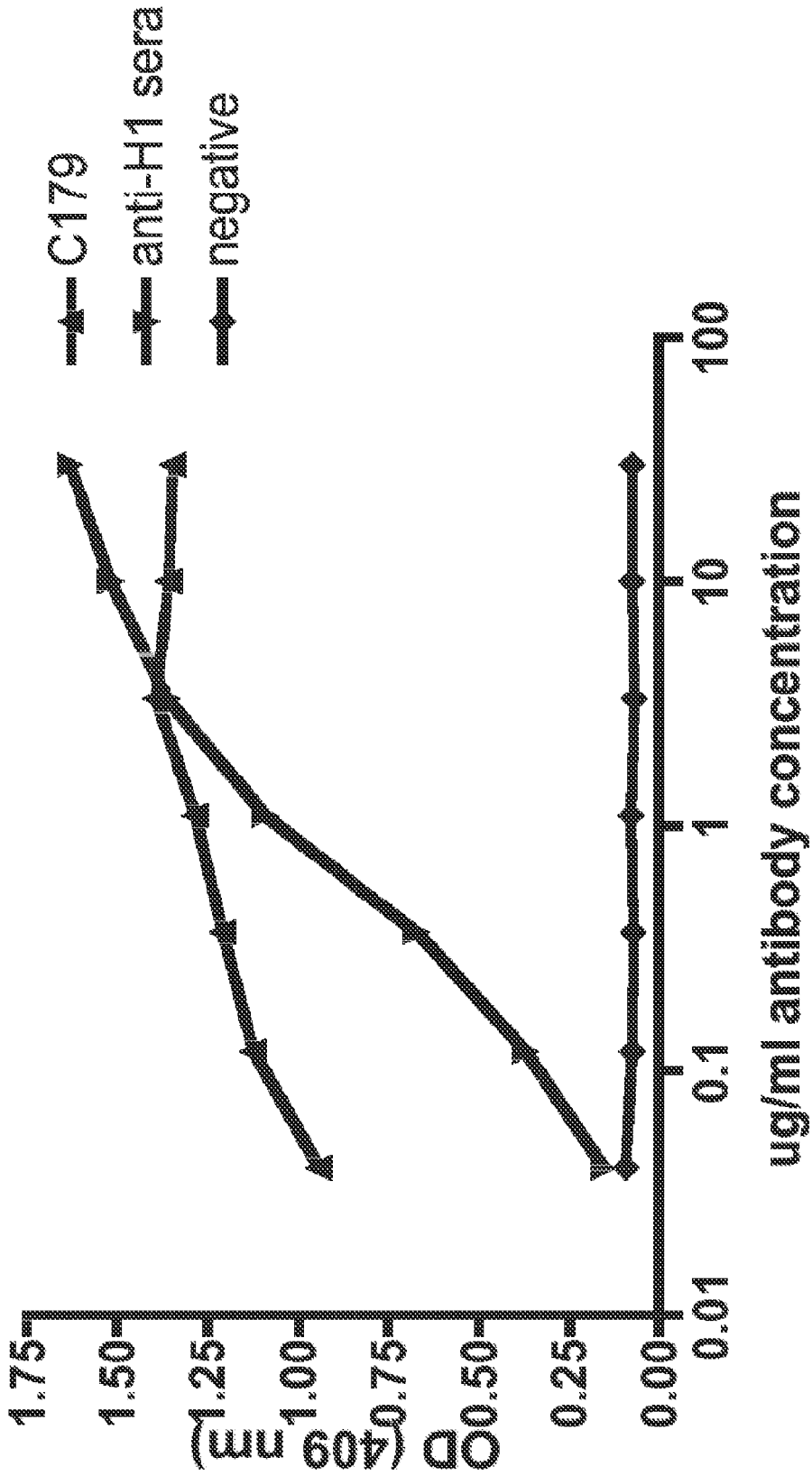
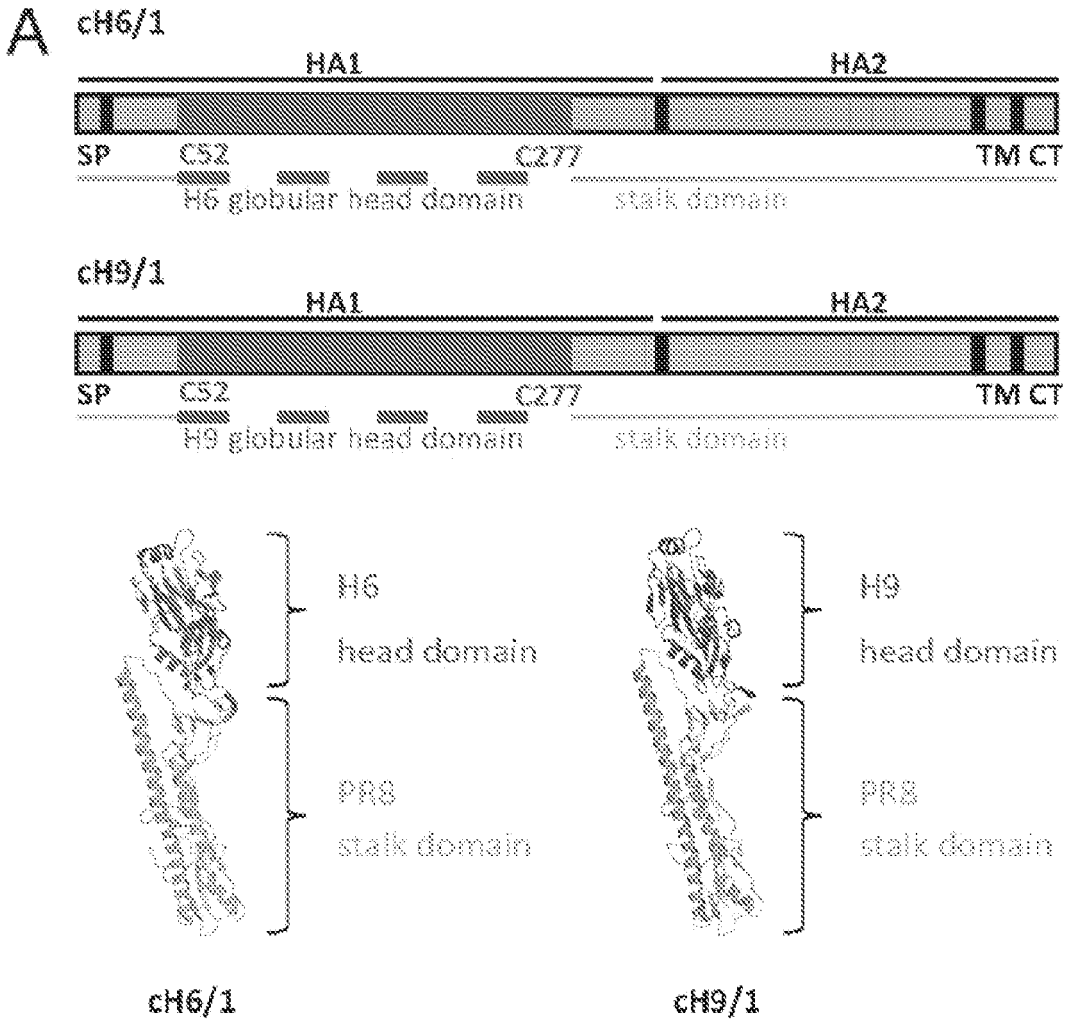

C179
anti-H1 sera
negative
OD (409 nm)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0.01
0.1
1
10
100
ug/ml antibody concentration

FIG. 13


A
cH6/1
HA1
HA2
SP
C52
C277
TM CT
H6 globular head domain
stalk domain
cH9/1
HA1
HA2
SP
C52
C277
TM CT
H9 globular head domain
stalk domain
H6
head domain
PR8
stalk domain
H9
head domain
PR8
stalk domain
cH6/1
cH9/1

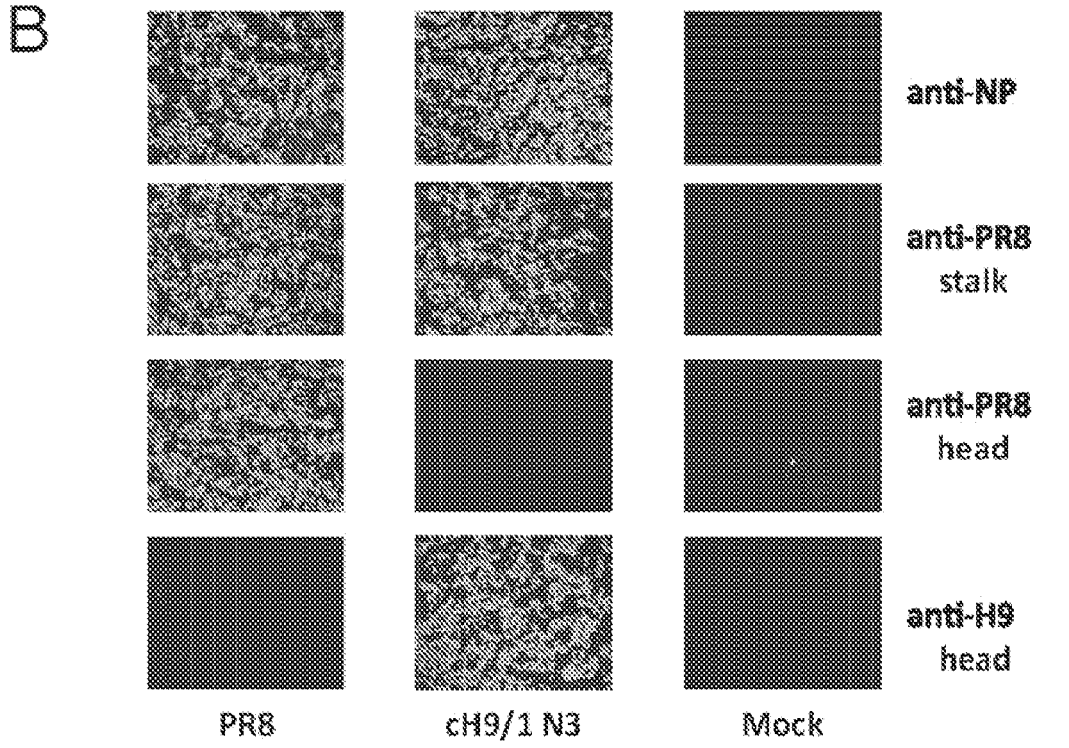

B
anti-NP
anti-PR8
stalk
anti-PR8
head
anti-H9
head
PR8
cH9/1 N3
Mock

FIG. 14

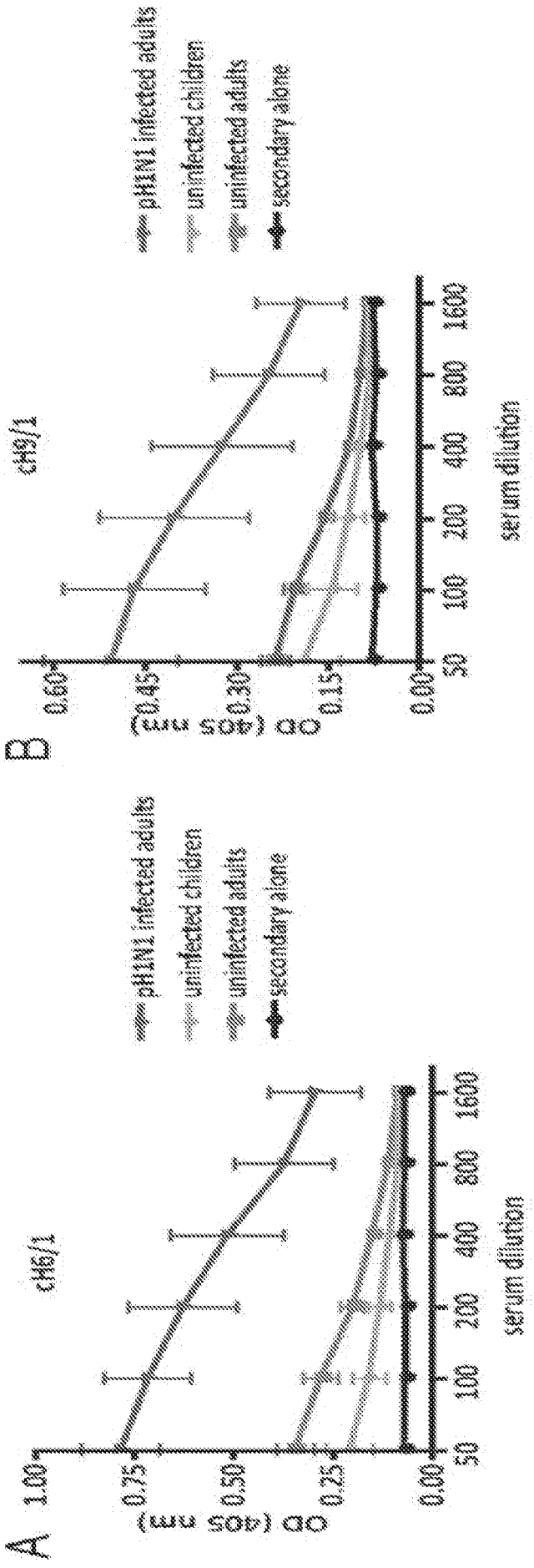

A
cH6/1
OD (405 nm)
1.00
0.75
0.50
0.25
0.00
50
100
200
400
800
1600
serum dilution
pH1N1 infected adults
uninfected children
uninfected adults
secondary alone
B
cH9/1
OD (405 nm)
0.60
0.45
0.30
0.15
0.00
50
100
200
400
800
1600
serum dilution
pH1N1 infected adults
uninfected children
uninfected adults
secondary alone

FIG. 15A - 15B

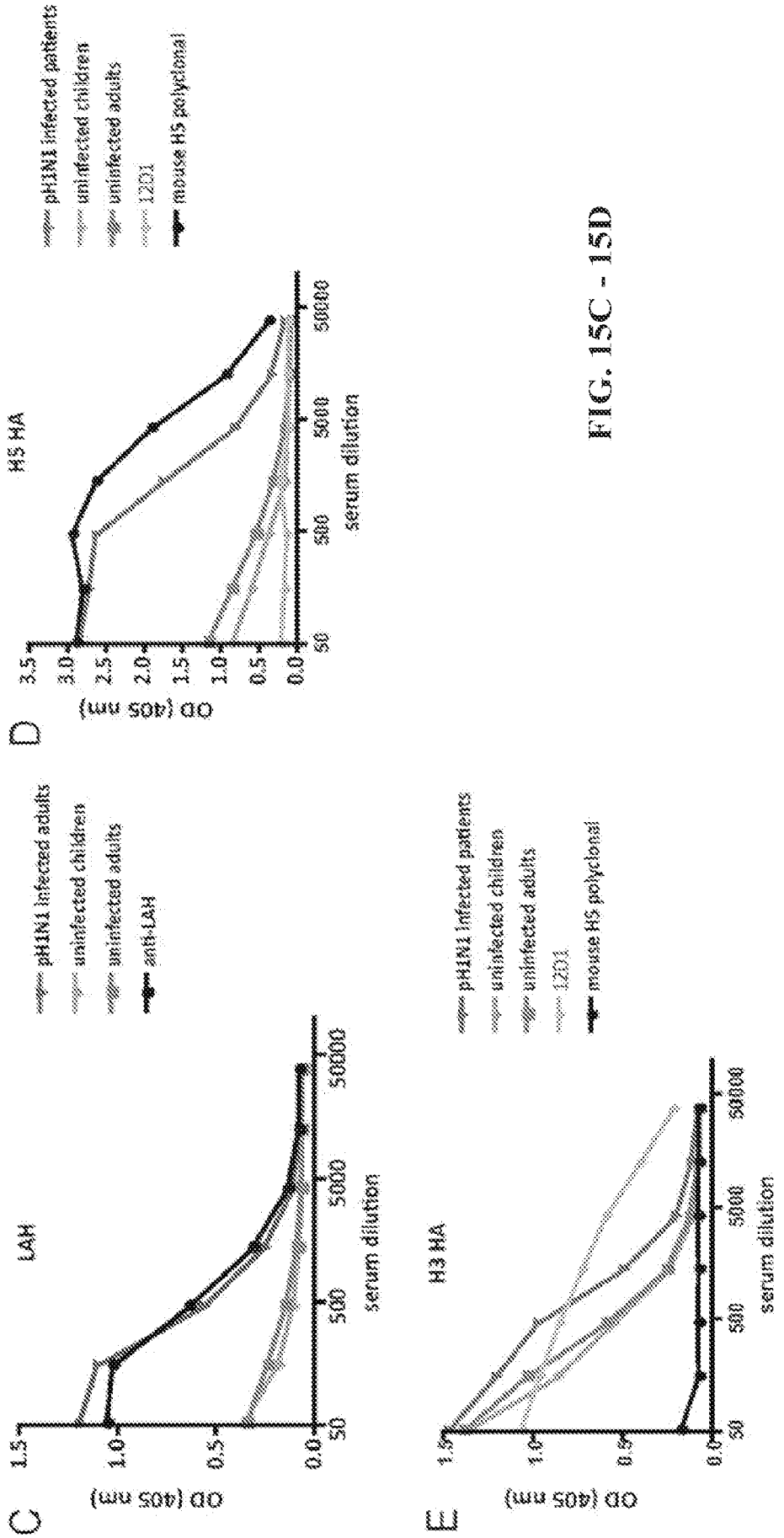

C
LAH
OD (405 nm)
serum dilution
pH1N1 infected adults
uninfected children
uninfected adults
anti-LAH
D
H5 HA
OD (405 nm)
serum dilution
pH1N1 infected patients
uninfected children
uninfected adults
1201
mouse H5 polyclonal
E
H3 HA
OD (405 nm)
serum dilution
pH1N1 infected patients
uninfected children
uninfected adults
1201
mouse H5 polyclonal

FIG. 15C - 15D

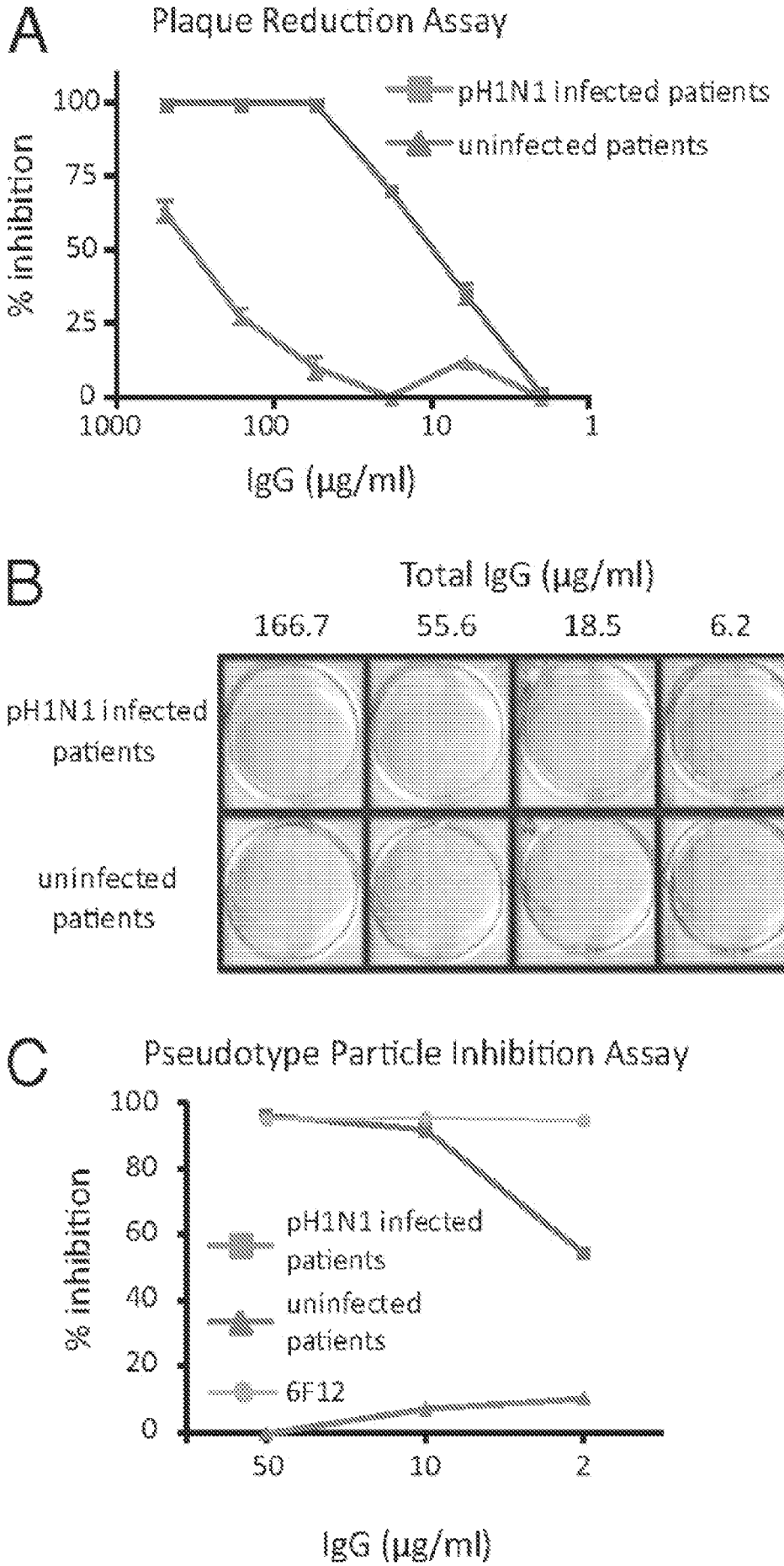

A
Plaque Reduction Assay
pH1N1 infected patients
uninfected patients
% inhibition
100
75
50
25
0
1000
100
10
1
IgG (µg/ml)
B
Total IgG (µg/ml)
166.7
55.6
18.5
6.2
pH1N1 infected patients
uninfected patients
C
Pseudotype Particle Inhibition Assay
% inhibition
100
80
60
40
20
0
pH1N1 infected patients
uninfected patients
6F12
50
10
2
IgG (µg/ml)

FIG. 16

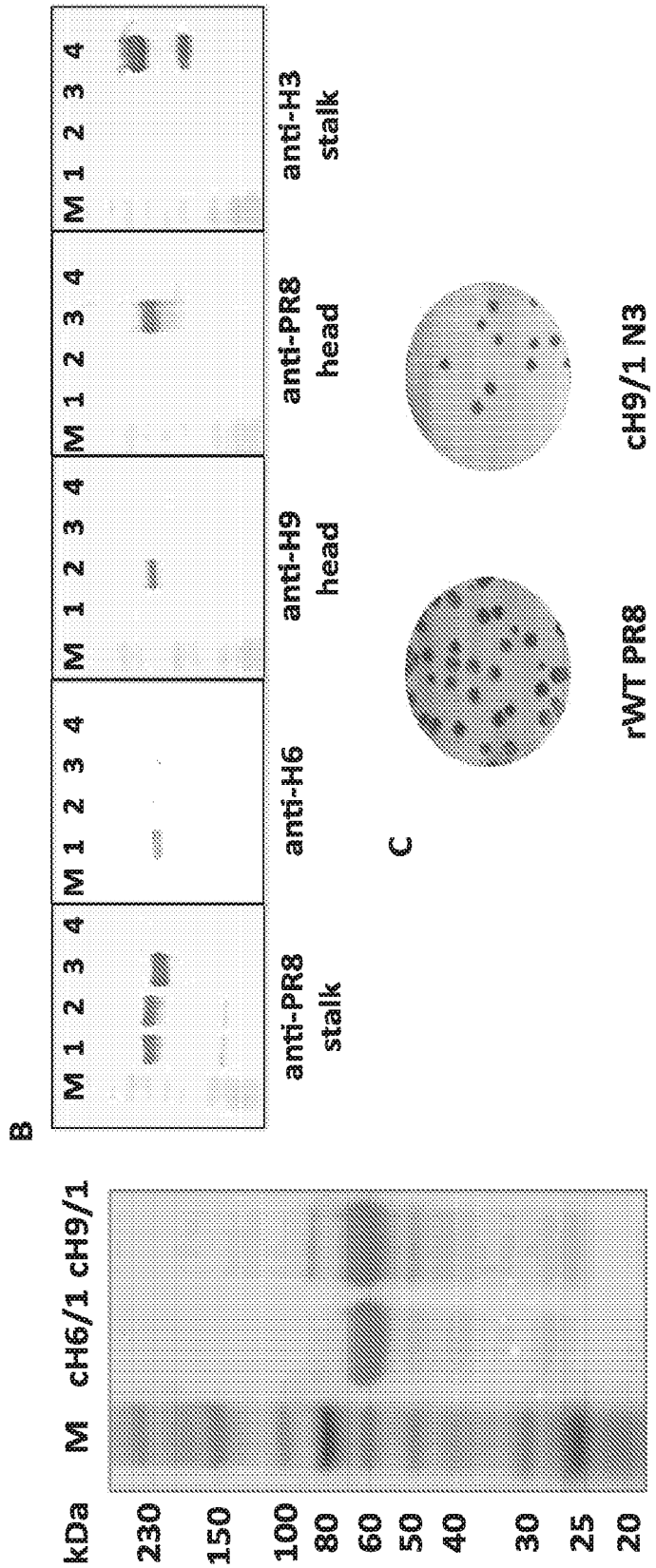

A
kDa
M
cH6/1
cH9/1
230
150
100
80
60
50
40
30
25
20
B
M 1 2 3 4
anti-PR8 stalk
anti-H6
anti-H9 head
anti-PR8 head
anti-H3 stalk
C
rWT PR8
cH9/1 N3

FIG. 17

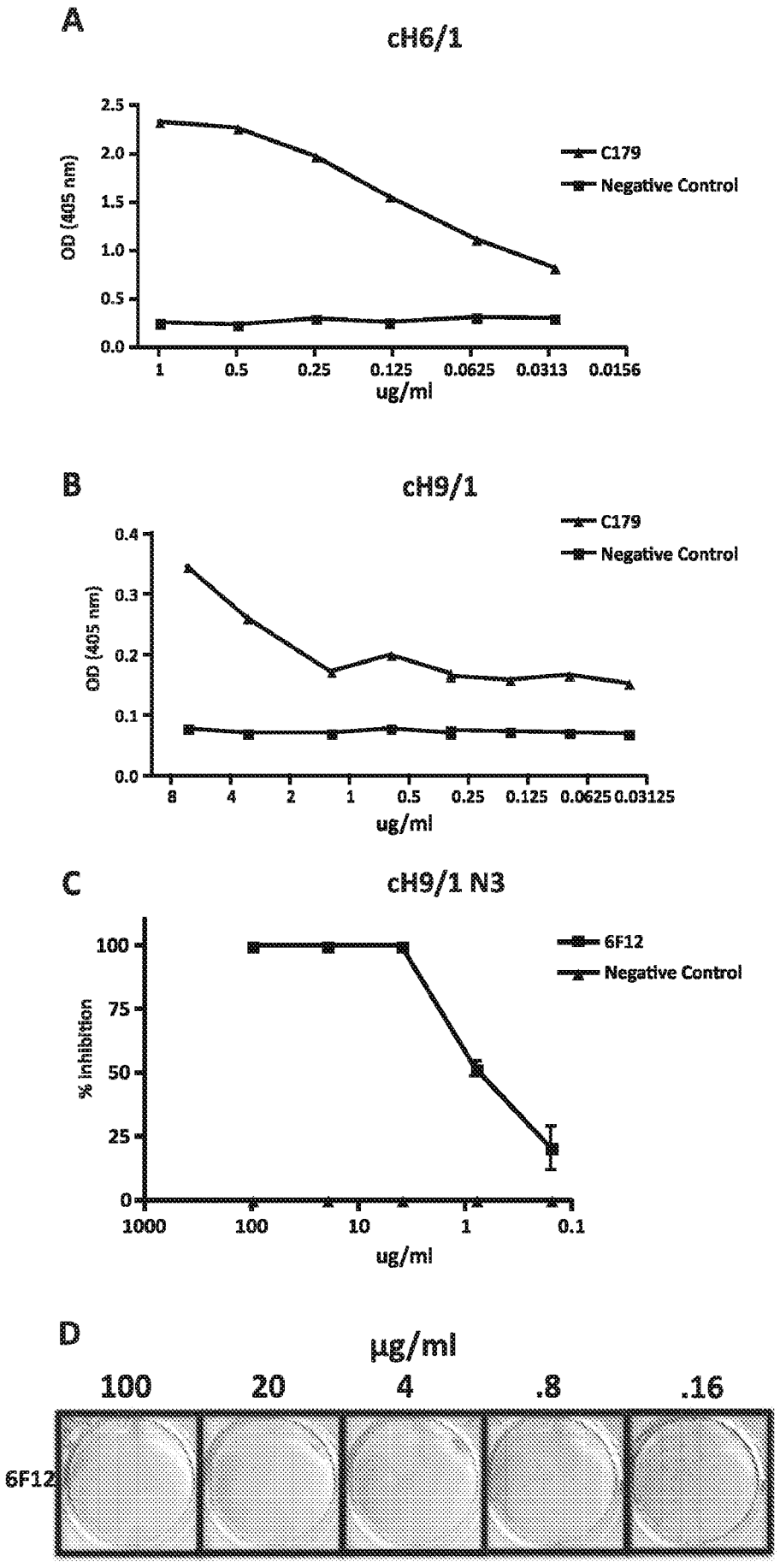

A
cH6/1
OD (405 nm)
2.5
2.0
1.5
1.0
0.5
0.0
1
0.5
0.25
0.125
0.0625
0.0313
0.0156
ug/ml
C179
Negative Control
B
cH9/1
OD (405 nm)
0.4
0.3
0.2
0.1
0.0
8
4
2
1
0.5
0.25
0.125
0.0625
0.03125
ug/ml
C179
Negative Control
C
cH9/1 N3
% inhibition
100
75
50
25
0
1000
100
10
1
0.1
ug/ml
6F12
Negative Control
D
µg/ml
100
20
4
.8
.16
6F12

FIG. 18

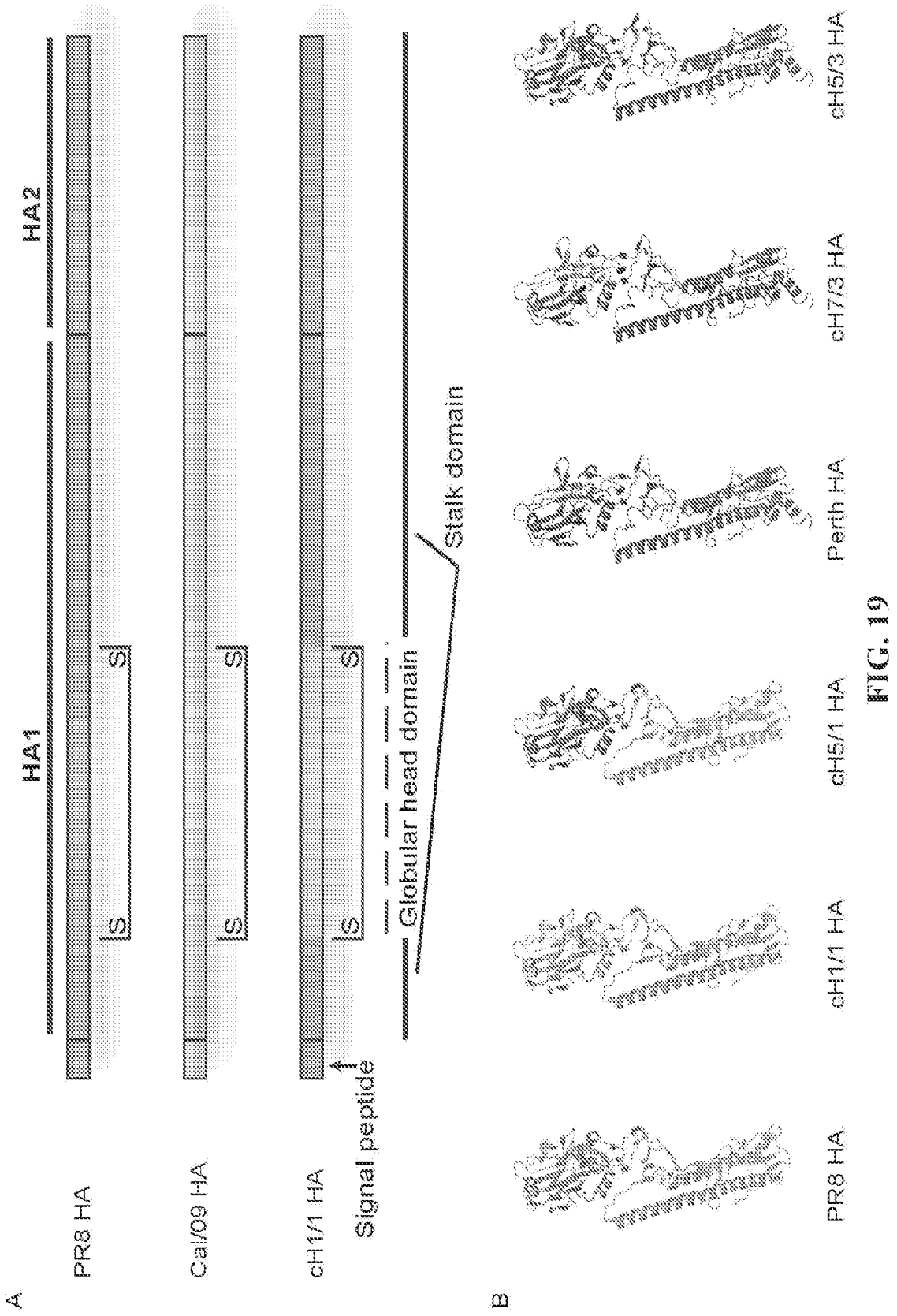

A
HA1
HA2
PR8 HA
Cal/09 HA
cH1/1 HA
S
S
S
S
S
S
Signal peptide
Globular head domain
Stalk domain
B
PR8 HA
cH1/1 HA
cH5/1 HA
Perth HA
cH7/3 HA
cH5/3 HA

FIG. 19

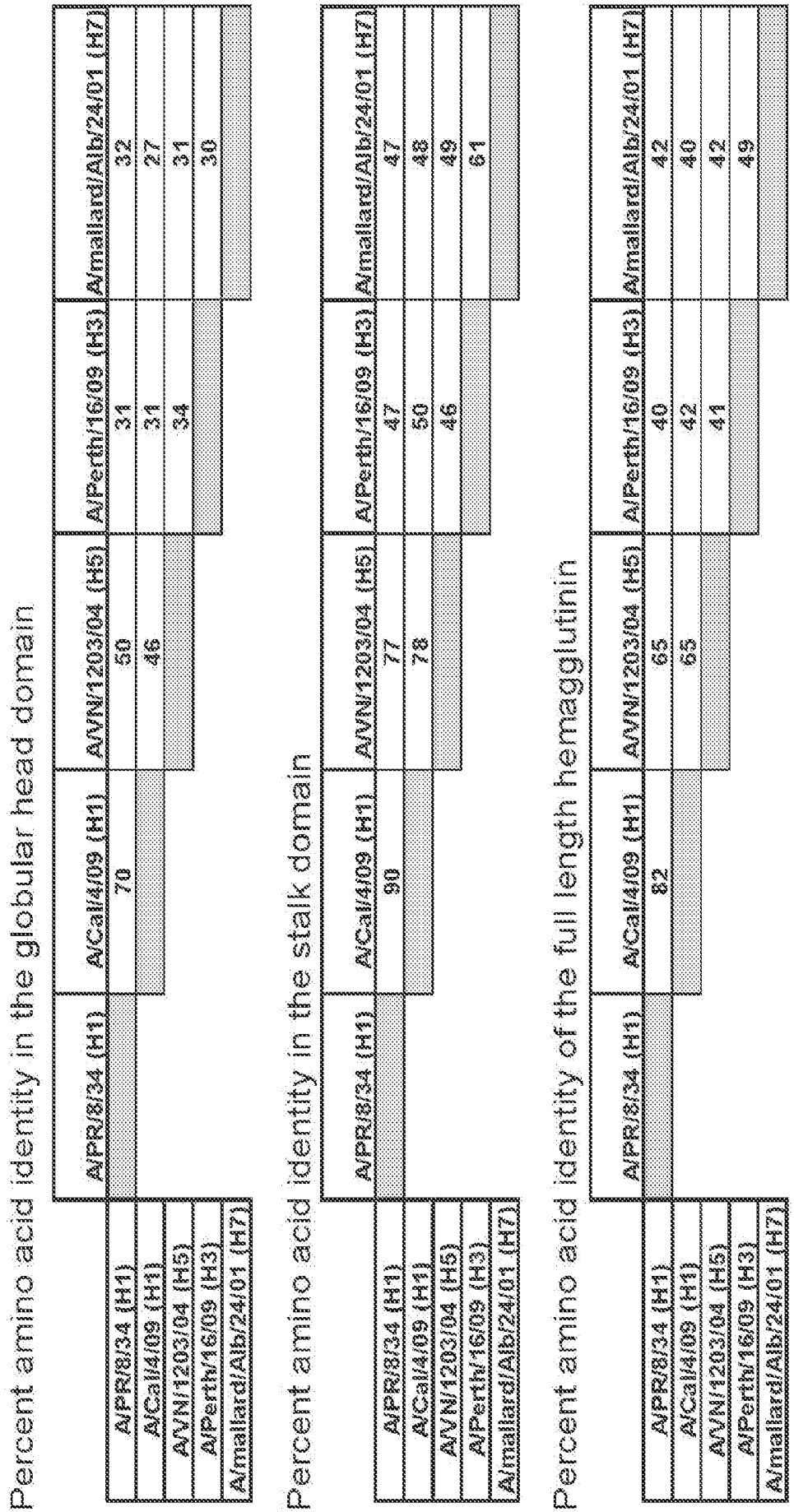

Percent amino acid identity in the globular head domain
A/PR/8/34 (H1)
A/Cal/4/09 (H1)
A/VN/1203/04 (H5)
A/Perth/16/09 (H3)
A/mallard/Alb/24/01 (H7)
70
50
46
31
31
34
32
27
31
30
Percent amino acid identity in the stalk domain
90
77
78
47
50
46
47
48
49
61
Percent amino acid identity of the full length hemagglutinin
82
65
65
40
42
41
42
40
42
49

FIG. 20

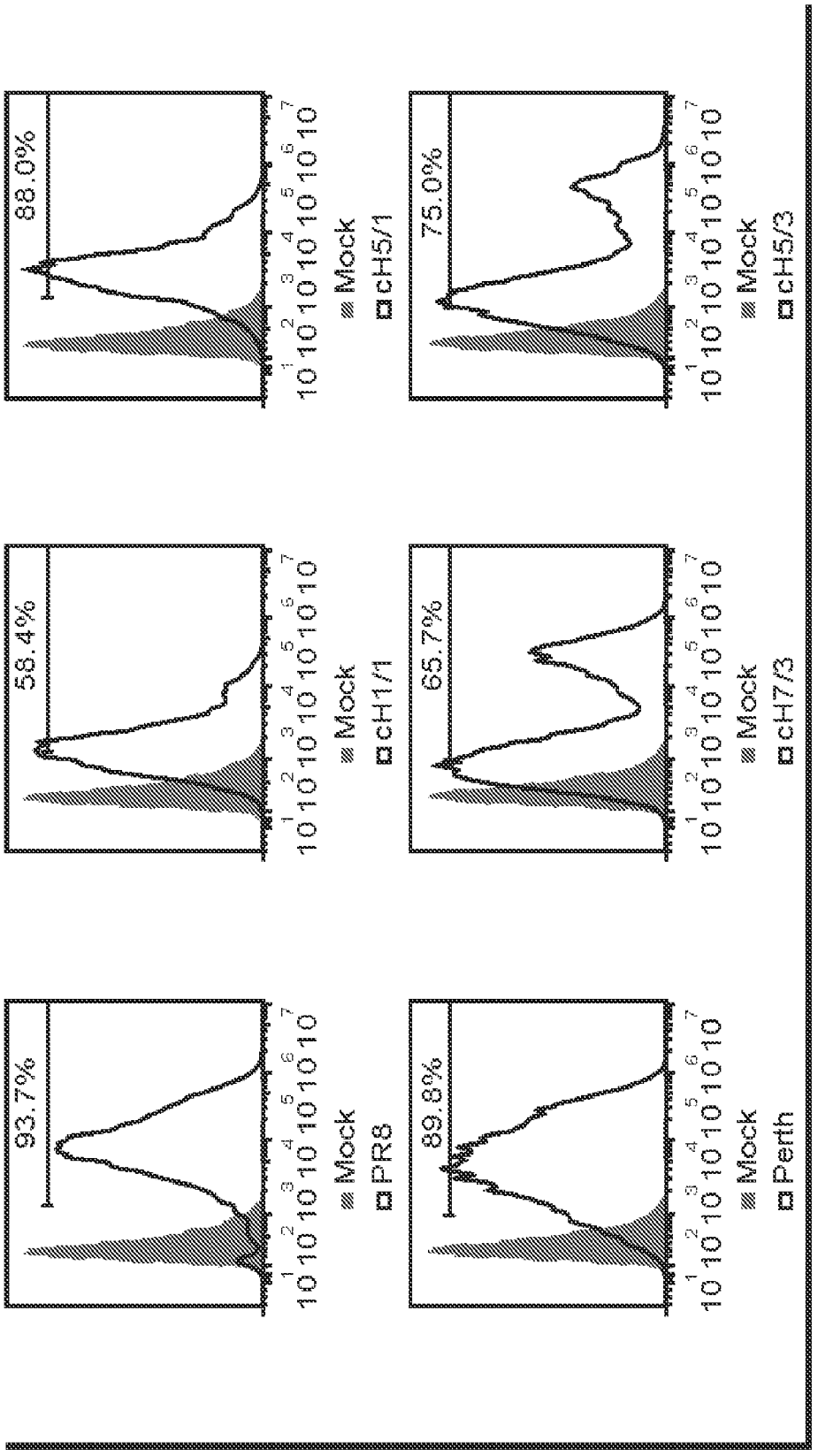

93.7%
89.8%
58.4%
65.7%
88.0%
75.0%
Mock
PR8
Perth
cH1/1
cH7/3
cH5/1
cH5/3
Cell Number
Hemagglutinin

FIG. 21

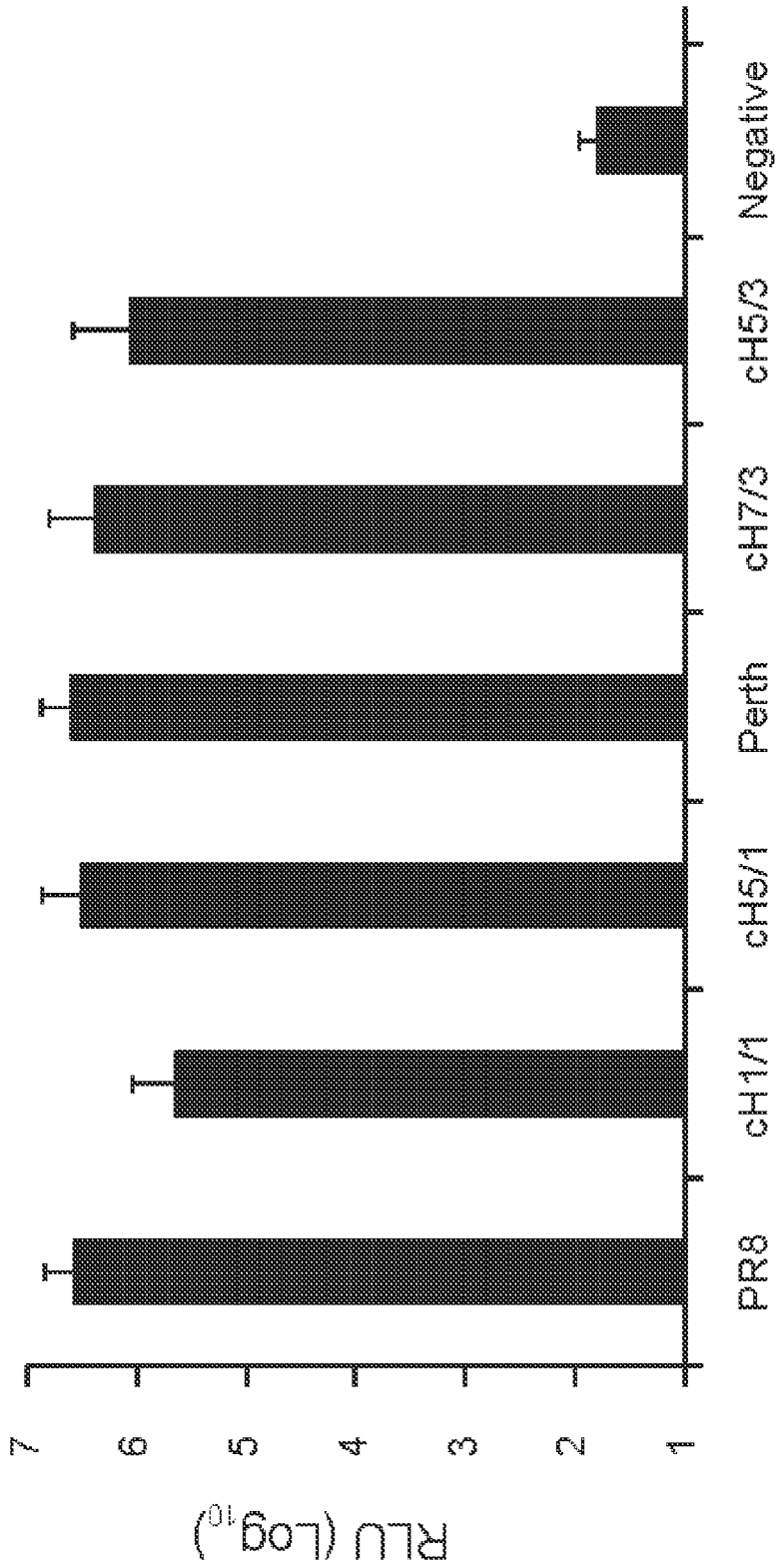


FIG. 22

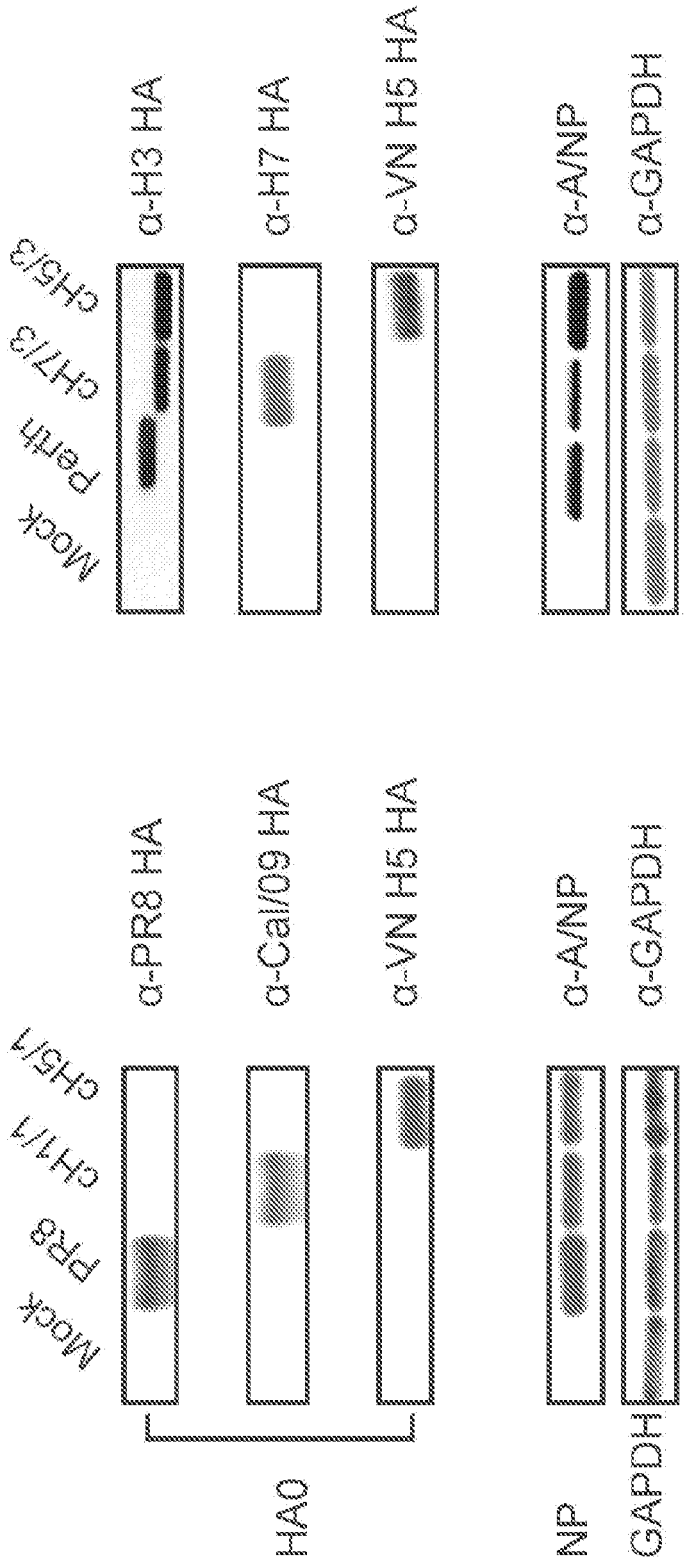

Mock
PR8
CH1/1
CH5/1
α-PR8 HA
α-Cal/09 HA
α-VN H5 HA
α-A/NP
α-GAPDH
HA0
NP
GAPDH
Mock
Perth
CH7/3
CH5/3
α-H3 HA
α-H7 HA
α-VN H5 HA
α-A/NP
α-GAPDH

FIG. 23

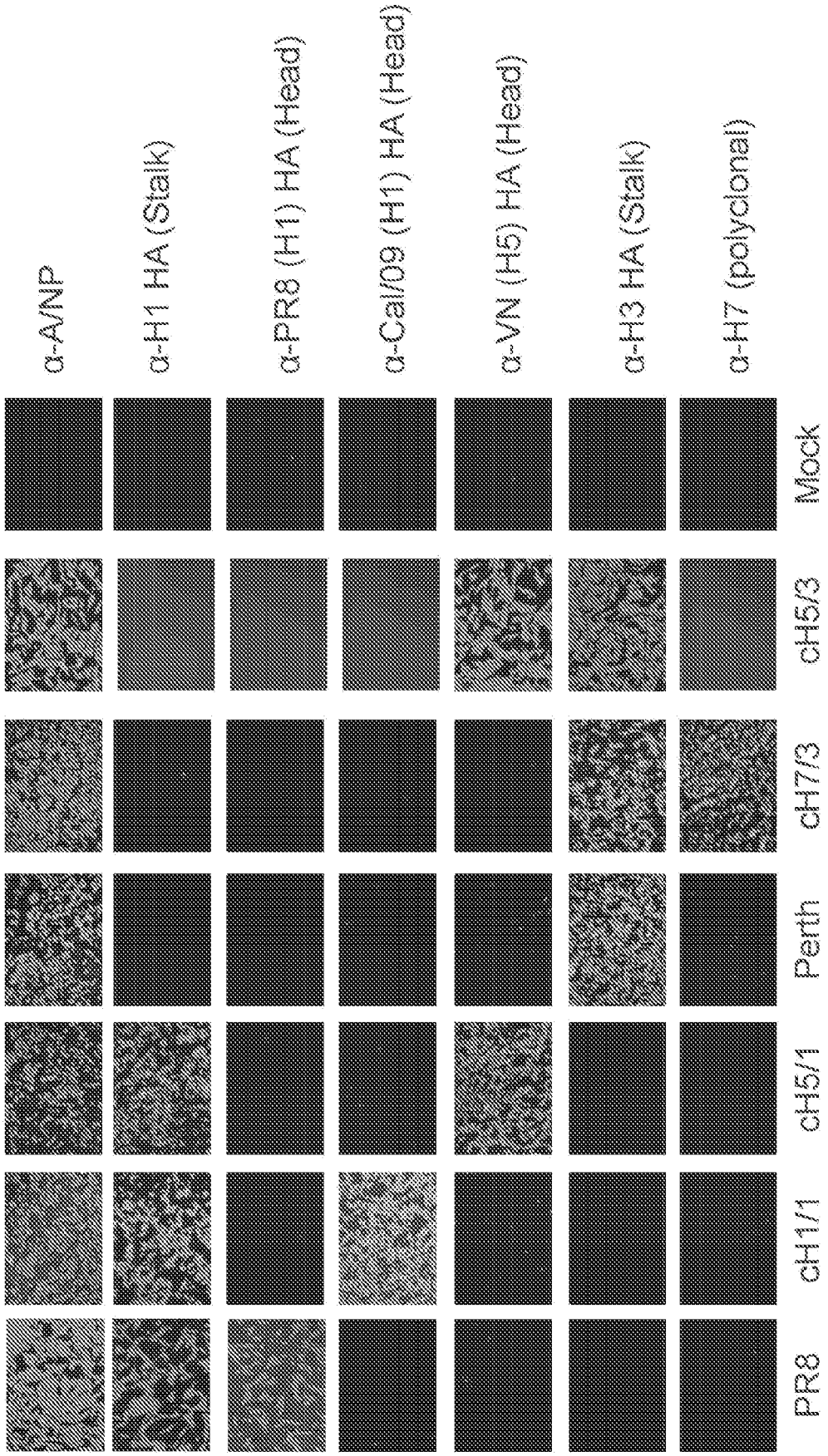

α-A/NP
α-H1 HA (Stalk)
α-PR8 (H1) HA (Head)
α-Cal/09 (H1) HA (Head)
α-VN (H5) HA (Head)
α-H3 HA (Stalk)
α-H7 (polyclonal)
PR8
cH1/1
cH5/1
Perth
cH7/3
cH5/3
Mock

FIG. 24

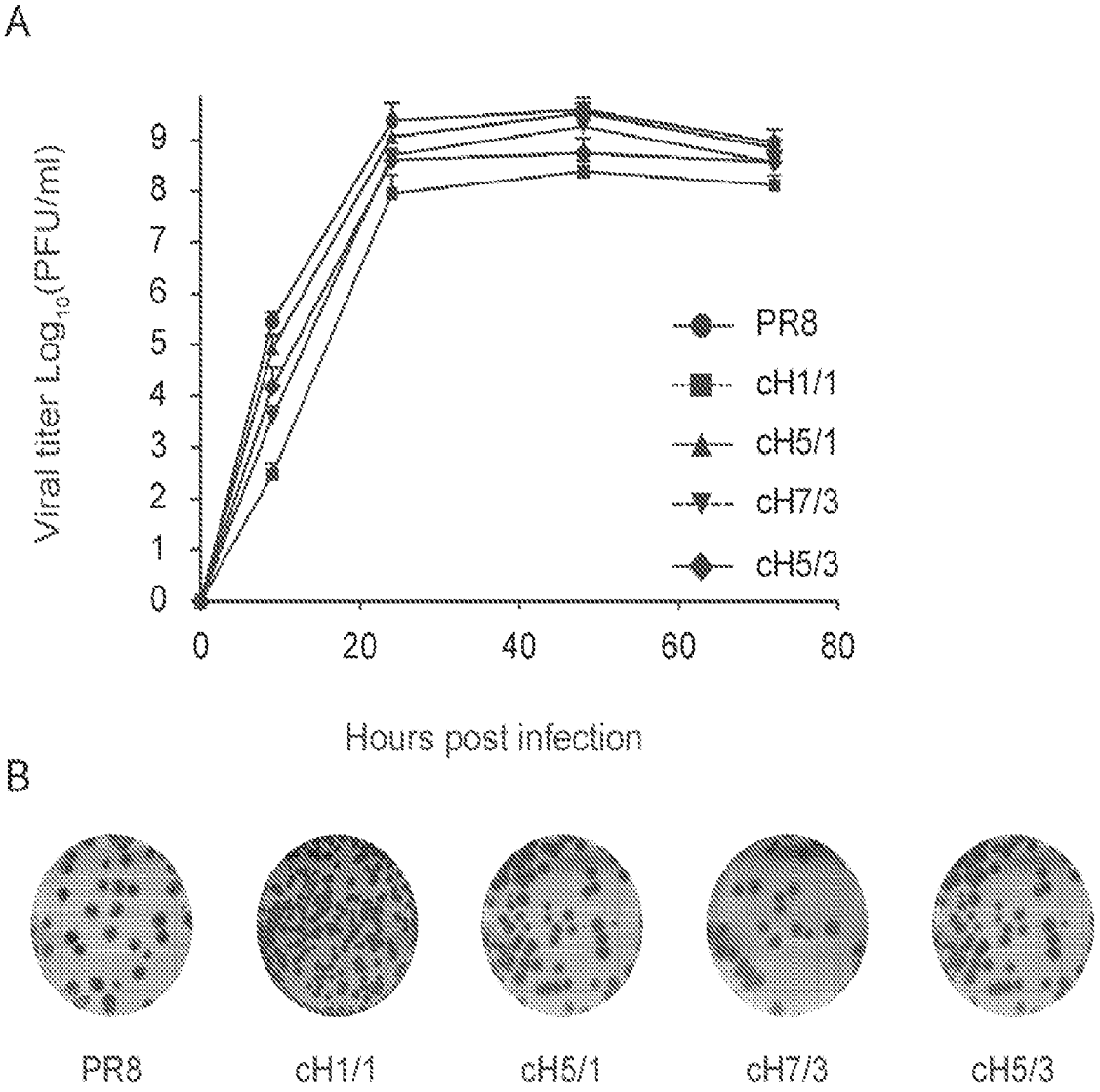

A
Viral titer Log10(PFU/ml)
0
1
2
3
4
5
6
7
8
9
0
20
40
60
80
Hours post infection
PR8
cH1/1
cH5/1
cH7/3
cH5/3
B
PR8
cH1/1
cH5/1
cH7/3
cH5/3

FIG. 25

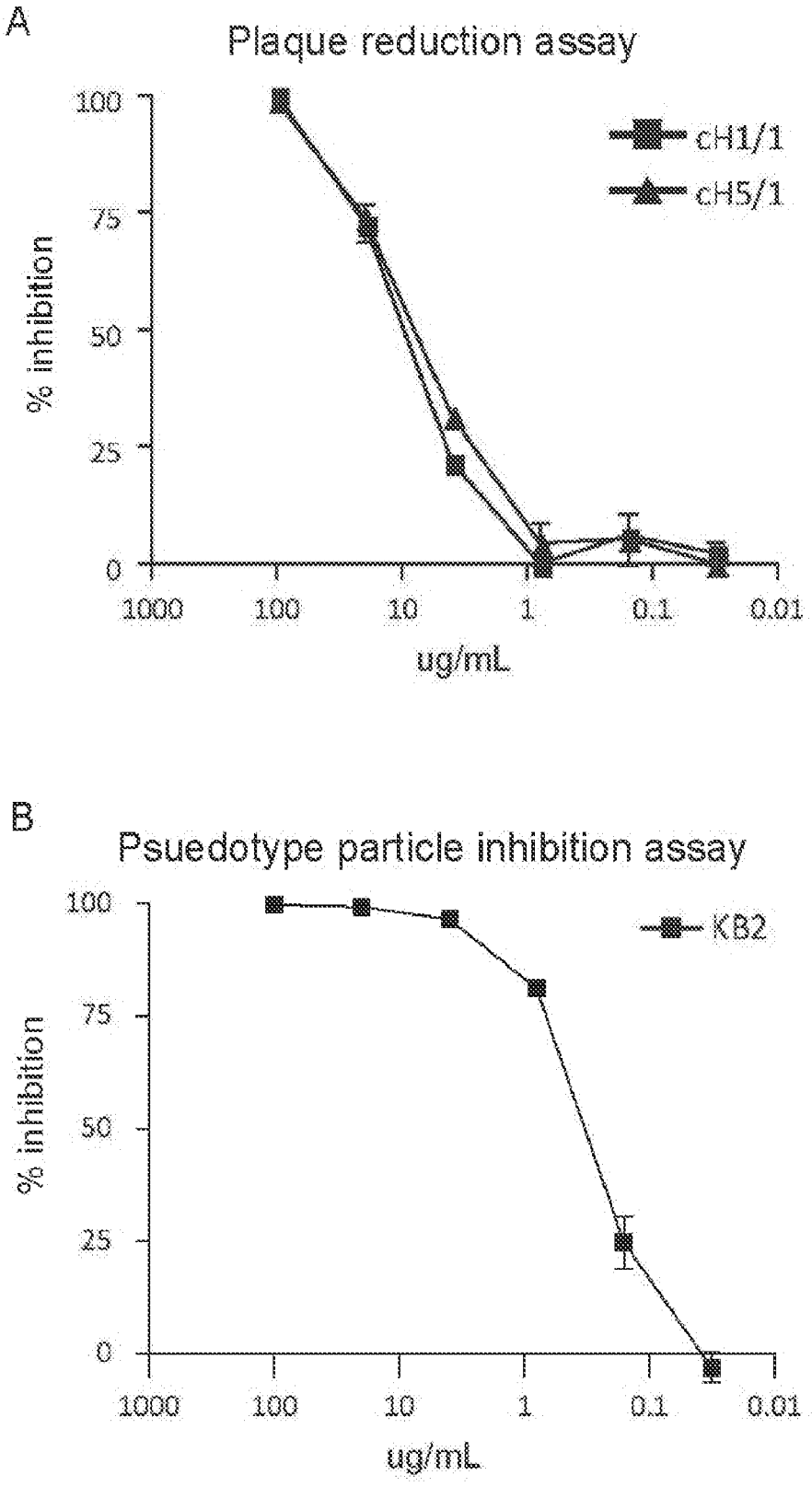

A
Plaque reduction assay
100
75
50
25
0
% inhibition
cH1/1
cH5/1
1000
100
10
1
0.1
0.01
ug/mL
B
Psuedotype particle inhibition assay
100
75
50
25
0
% inhibition
KB2
1000
100
10
1
0.1
0.01
ug/mL

FIG. 26

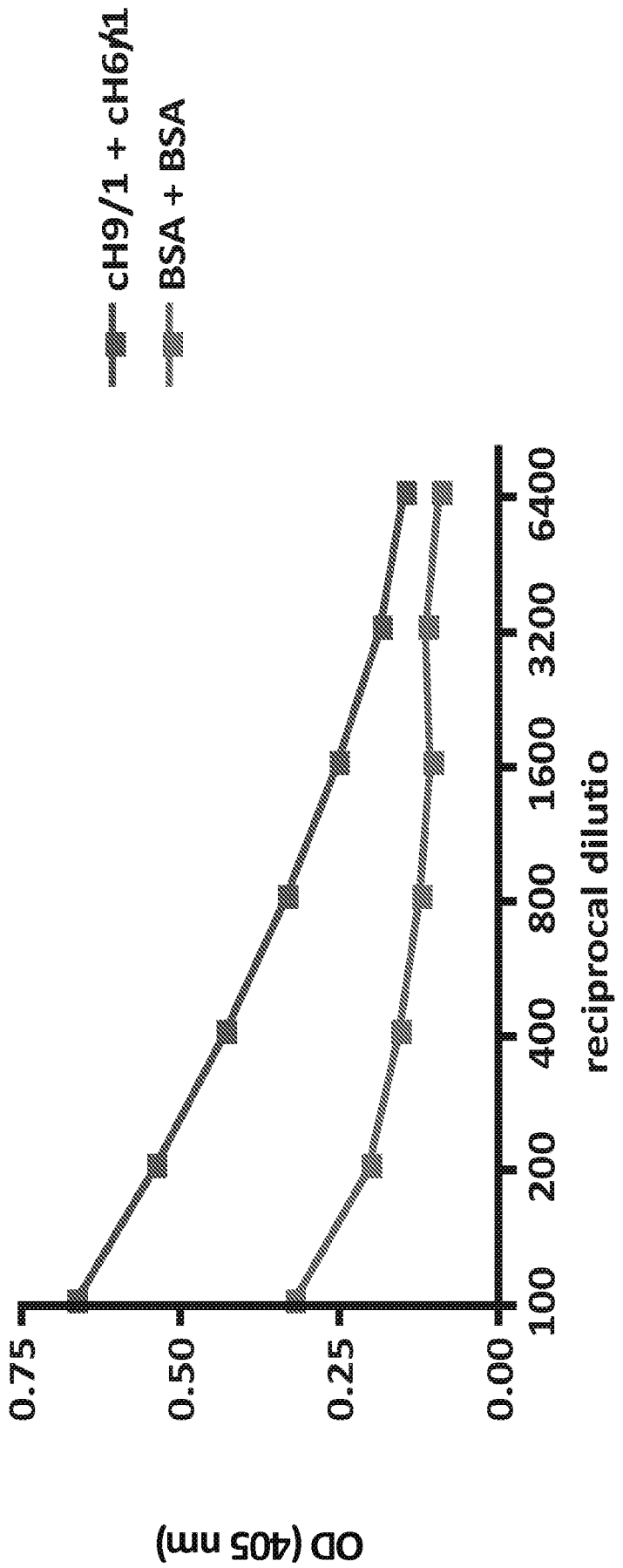

cH9/1 + cH6/1
BSA + BSA
OD (405 nm)
0.75
0.50
0.25
0.00
100
200
400
800
1600
3200
6400
reciprocal dilutio

FIG. 27A

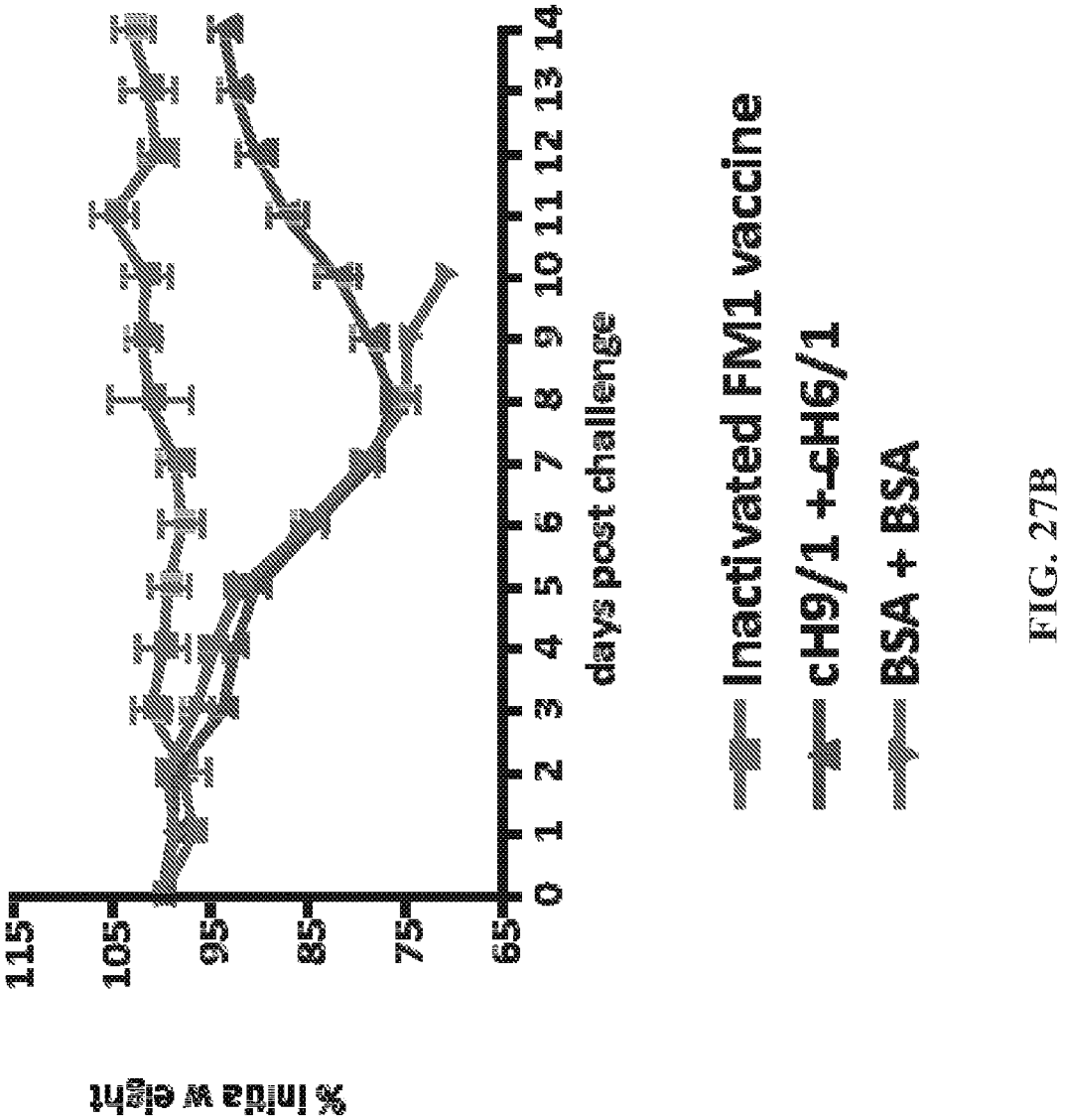

% Initial w eight
115
105
95
85
75
65
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
days post challenge
Inactivated FM1 vaccine
cH9/1 +cH6/1
BSA + BSA

FIG. 27B

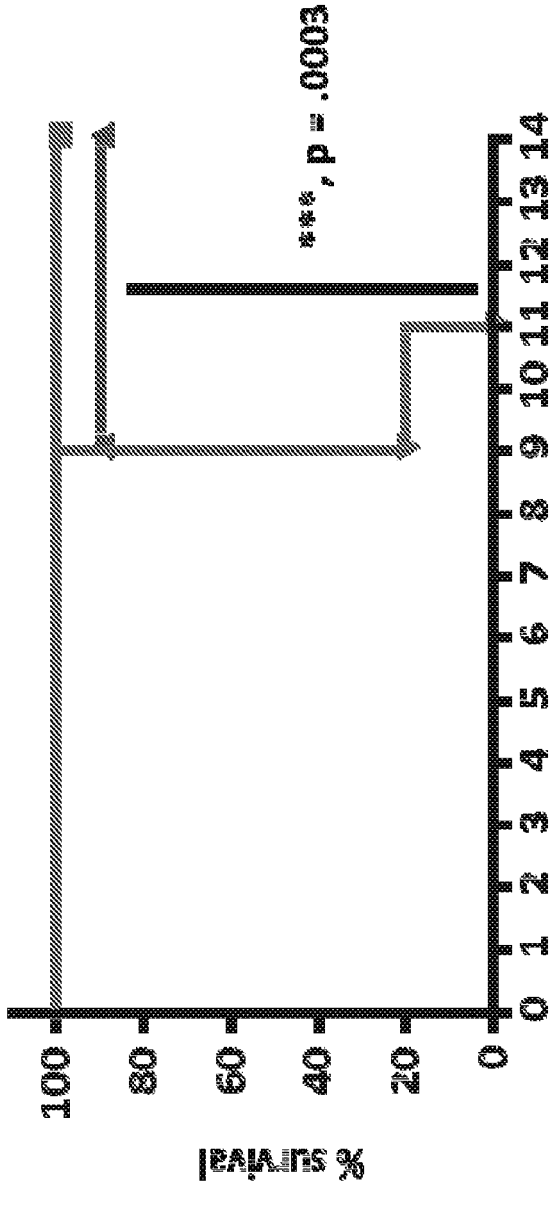

% survival
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
days post challenge
***, p = .0003
Inactivated FM1 vaccine
cH9/1 +cH6/1
BSA + BSA

FIG. 27C

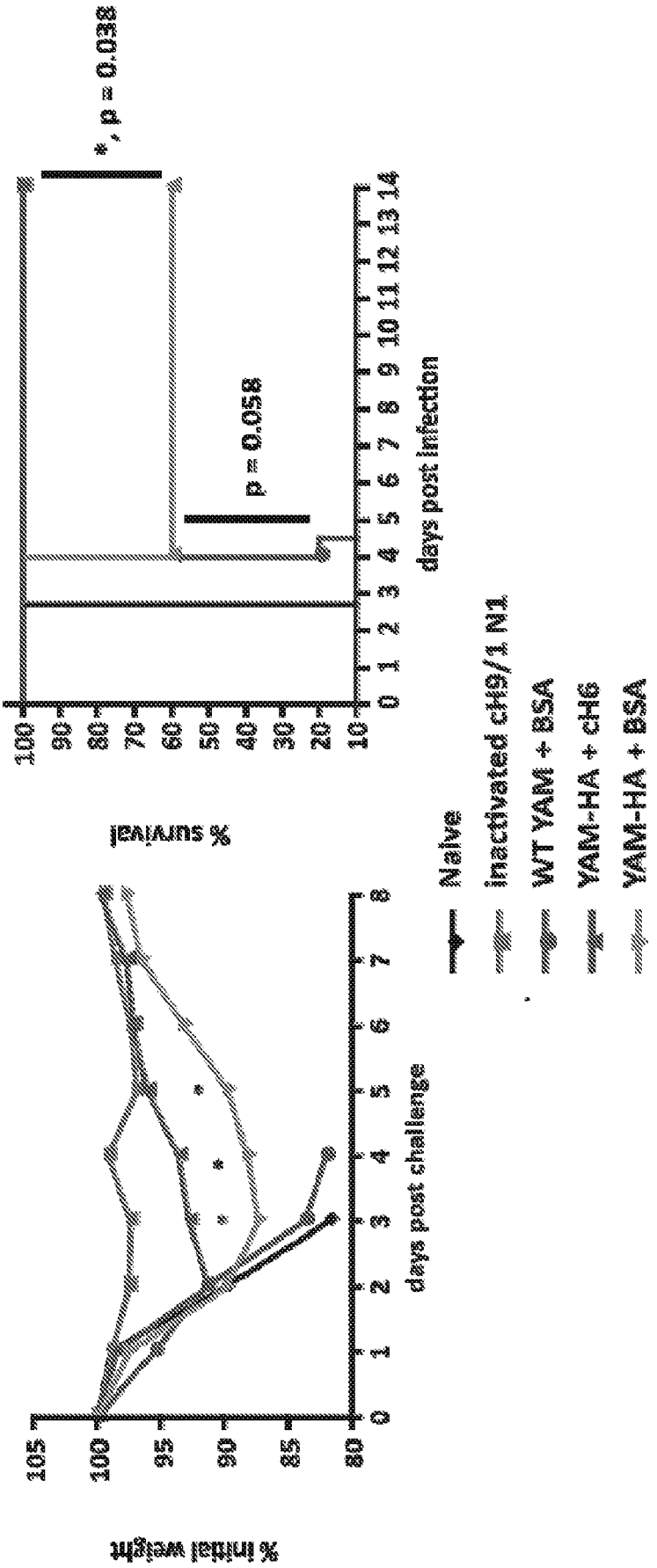

*, p = 0.038
p = 0.058
% survival
days post infection
% initial weight
days post challenge
Naive
inactivated cH9/1 N1
WT YAM + BSA
YAM-HA + cH6
YAM-HA + BSA

FIG. 28A - 28B

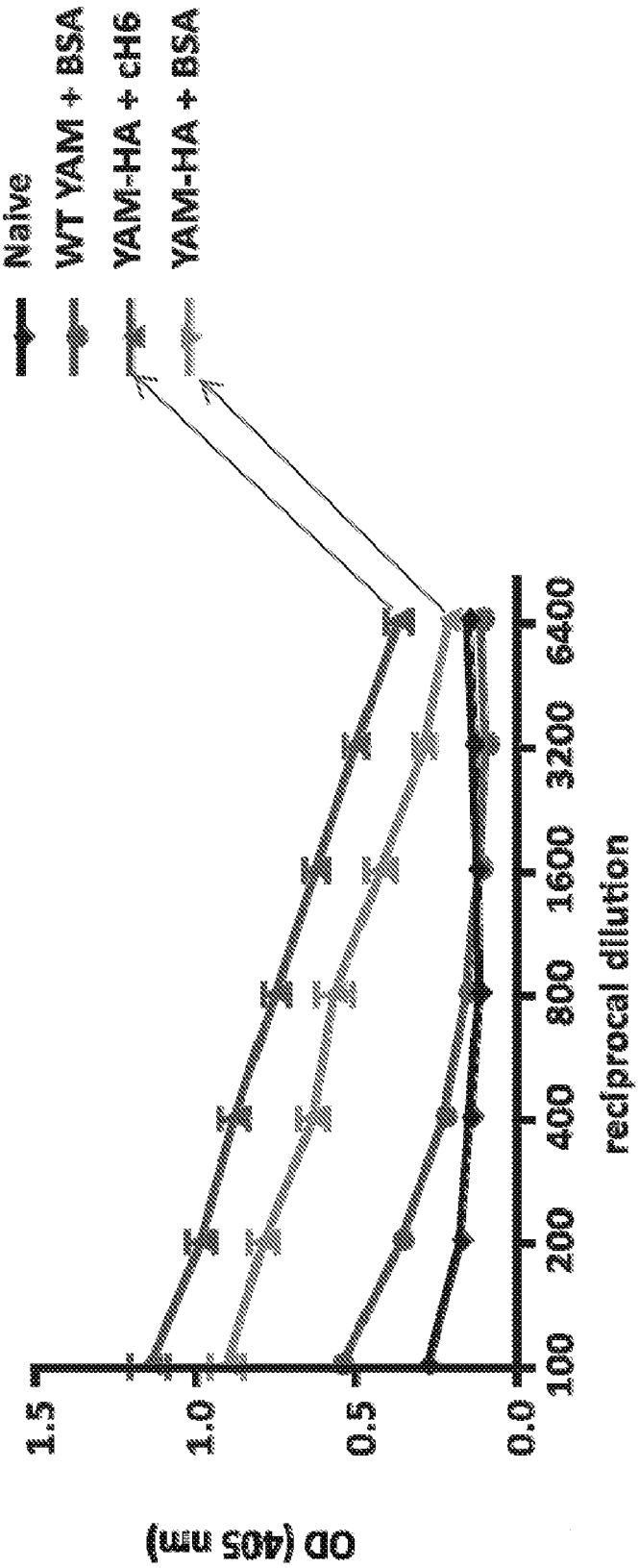

Naive
WT YAM + BSA
YAM-HA + cH6
YAM-HA + BSA
OD (405 nm)
1.5
1.0
0.5
0.0
100
200
400
800
1600
3200
6400
reciprocal dilution

FIG. 28C

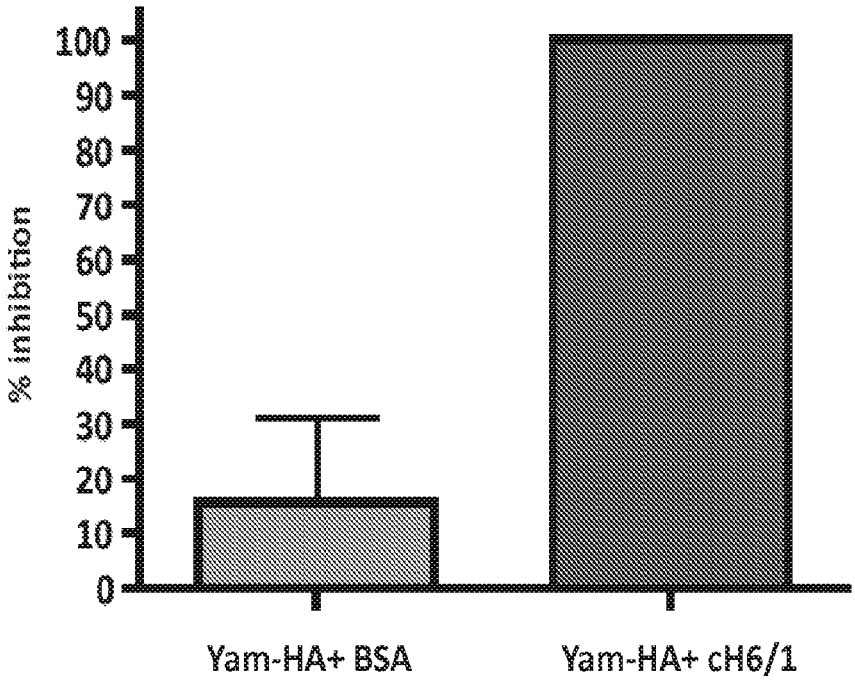


FIG. 28D

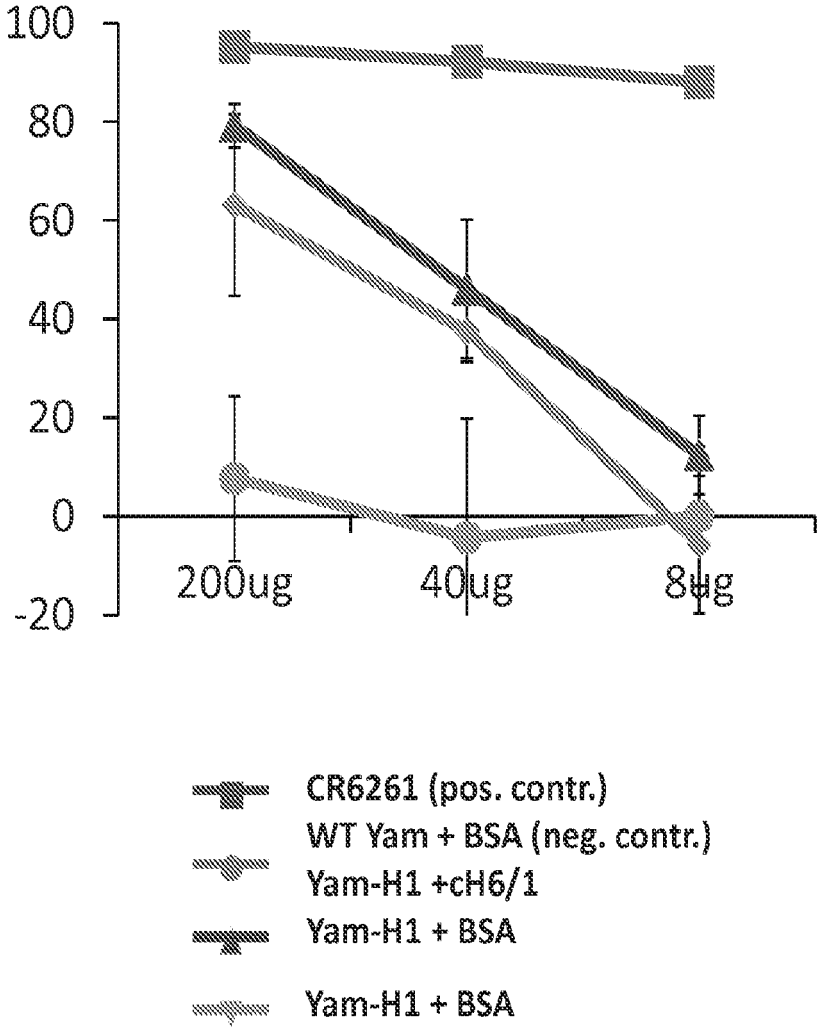

100
80
60
40
20
0
-20
200ug
40ug
8ug
CR6261 (pos. contr.)
WT Yam + BSA (neg. contr.)
Yam-H1 +cH6/1
Yam-H1 + BSA
Yam-H1 + BSA

FIG. 28E

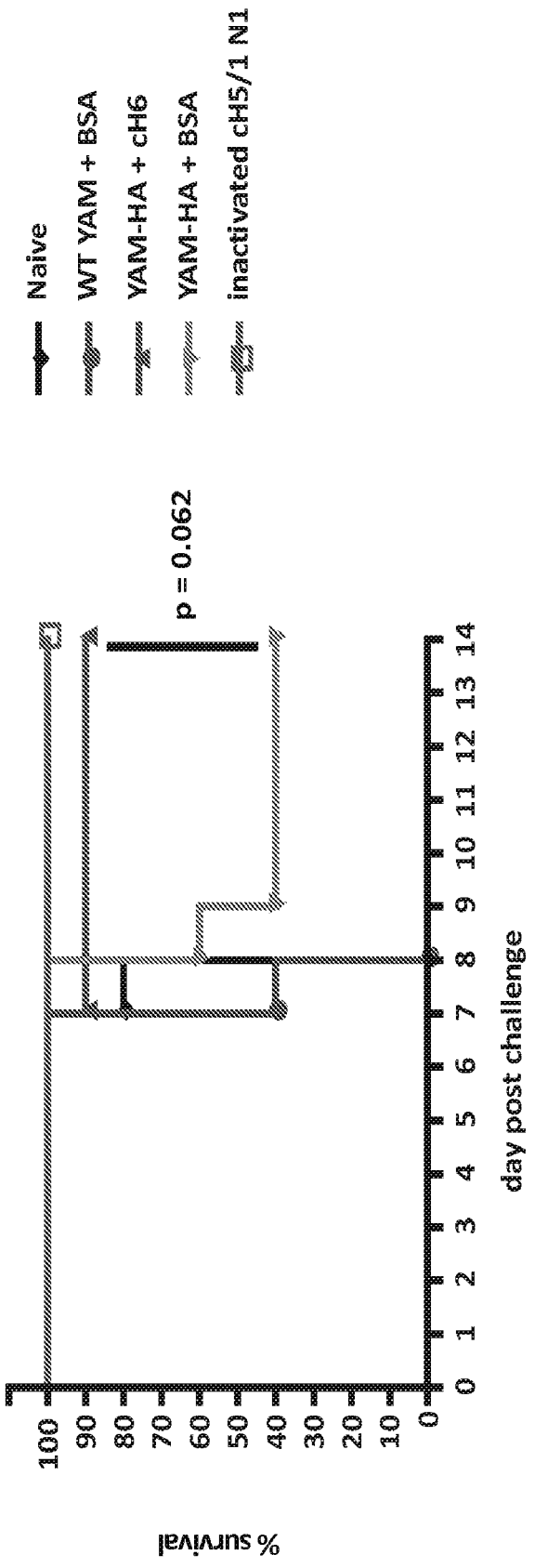

Naive
WT YAM + BSA
YAM-HA + cH6
YAM-HA + BSA
inactivated cH5/1 N1
p = 0.062
% survival
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
day post challenge

FIG. 29A

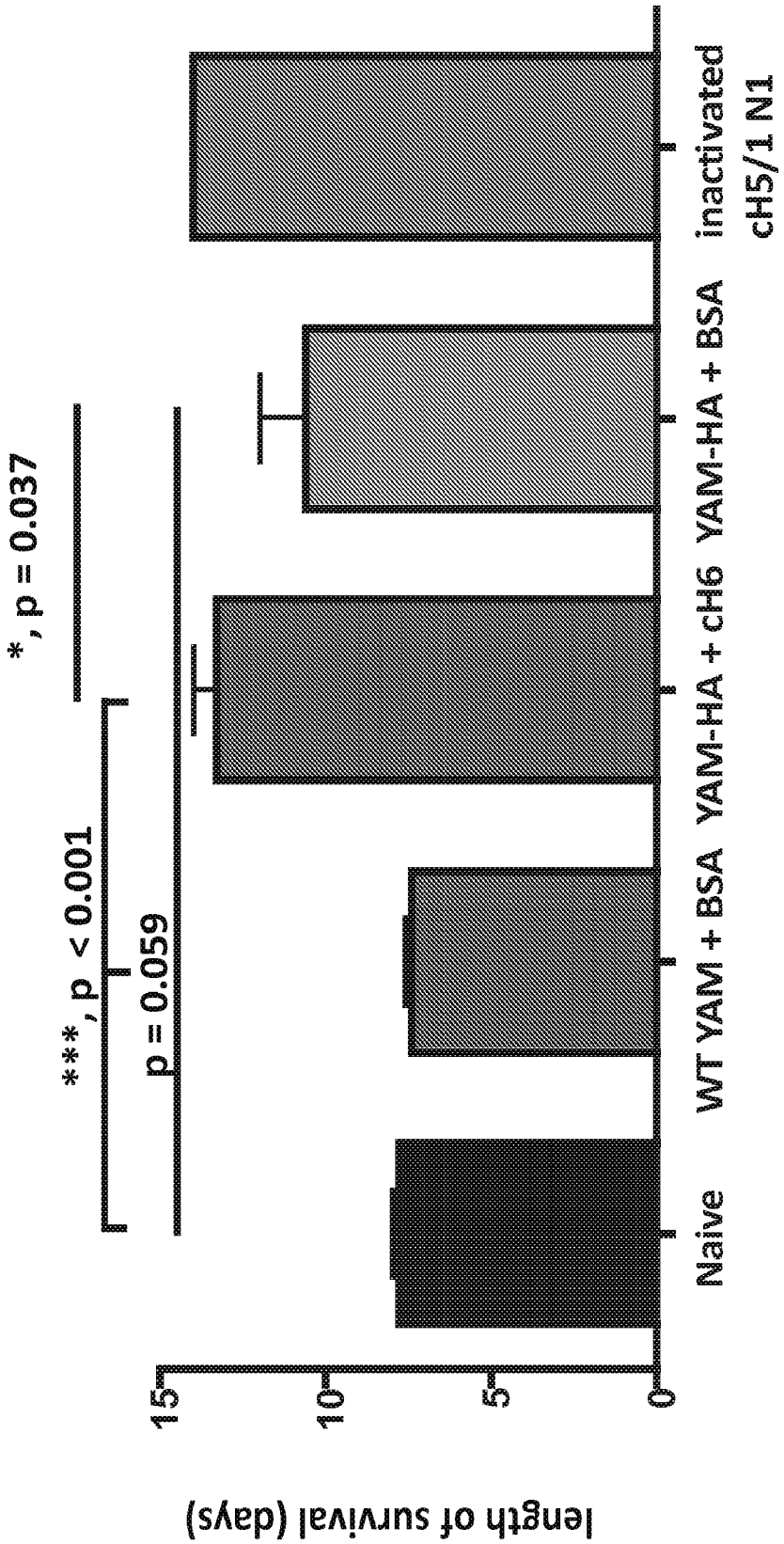

length of survival (days)
15
10
5
0
Naive
WT YAM + BSA
YAM-HA + cH6
YAM-HA + BSA
inactivated cH5/1 N1
*, p = 0.037
***, p < 0.001
p = 0.059

FIG. 29B

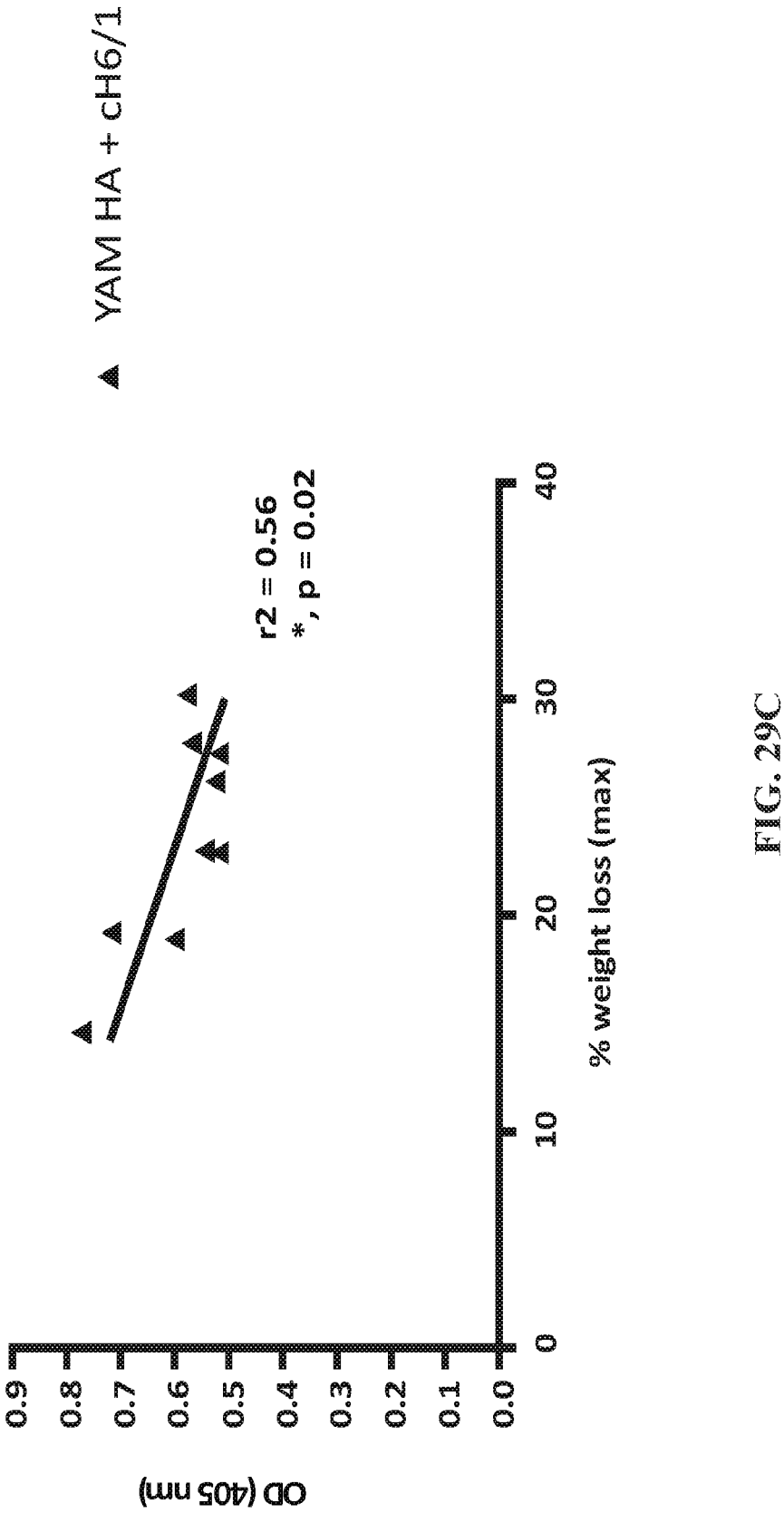

YAM HA + cH6/1
r2 = 0.56
*, p = 0.02
OD (405 nm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
0
10
20
30
40
% weight loss (max)

FIG. 29C

FIG. 30A

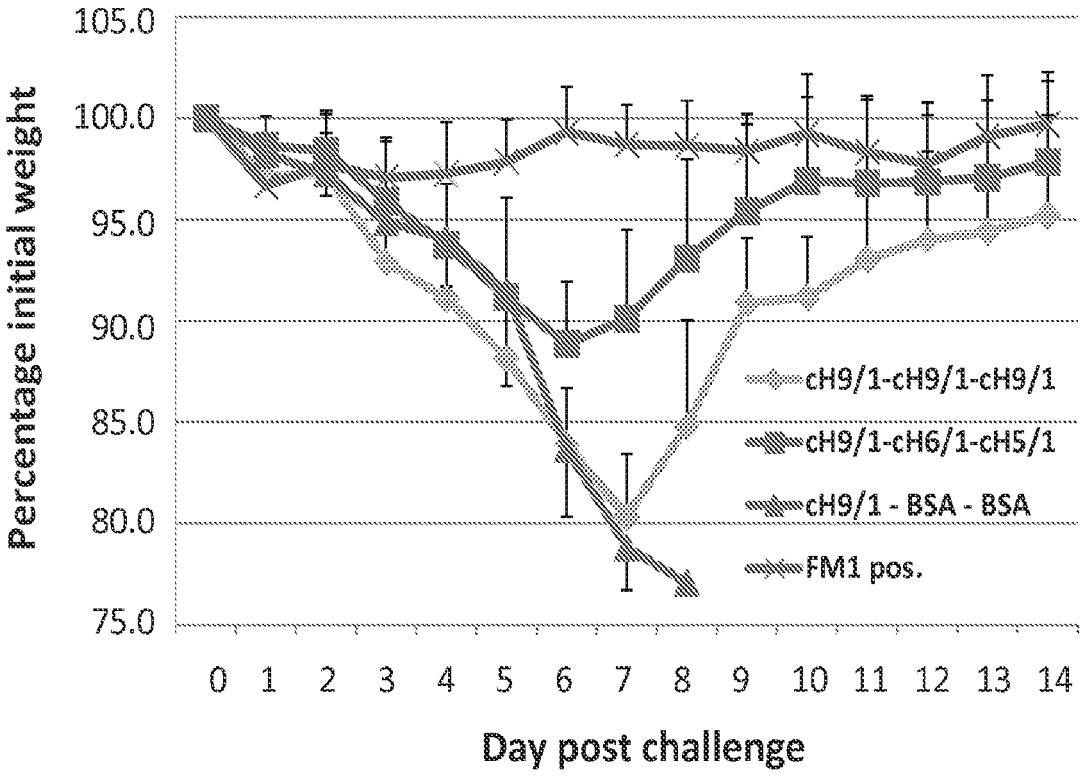


FIG. 30B

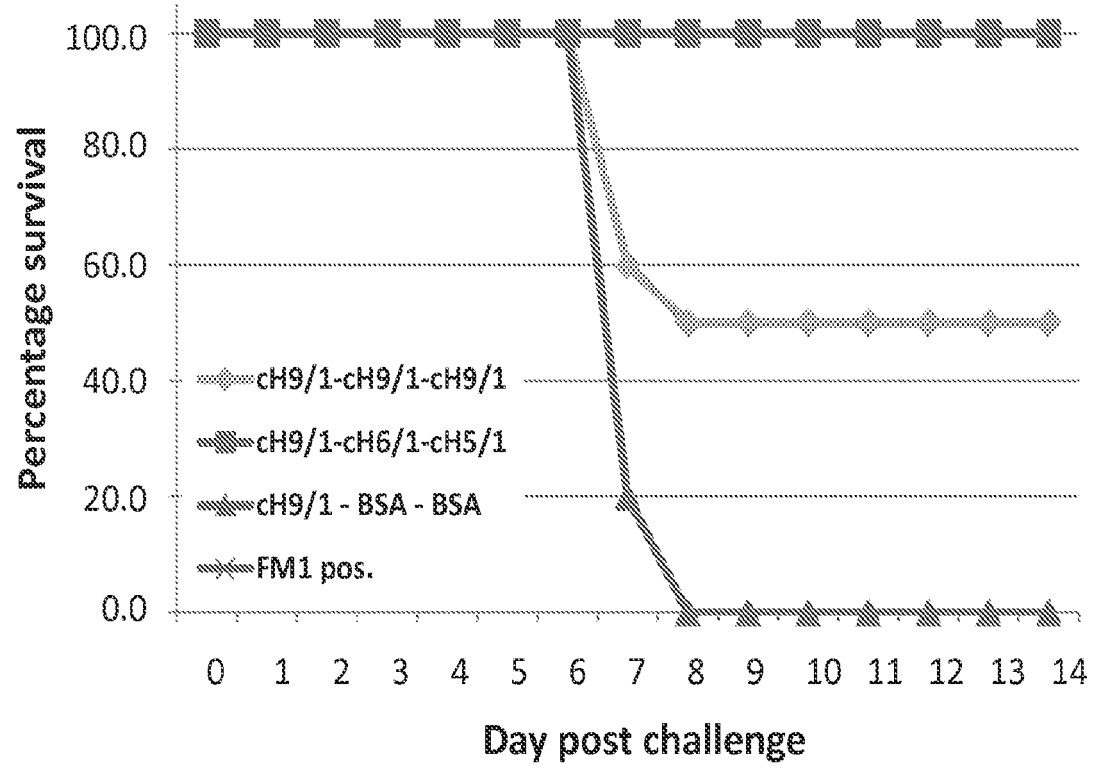


FIG. 30C

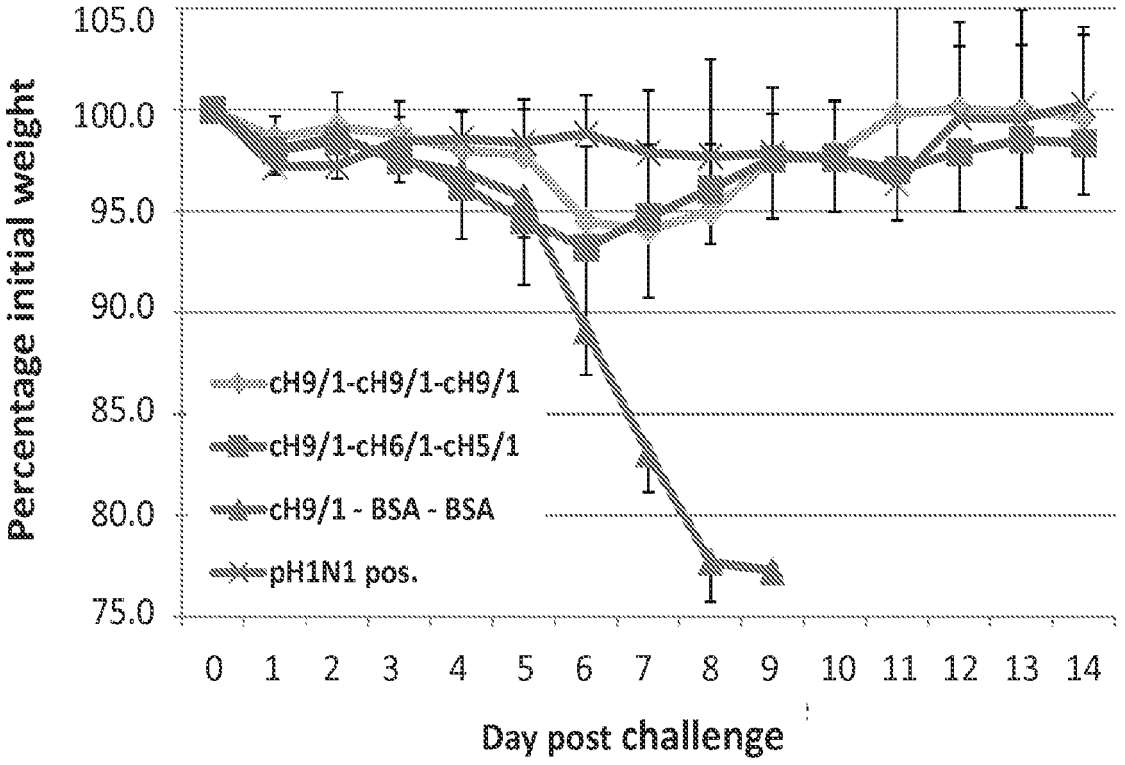


FIG. 30D

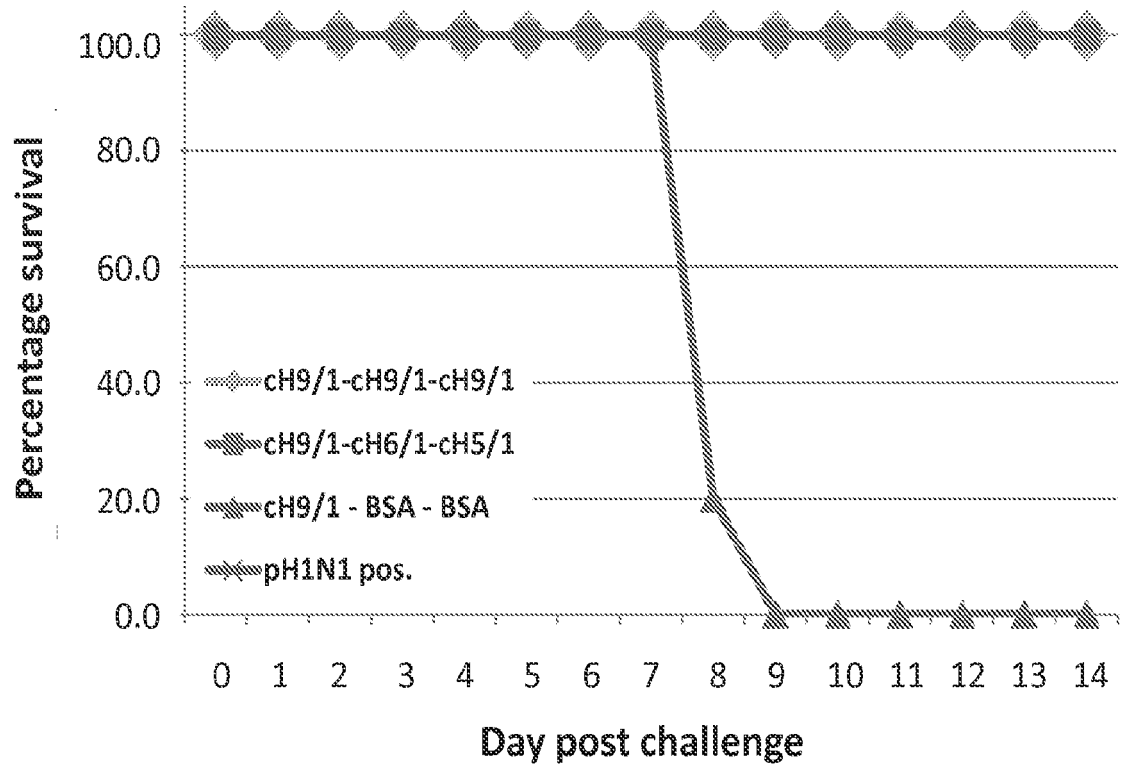


FIG. 30E

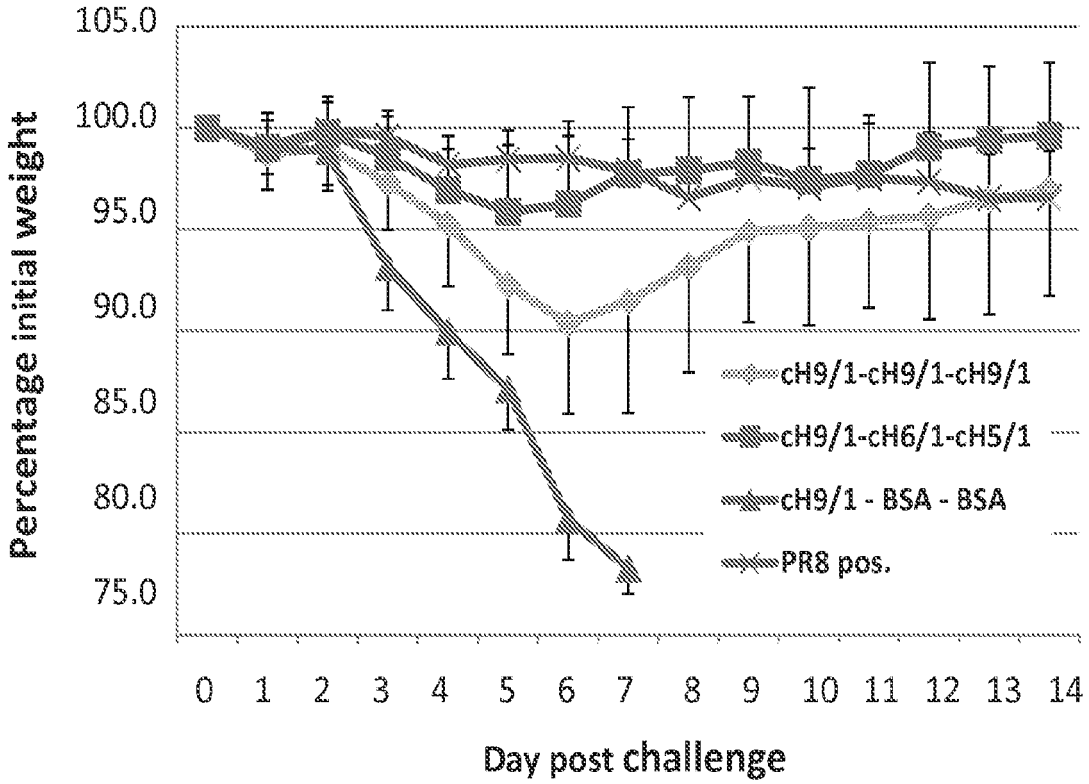
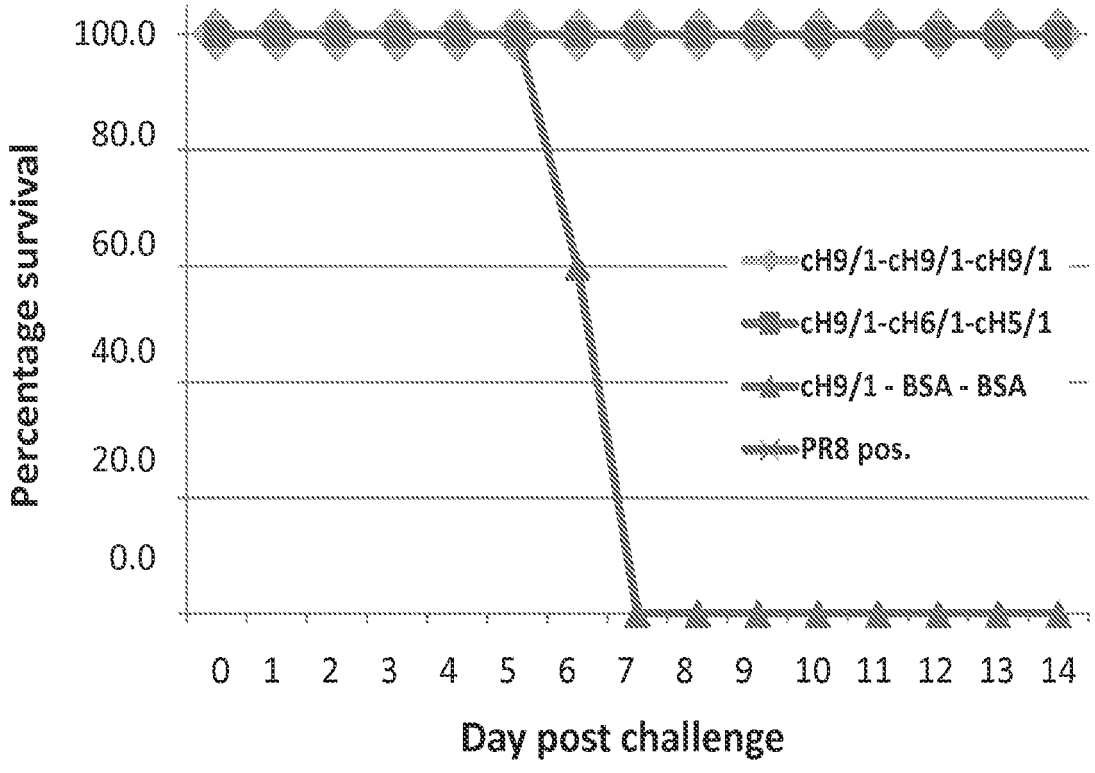


FIG. 30F



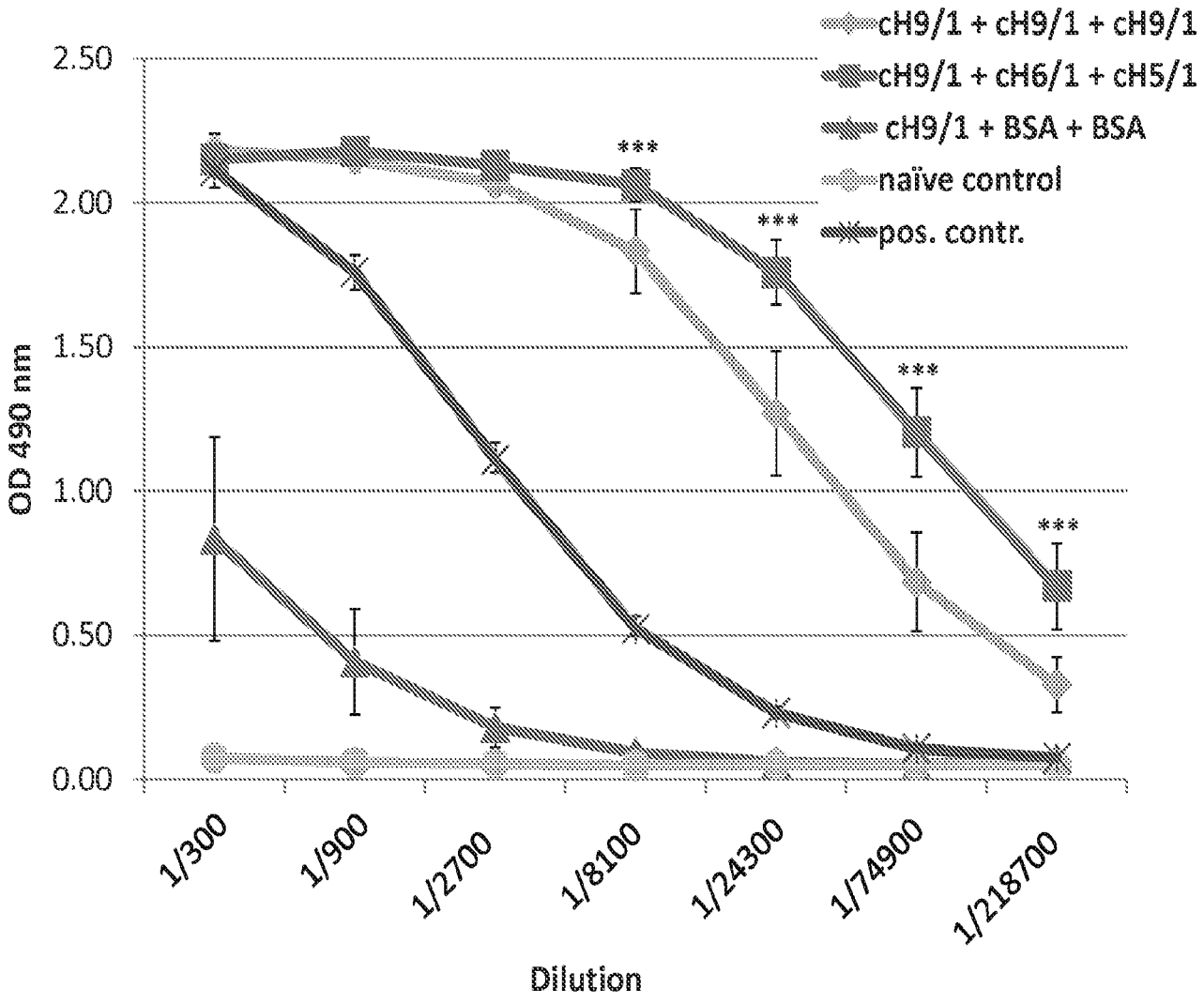

cH9/1 + cH9/1 + cH9/1
cH9/1 + cH6/1 + cH5/1
cH9/1 + BSA + BSA
naïve control
pos. contr.
2.50
2.00
1.50
1.00
0.50
0.00
OD 490 nm
***
***
***
***
1/300
1/900
1/2700
1/8100
1/24300
1/74900
1/218700
Dilution

FIG. 30G

FIG. 30H

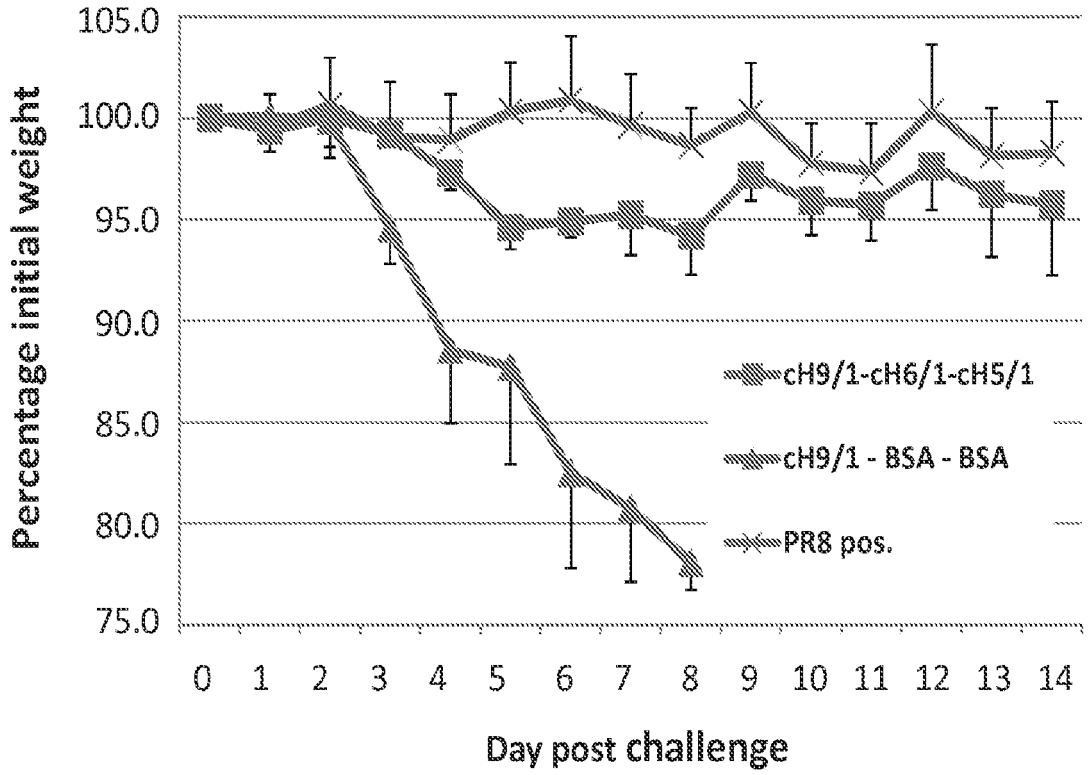
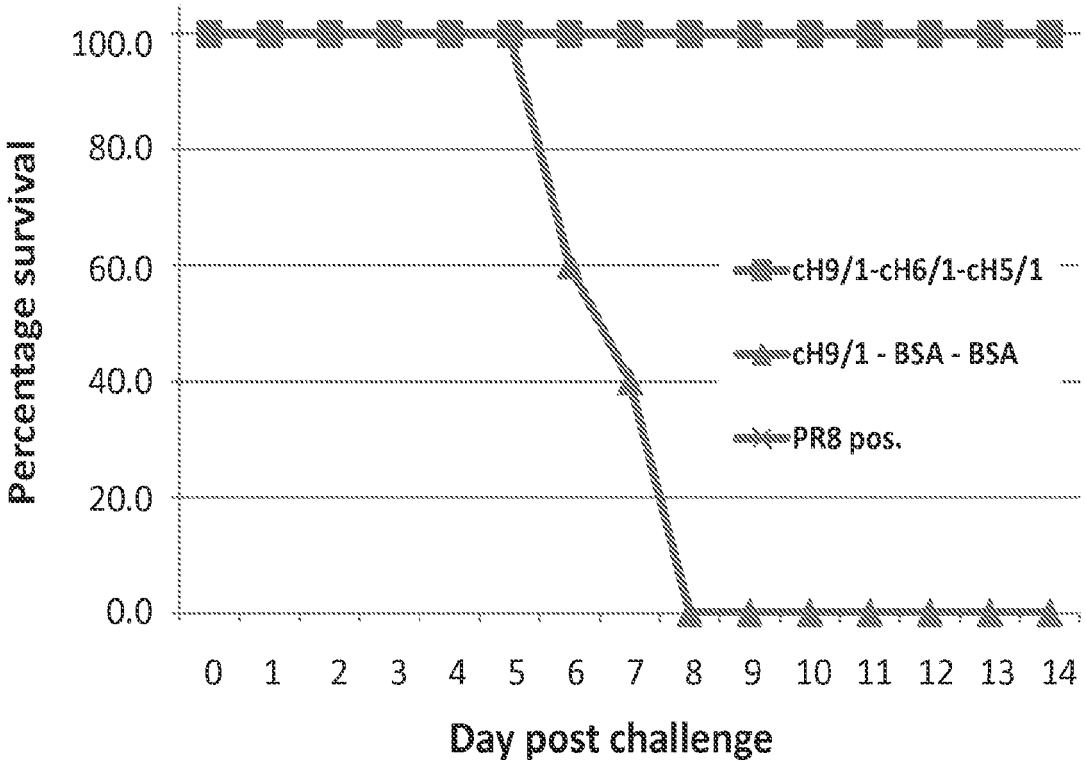


FIG. 30I



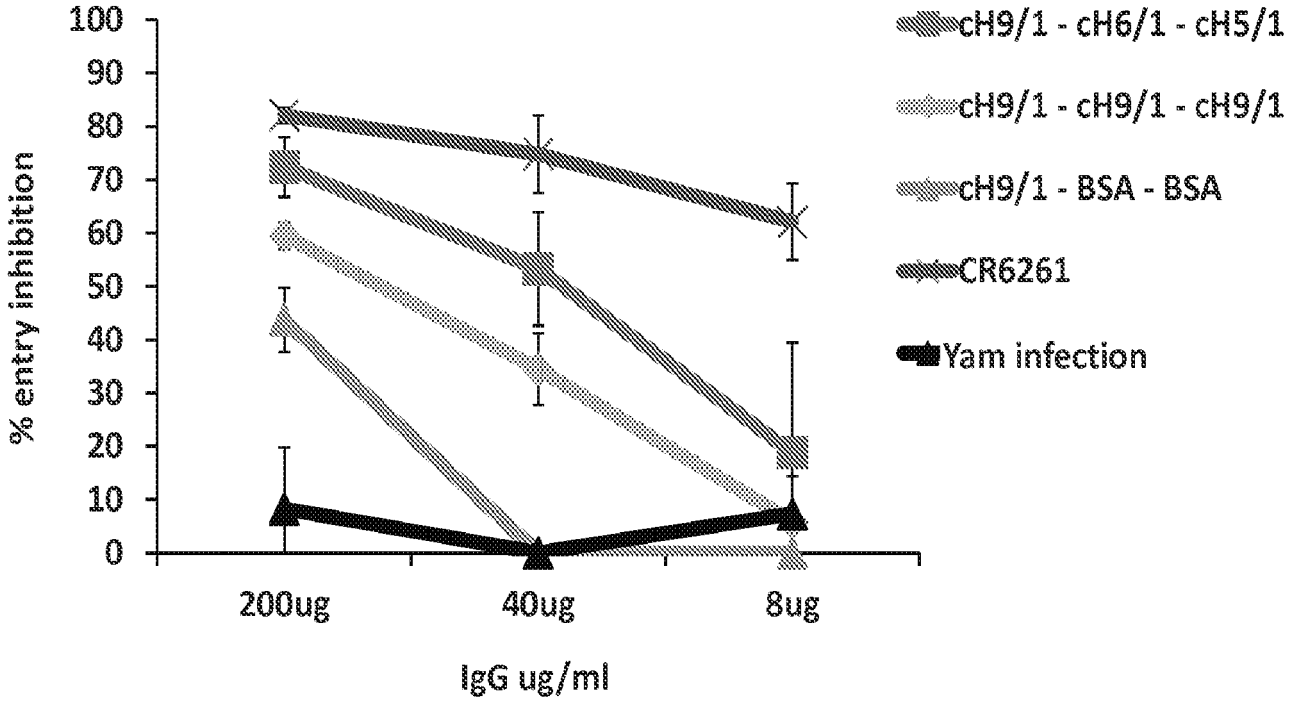

100
90
80
70
60
50
40
30
20
10
0
% entry inhibition
200ug
40ug
8ug
IgG ug/ml
cH9/1 - cH6/1 - cH5/1
cH9/1 - cH9/1 - cH9/1
cH9/1 - BSA - BSA
CR6261
Yam infection

FIG. 30J

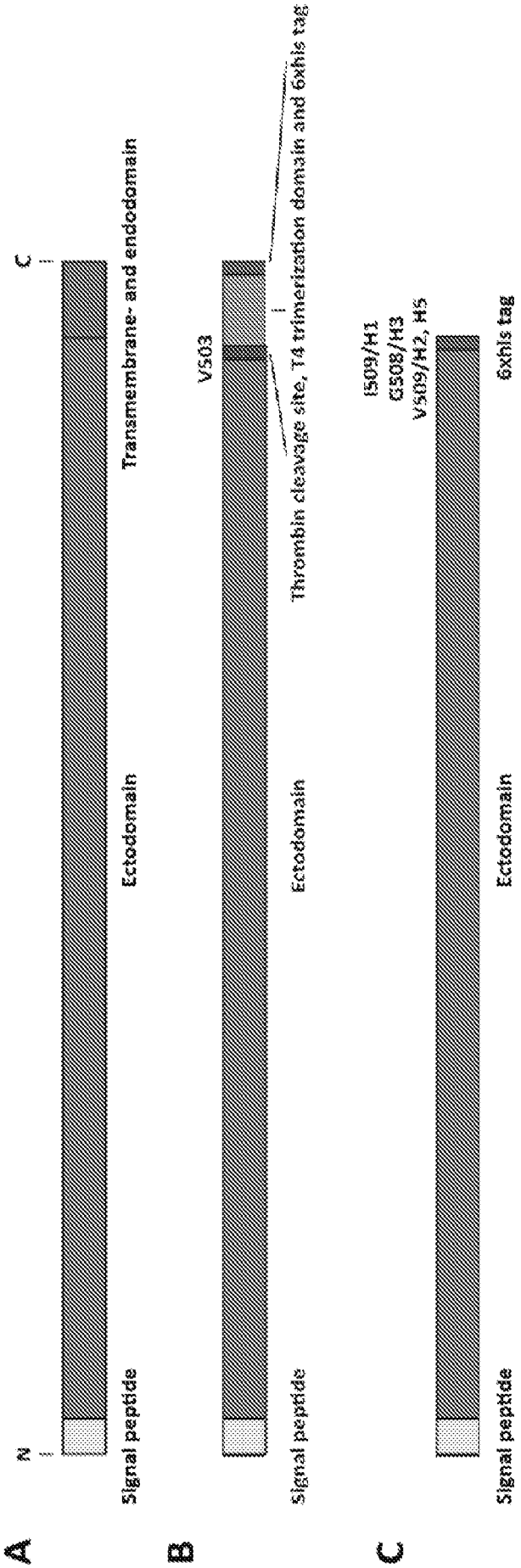

A
N
C
Signal peptide
Ectodomain
Transmembrane- and endodomain
B
Signal peptide
Ectodomain
V503
Thrombin cleavage site, T4 trimerization domain and 6xhis tag
C
Signal peptide
Ectodomain
I509/H1
G508/H3
V509/H2, H5
6xhis tag

FIG. 31A -31C

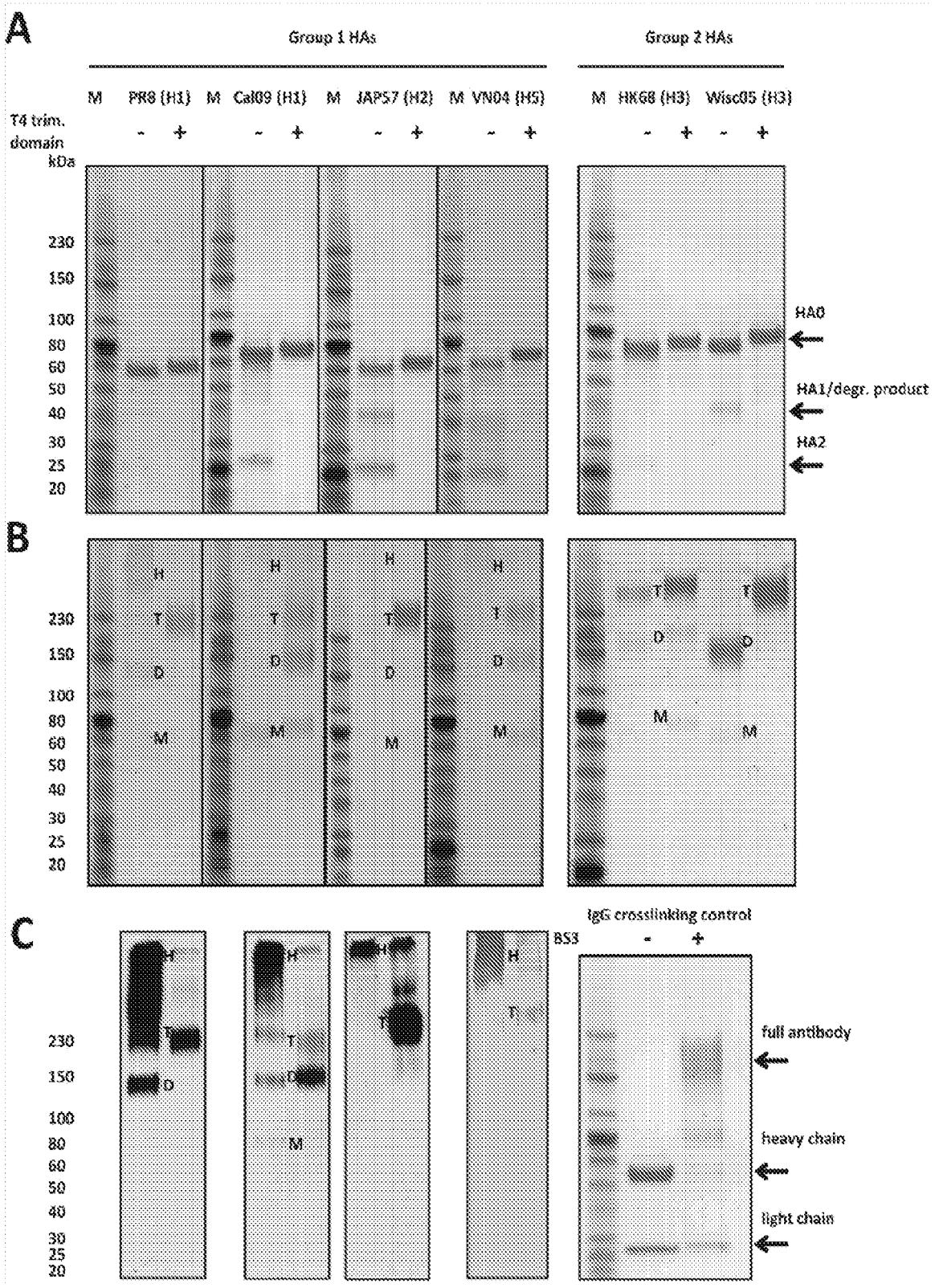

A
Group 1 HAs
Group 2 HAs
M PR8 (H1) M Cal09 (H1) M JAPS7 (H2) M VN04 (H5) M HK68 (H3) Wisc05 (H3)
T4 trim. domain
- + - + - + - + - + - +
kDa
230
150
100
80
60
50
40
30
25
20
HA0
HA1/degr. product
HA2
B
H
T
D
M
C
IgG crosslinking control
BS3 - +
full antibody
heavy chain
light chain

FIG. 32A - 32C

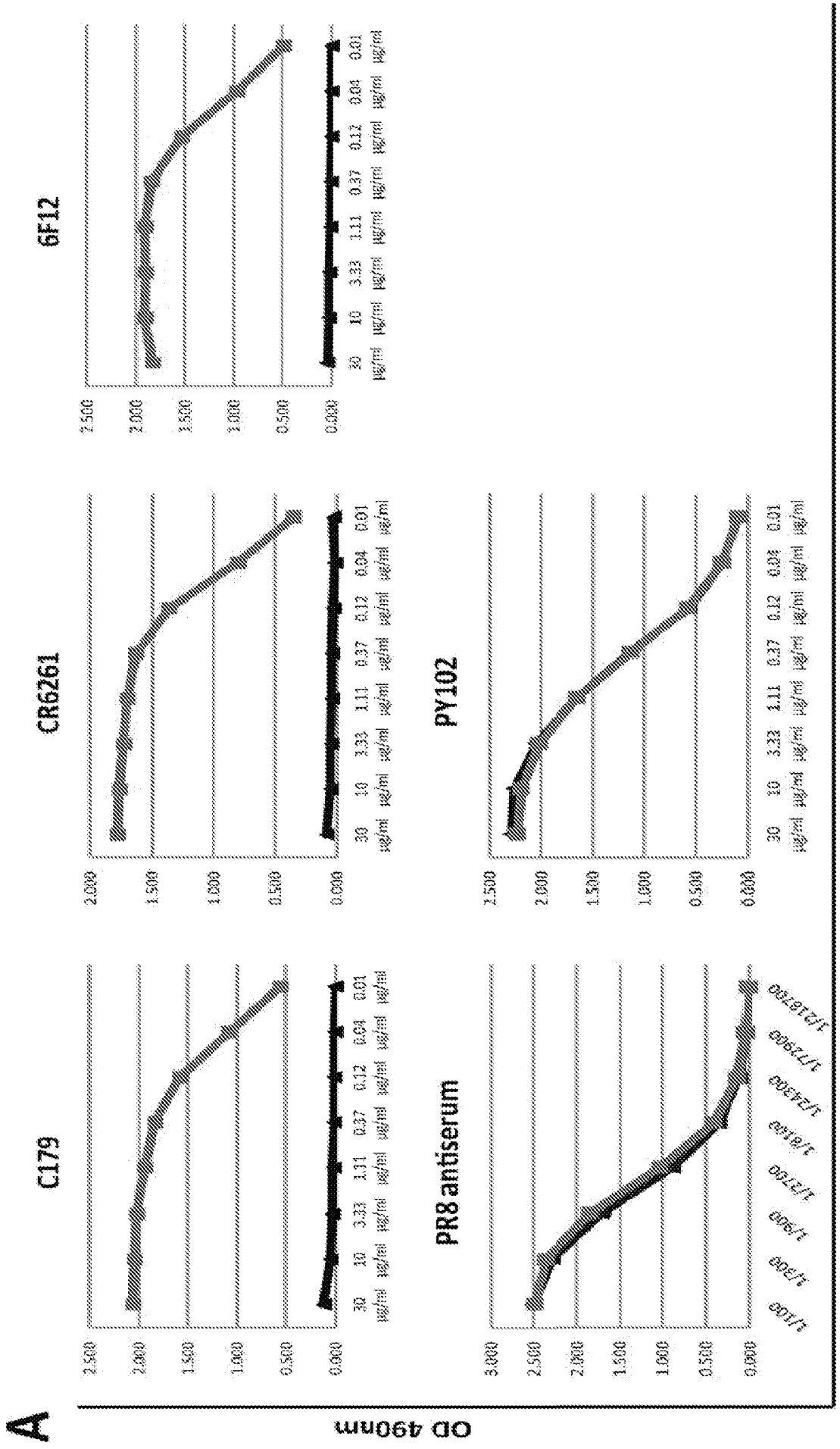

A
OD 490nm
C179
CR6261
6F12
PR8 antiserum
PY102
3.000
2.500
2.000
1.500
1.000
0.500
0.000
1/10
1/30
1/90
1/270
1/810
1/2430
1/7290
1/21870
30 10 3.33 1.11 0.37 0.12 0.04 0.01
μg/ml

FIG. 33A

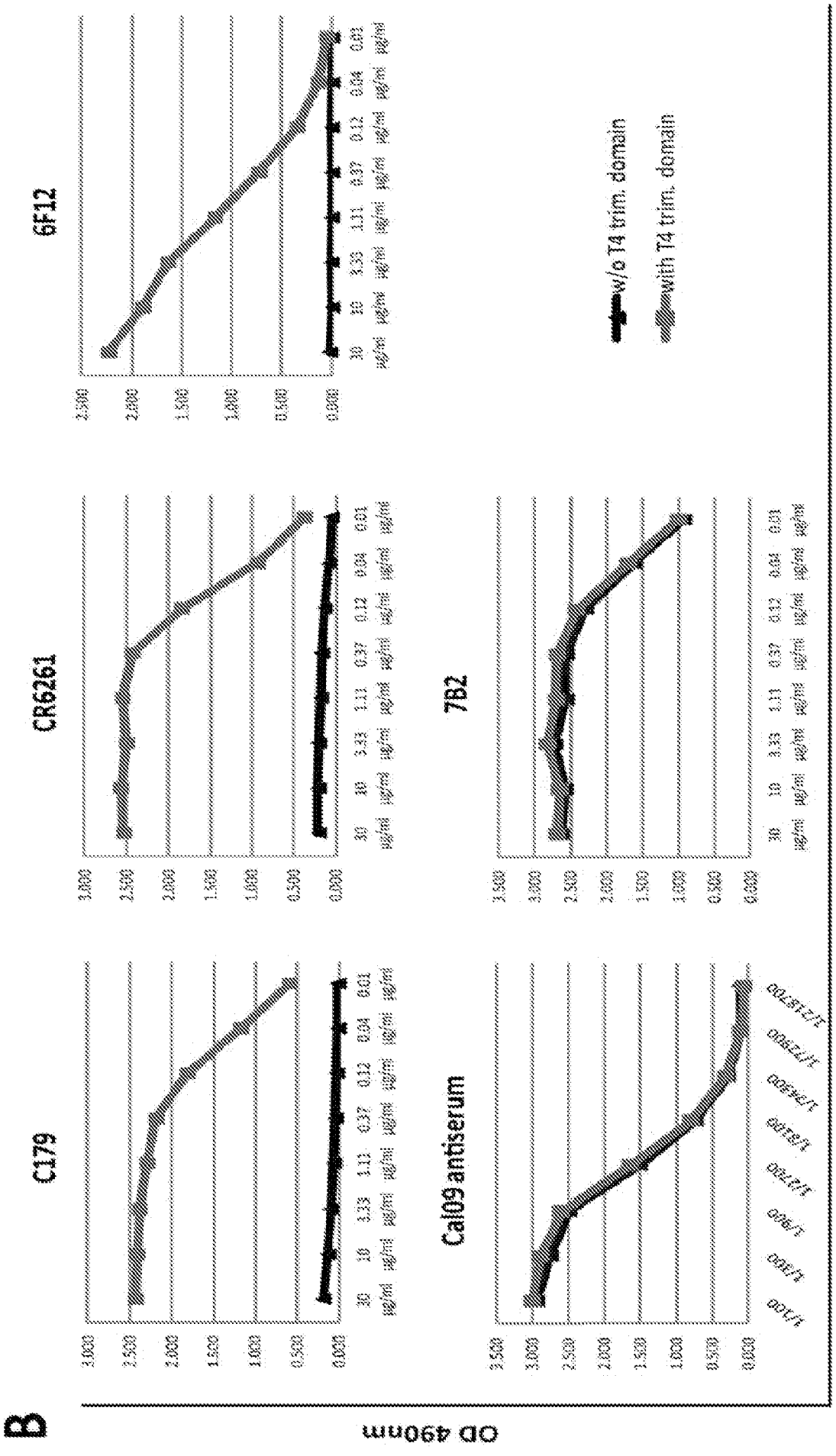

B
OD 490nm
C179
CR6261
6F12
Cal09 antiserum
7B2
w/o T4 trim. domain
with T4 trim. domain
Dilution

FIG. 33B

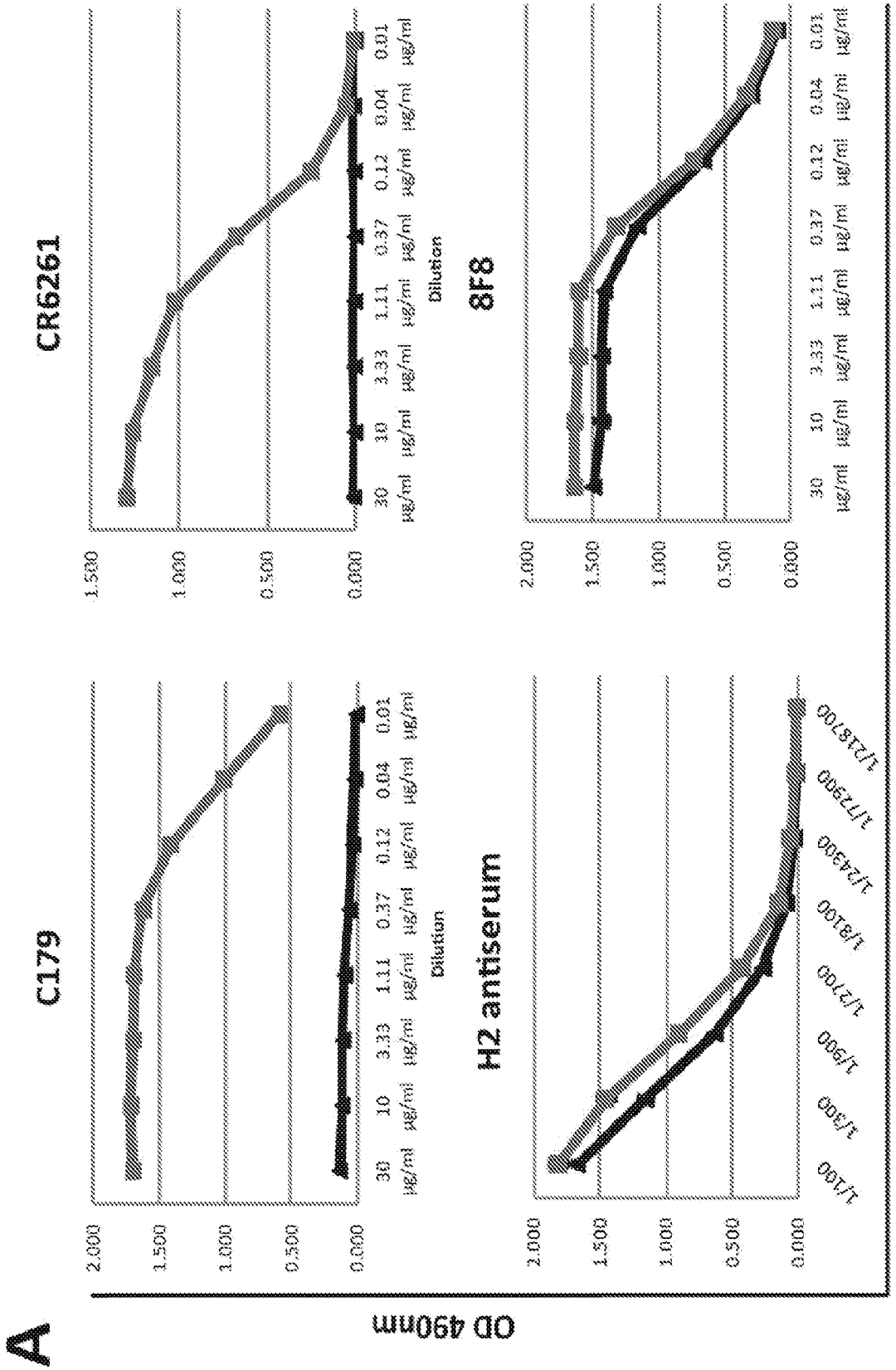

A
OD 490nm
C179
CR6261
H2 antiserum
8F8
2.000
1.500
1.000
0.500
0.000
30 µg/ml
10 µg/ml
3.33 µg/ml
1.11 µg/ml
0.37 µg/ml
0.12 µg/ml
0.04 µg/ml
0.01 µg/ml
Dilution
1/100
1/300
1/900
1/2700
1/8100
1/24300
1/72900
1/218700
Dilution

FIG. 34A

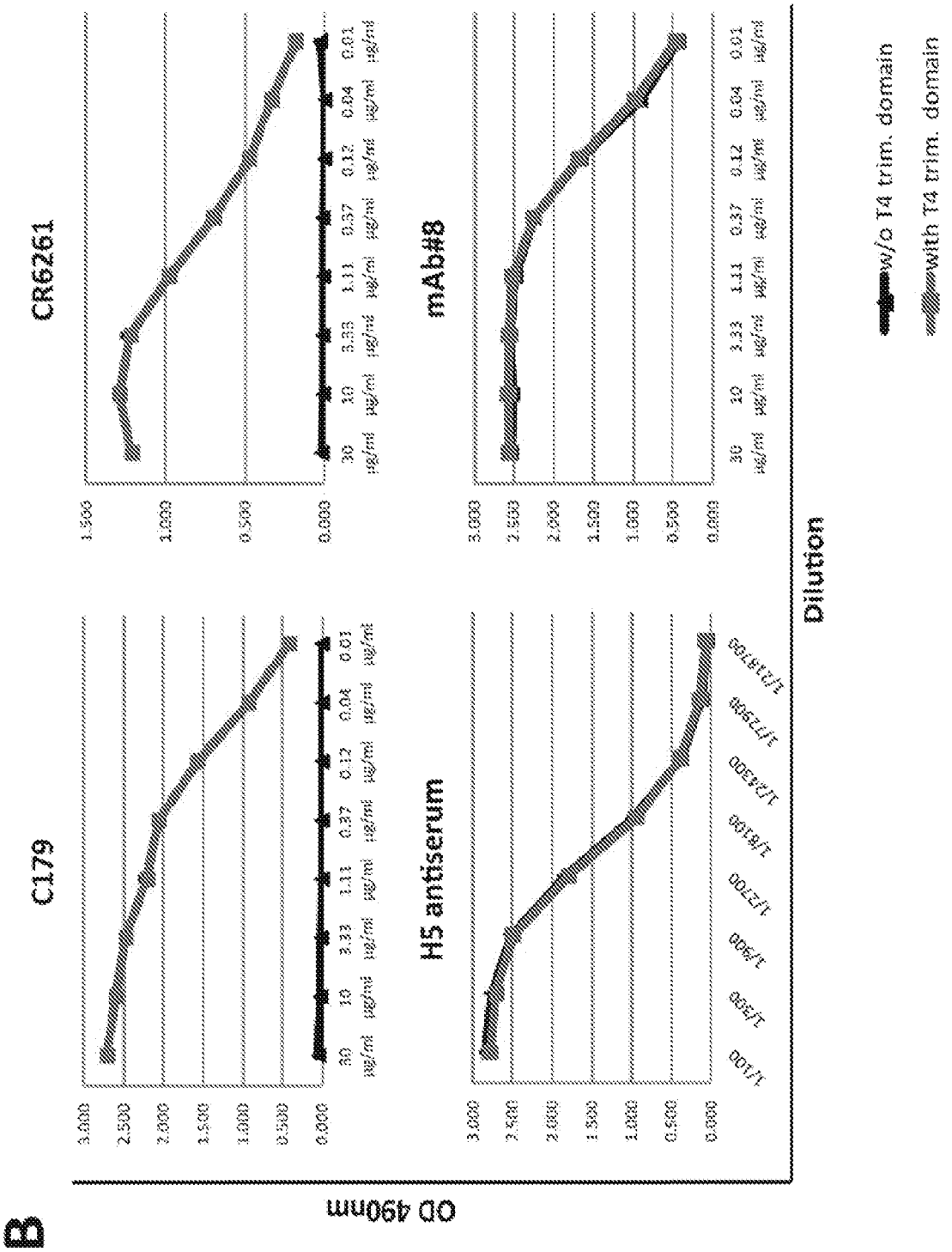

B
C179
CR6261
H5 antiserum
mAb#8
OD 490nm
Dilution
w/o T4 trim. domain
with T4 trim. domain

FIG. 34B

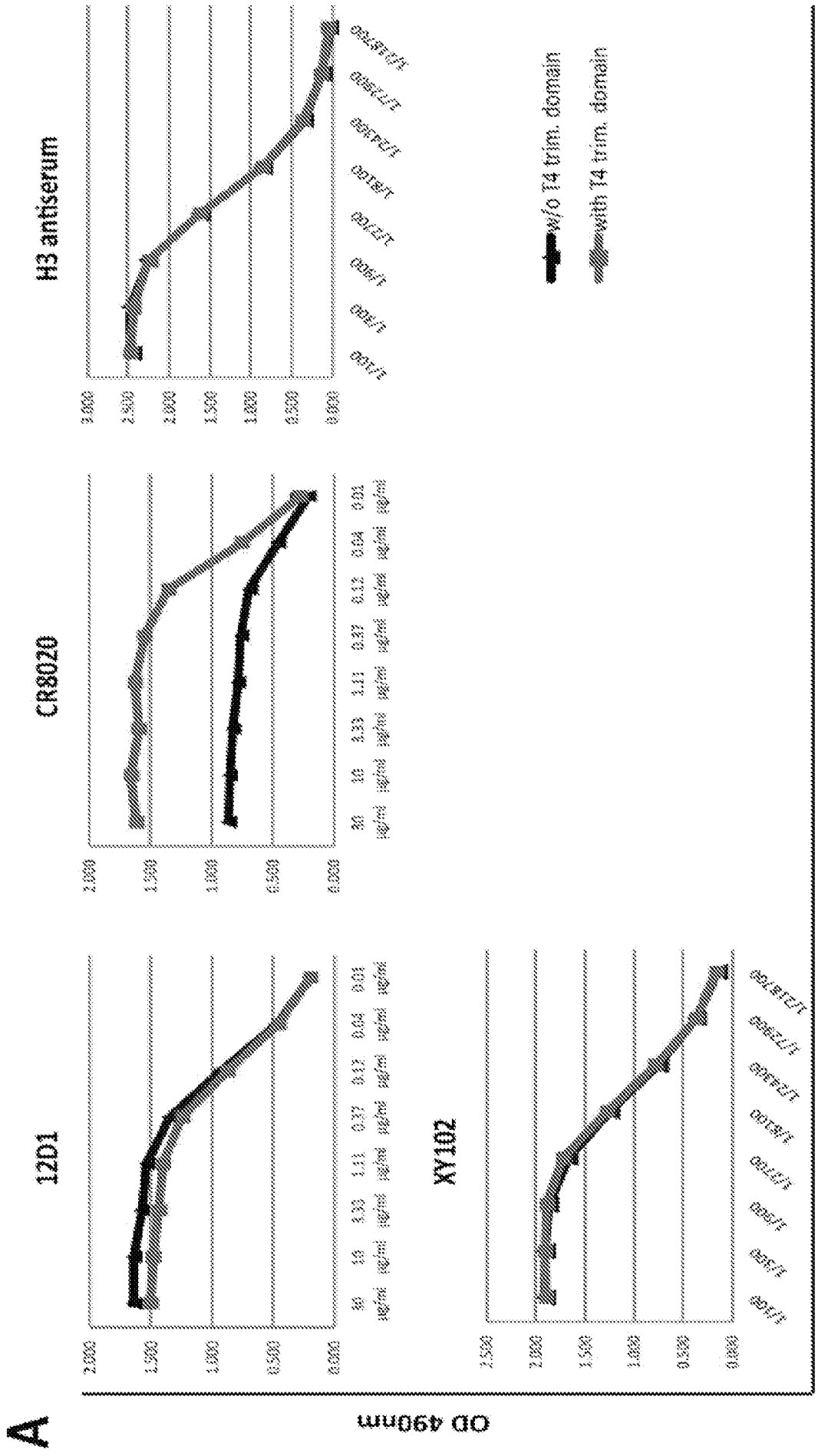

A
OD 490nm
12D1
CR8020
H3 antiserum
XY102
w/o T4 trim. domain
with T4 trim. domain
Dilution

FIG. 35A

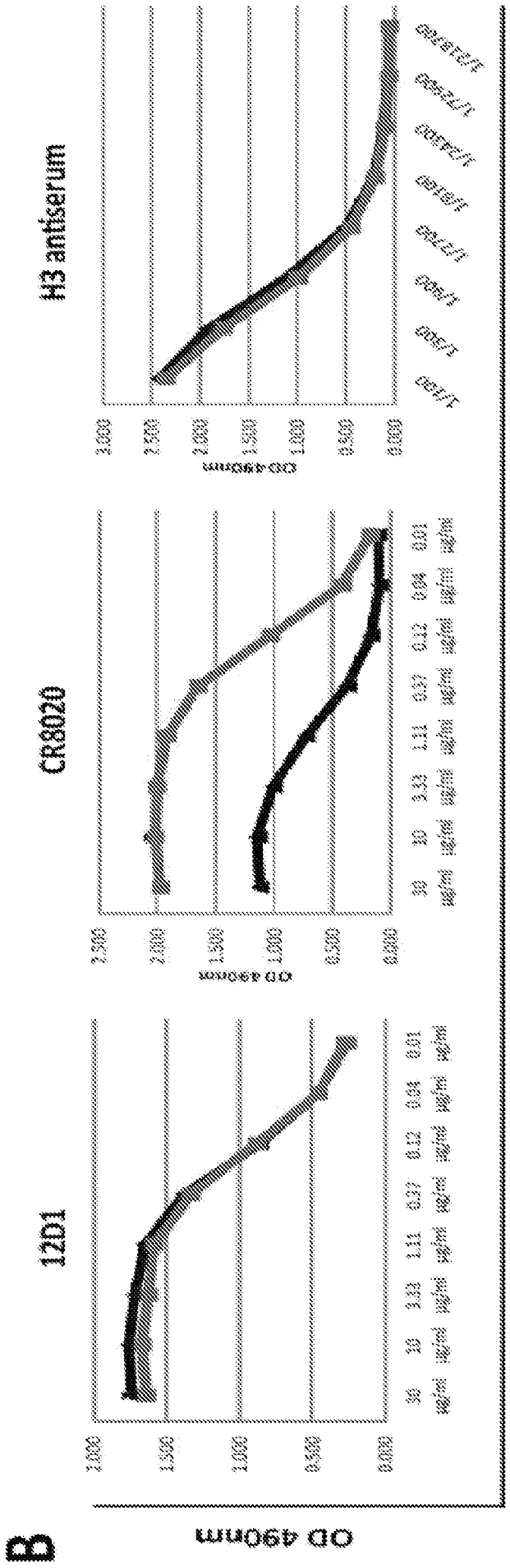


FIG. 35B

MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTG
VIPLTTTPTKSYFA*C**DLDGVKPLILRDCSVAGWLLGNPMCDEFINV*
PEWSYIVEKANPVNDLCYPGDFNDYEELKHLLSRINHFEKIQIIPKSSW
SSHEASLGVSSACPYQGKSSFFRNVVWLIKKNSTYPTIKRSYNNTNQED
LLVLWGIHHPNDAAEQTKLYQNPTTYISVGTSTLNQRLVPRIATRSKVN
GQSGRMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSTIMKSE
*LEYGN**C***ADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTP**
LKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAGWH
GYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQ
RLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGII
NSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLD
RIAAGTFNAGEFSLPTFDSLNITAASLNDDGLDNHTILLYYSTA
ASSLAVTLMLAIFIVYMVSRDNVSCSICL

Bold Text: B/Florida/4/2006 stalk domain

Italicized Text: A/Vietnam/1203/2004 H5 globular head domain

Enlarged Text: Cysteines from H5 globular head domain which may guide insertion location of the globular head domain

FIG. 36

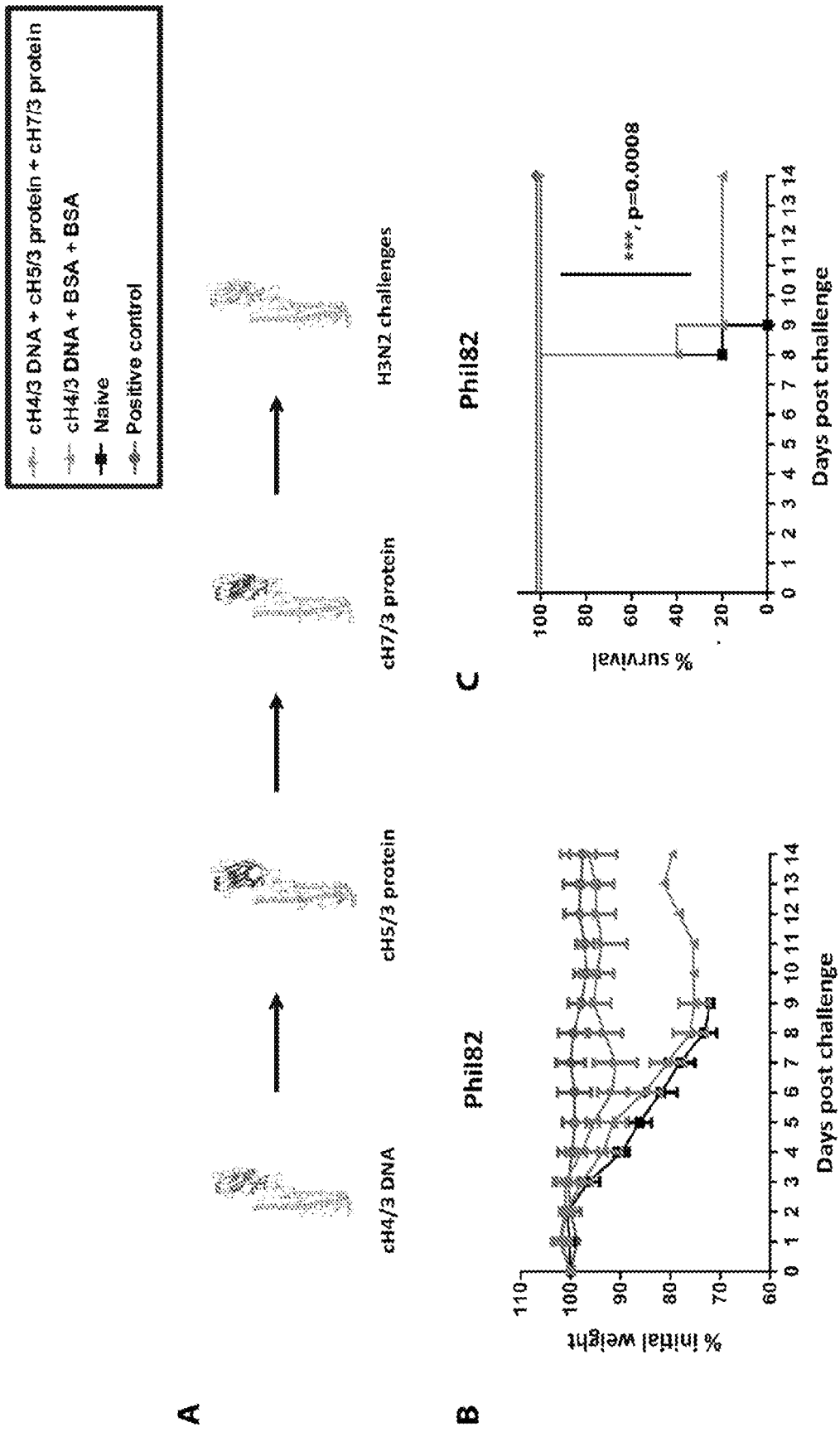

cH4/3 DNA + cH5/3 protein + cH7/3 protein
cH4/3 DNA + BSA + BSA
Naive
Positive control
A
cH4/3 DNA
cH5/3 protein
cH7/3 protein
H3N2 challenges
B
Phil82
% initial weight
Days post challenge
C
Phil82
% survival
***, p=0.0008
Days post challenge

Fig. 37 A-C

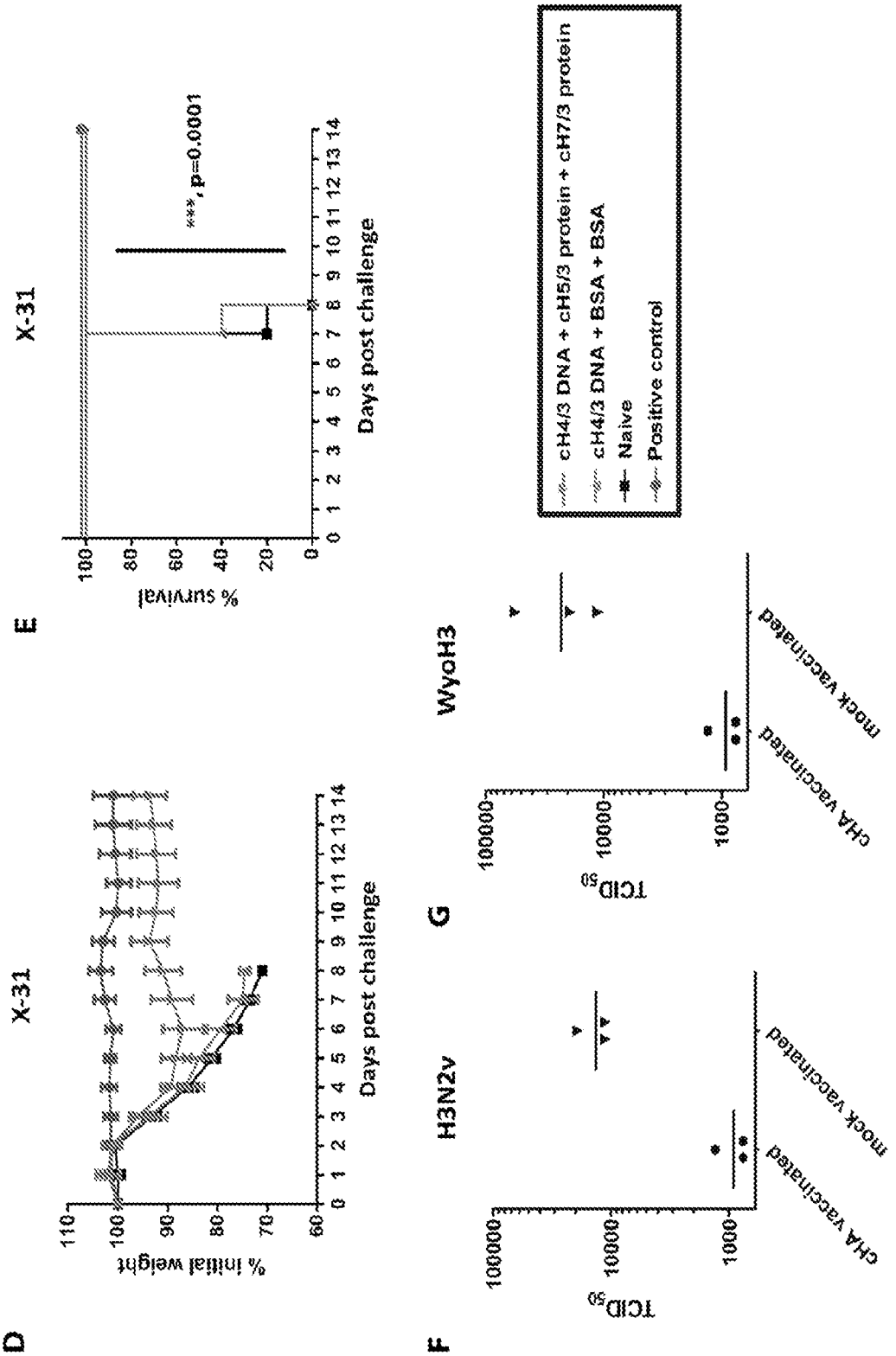

D
X-31
% initial weight
Days post challenge
E
X-31
% survival
***, p=0.0001
Days post challenge
cH4/3 DNA + cH5/3 protein + cH7/3 protein
cH4/3 DNA + BSA + BSA
Naive
Positive control
F
H3N2v
TCID50
cHA Vaccinated
mock Vaccinated
G
WyoH3
TCID50
cHA Vaccinated
mock Vaccinated

Fig. 37 D-G

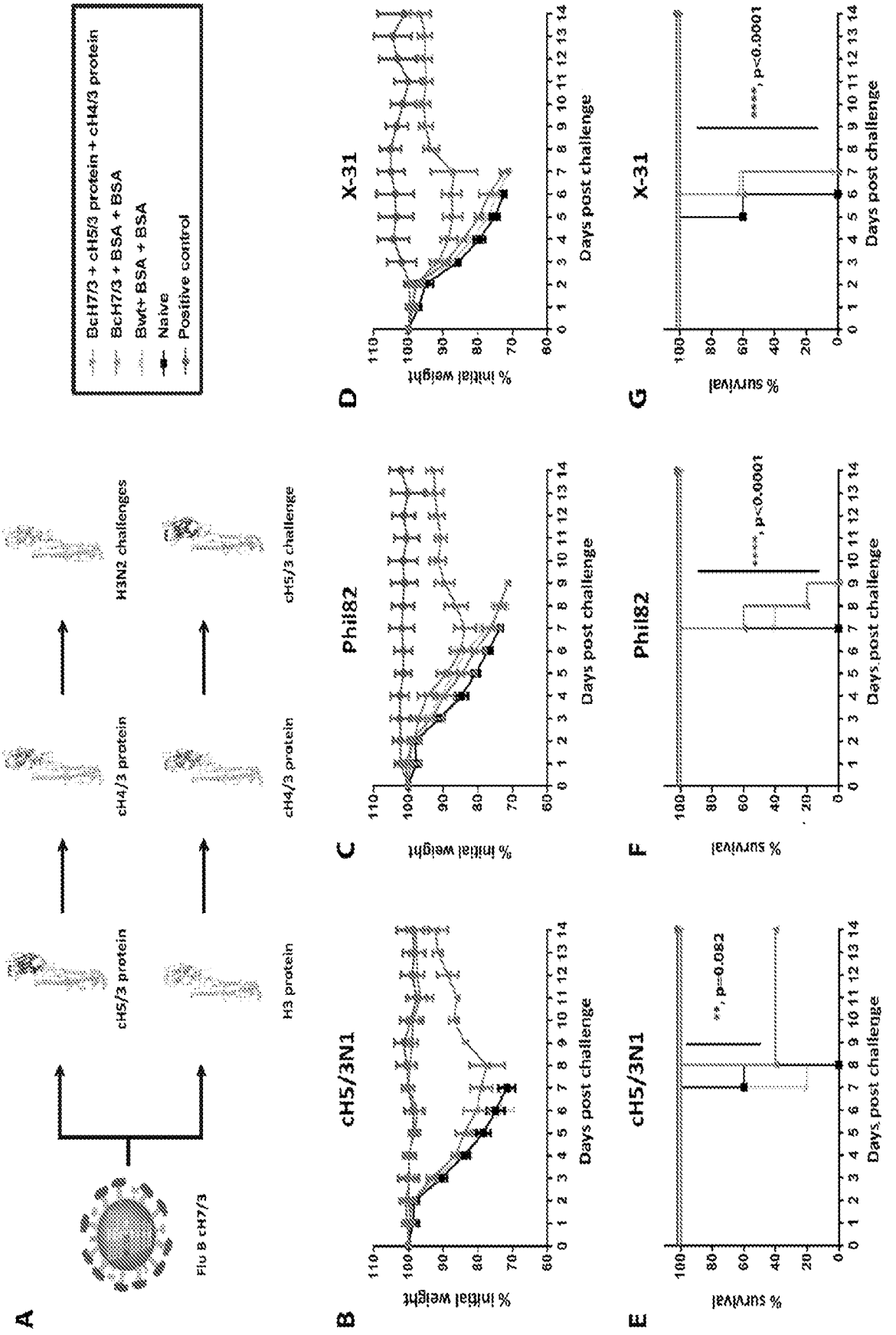

A
cH5/3 protein
H3 protein
Flu B cH7/3
cH4/3 protein
cH4/3 protein
H3N2 challenges
cH5/3 challenge
BcH7/3 + cH5/3 protein + cH4/3 protein
BcH7/3 + BSA + BSA
Bwt+ BSA + BSA
Naive
Positive control
B
cH5/3N1
% initial weight
Days post challenge
C
Phil82
% initial weight
Days post challenge
D
X-31
% initial weight
Days post challenge
E
cH5/3N1
% survival
**, p=0.082
Days post challenge
F
Phil82
% survival
****, p<0.0001
Days post challenge
G
X-31
% survival
****, p<0.0001
Days post challenge

Fig. 38 A-G

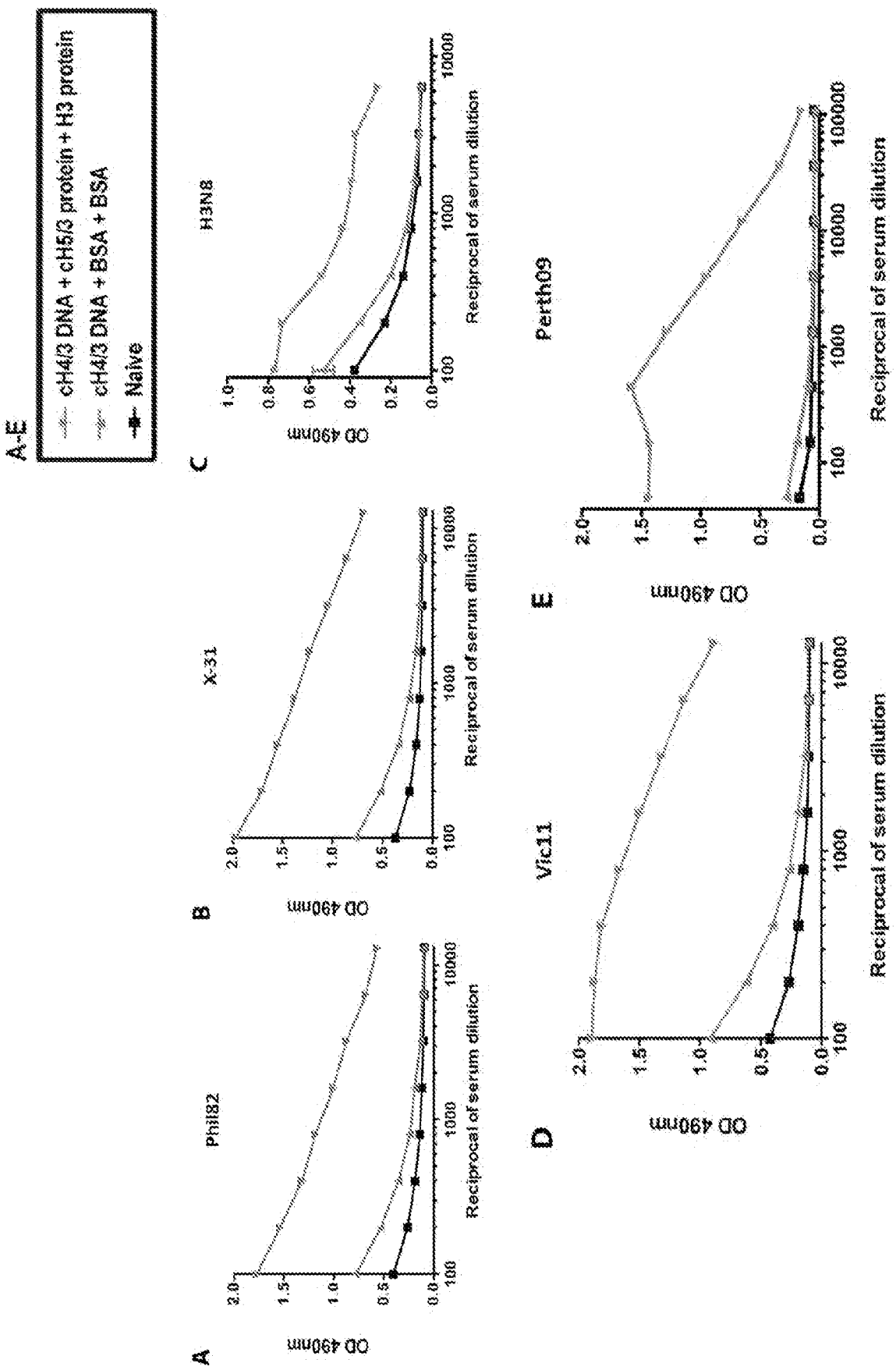

A-E
cH4/3 DNA + cH5/3 protein + H3 protein
cH4/3 DNA + BSA + BSA
Naive
A
Phil82
B
X-31
C
H3N8
D
Vic11
E
Perth09
OD 490nm
Reciprocal of serum dilution

Fig. 39 A-E

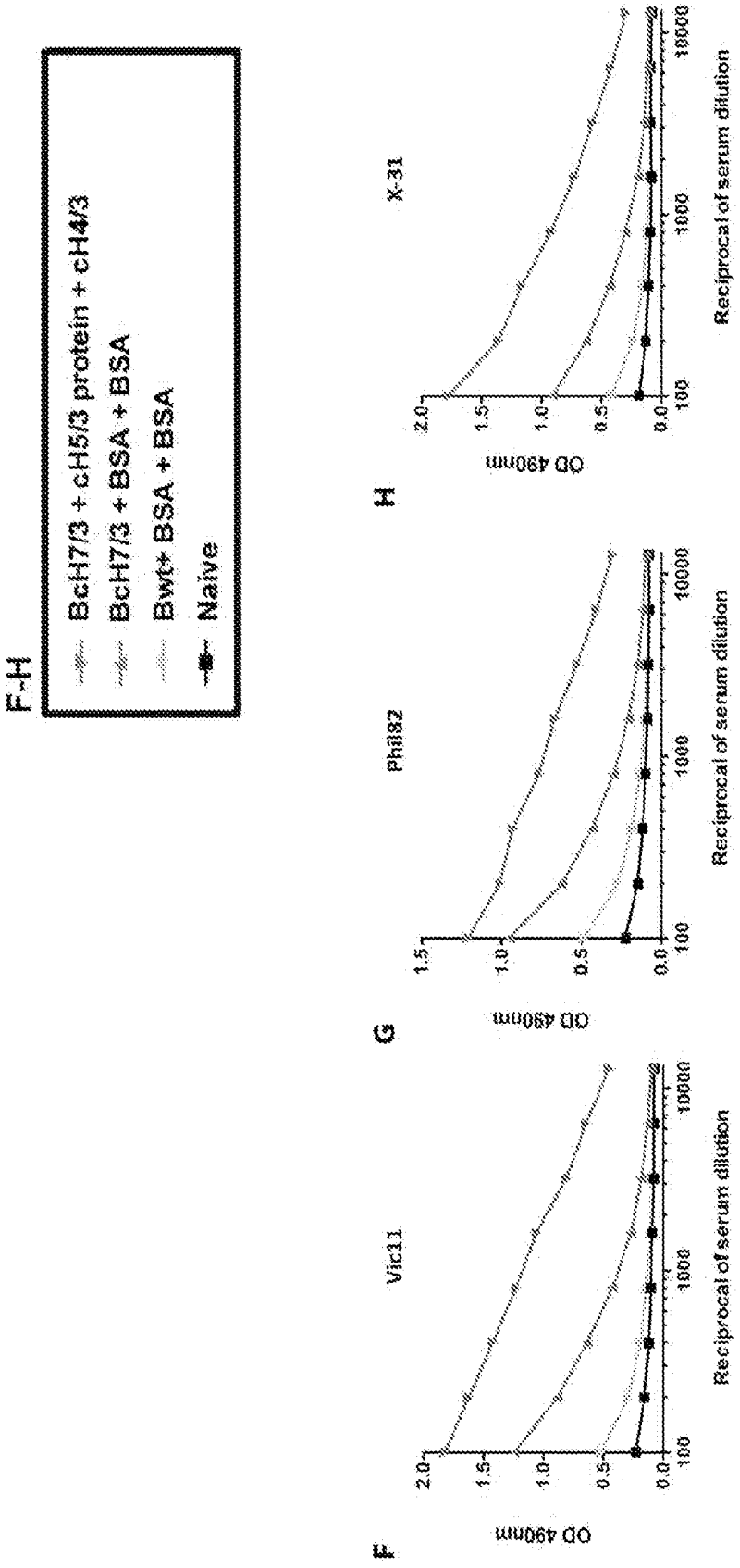

F-H
BcH7/3 + cH5/3 protein + cH4/3
BcH7/3 + BSA + BSA
Bwt+ BSA + BSA
Naive
F
Vic11
G
Phil82
H
X-31
OD 490nm
Reciprocal of serum dilution

Fig. 39 F-H

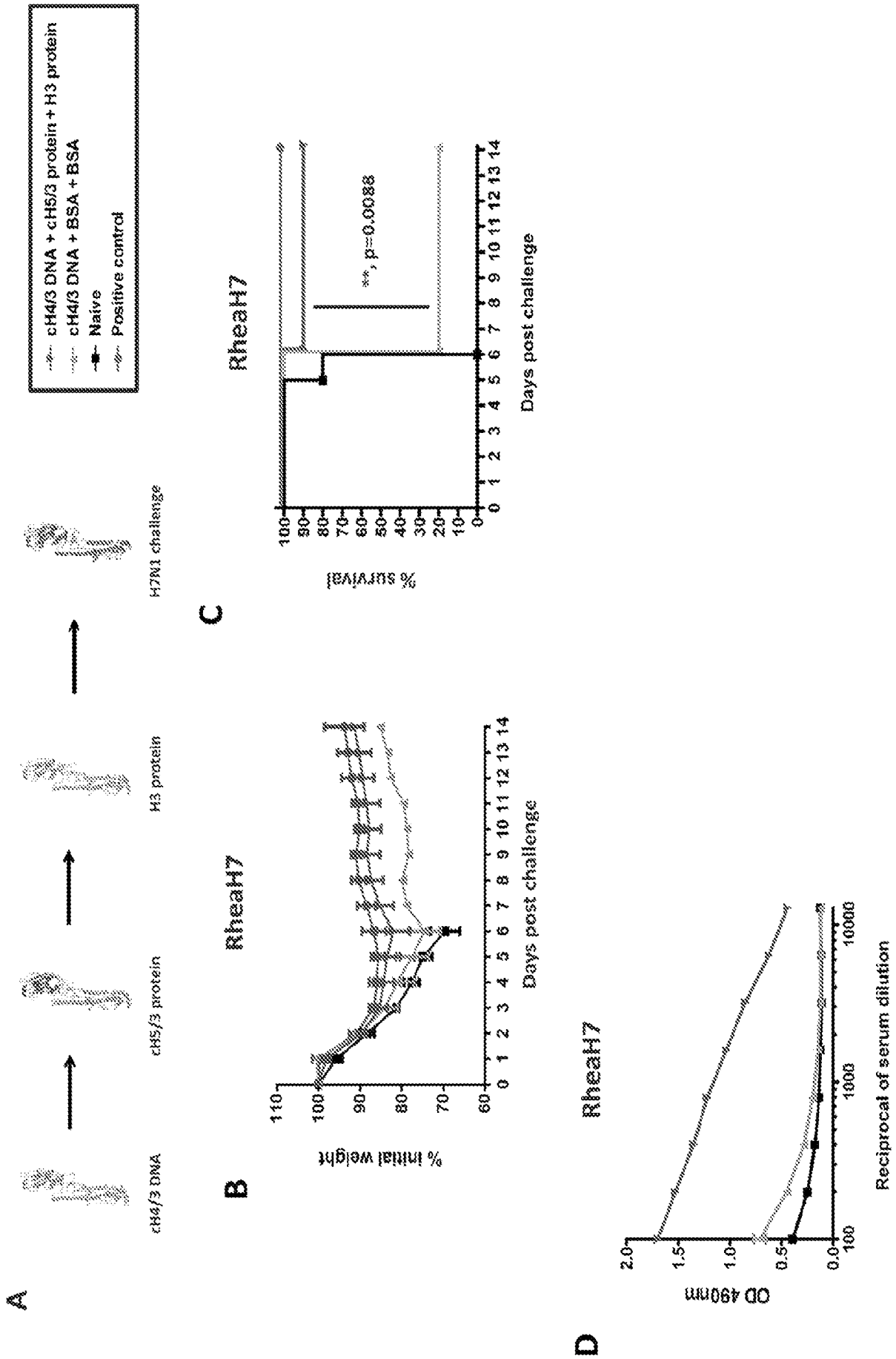

A
cH4/3 DNA
cH5/3 protein
H3 protein
H7N1 challenge
cH4/3 DNA + cH5/3 protein + H3 protein
cH4/3 DNA + BSA + BSA
Naive
Positive control
B
RheaH7
% initial weight
Days post challenge
C
RheaH7
% survival
**, p=0.0088
Days post challenge
D
RheaH7
OD 490nm
Reciprocal of serum dilution

Fig. 40 A-D

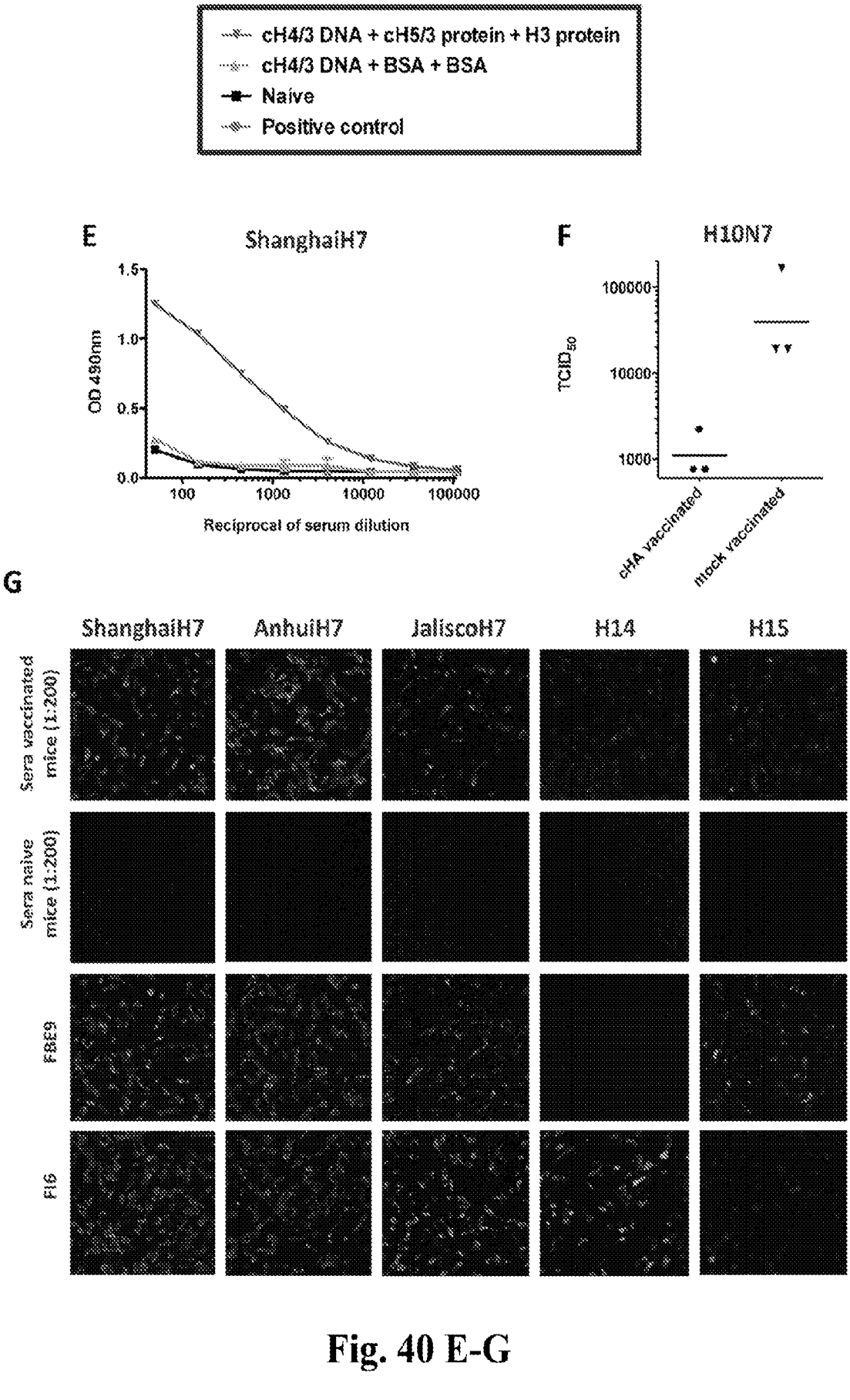

cH4/3 DNA + cH5/3 protein + H3 protein
cH4/3 DNA + BSA + BSA
Naive
Positive control
E
ShanghaiH7
OD 490nm
1.5
1.0
0.5
0.0
100
1000
10000
100000
Reciprocal of serum dilution
F
H10N7
TCID50
100000
10000
1000
cHA vaccinated
mock vaccinated
G
ShanghaiH7
AnhuiH7
JaliscoH7
H14
H15
Sera vaccinated mice (1:200)
Sera naive mice (1:200)
FBE9
F16

Fig. 40 E-G

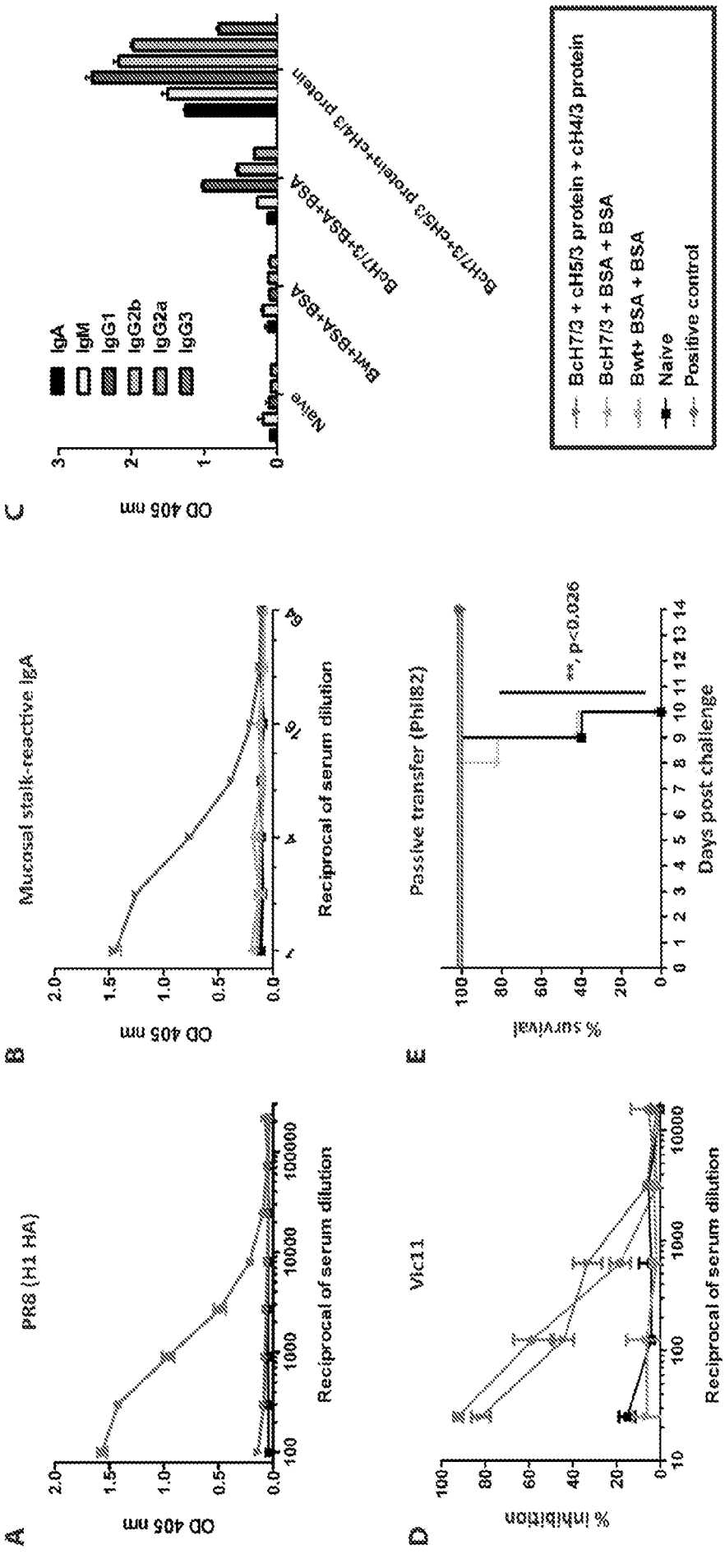

A
PR8 (H1 HA)
OD 405 nm
Reciprocal of serum dilution
B
Mucosal stalk-reactive IgA
OD 405 nm
Reciprocal of serum dilution
C
IgA
IgM
IgG1
IgG2b
IgG2a
IgG3
OD 405 nm
Naive
Bwt+BSA+BSA
BcH7/3+BSA+BSA
BcH7/3+cHs3 protein+cH4/3 protein
BcH7/3 + cH5/3 protein + cH4/3 protein
BcH7/3 + BSA + BSA
Bwt+ BSA + BSA
Naive
Positive control
D
Vic11
% inhibition
Reciprocal of serum dilution
E
Passive transfer (Phil82)
**, p<0.026
% survival
Days post challenge

Figure 41 A-E

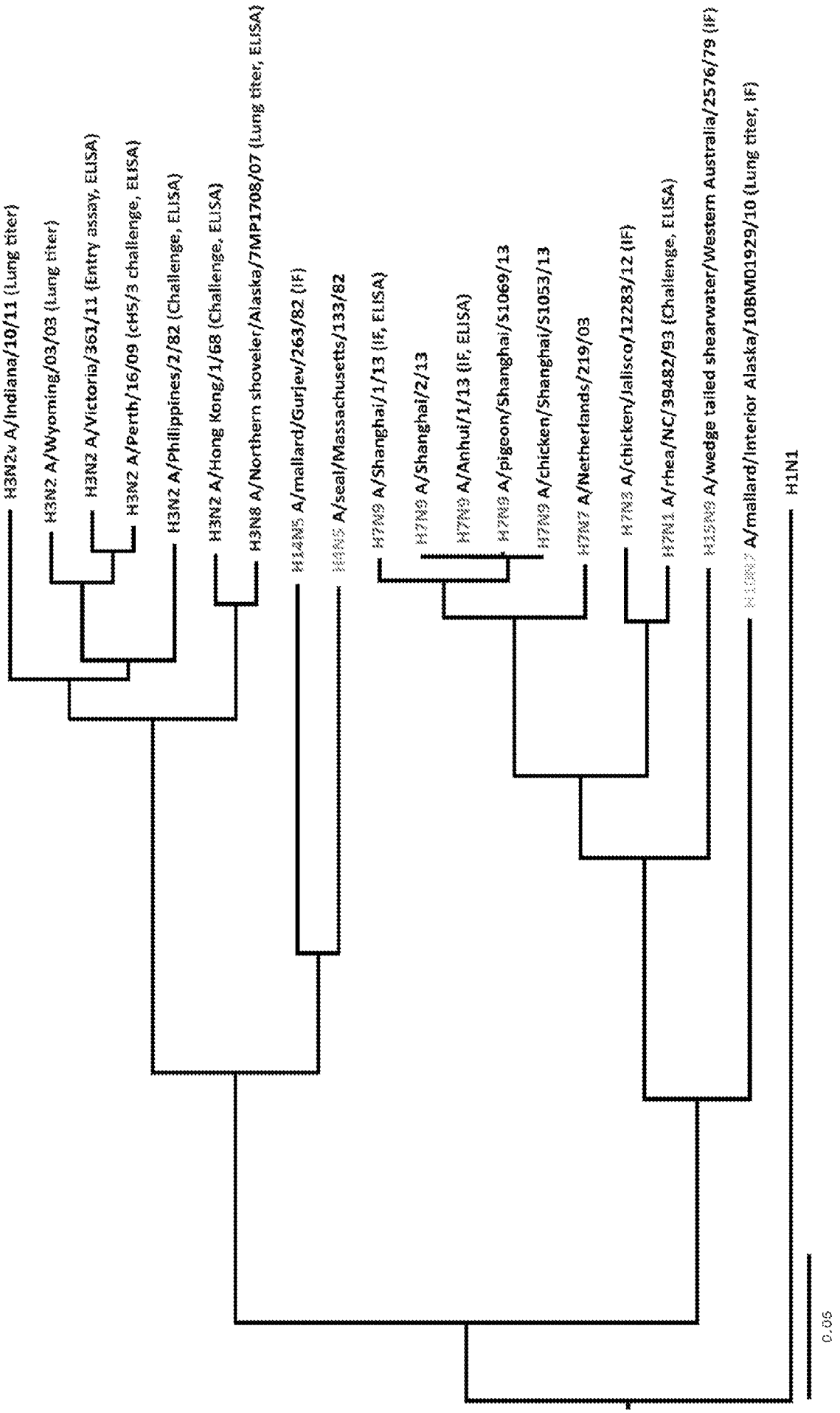

H3N2v A/Indiana/10/11 (Lung titer)
H3N2 A/Wyoming/03/03 (Lung titer)
H3N2 A/Victoria/361/11 (Entry assay, ELISA)
H3N2 A/Perth/16/09 (cH5/3 challenge, ELISA)
H3N2 A/Philippines/2/82 (Challenge, ELISA)
H3N2 A/Hong Kong/1/68 (Challenge, ELISA)
H3N8 A/Northern shoveler/Alaska/7MP1708/07 (Lung titer, ELISA)
H1N1 A/mallard/Gurjev/263/82 (IF)
H1N1 A/seal/Massachusetts/133/82
H7N9 A/Shanghai/1/13 (IF, ELISA)
H7N9 A/Shanghai/2/13
H7N9 A/Anhui/1/13 (IF, ELISA)
H7N9 A/pigeon/Shanghai/S1069/13
H7N9 A/chicken/Shanghai/S1053/13
H7N7 A/Netherlands/219/03
H7N3 A/chicken/Jalisco/12283/12 (IF)
H7N1 A/rhea/NC/39482/93 (Challenge, ELISA)
H1N1 A/wedge tailed shearwater/Western Australia/2576/79 (IF)
H1N1 A/mallard/Interior Alaska/10BM01929/10 (Lung titer, IF)
H1N1
0.05

Figure 42

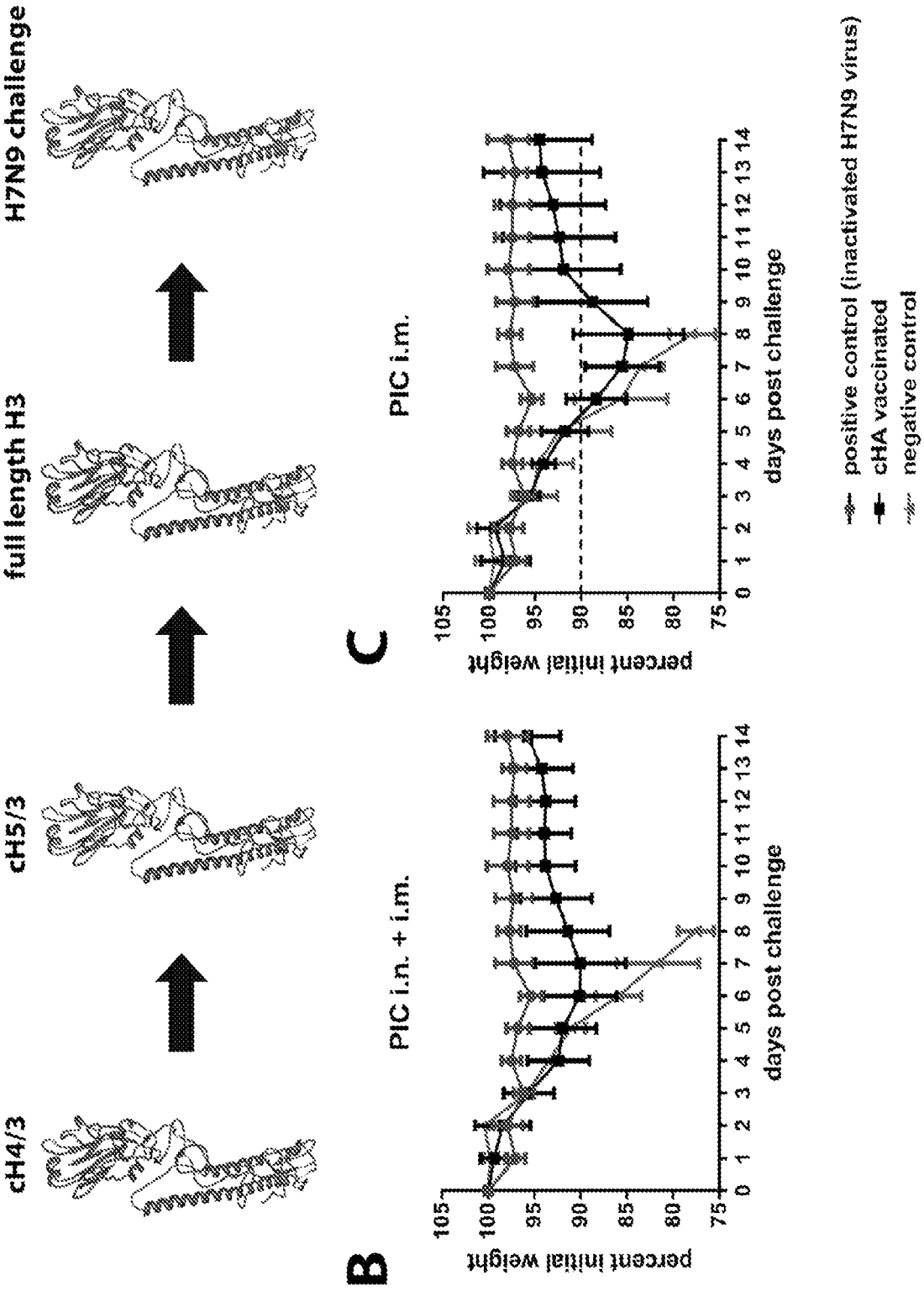

A
cH4/3
cH5/3
full length H3
H7N9 challenge
B
PIC i.n. + i.m.
percent initial weight
days post challenge
C
PIC i.m.
percent initial weight
days post challenge
positive control (inactivated H7N9 virus)
cHA vaccinated
negative control

Figure 43 A-C

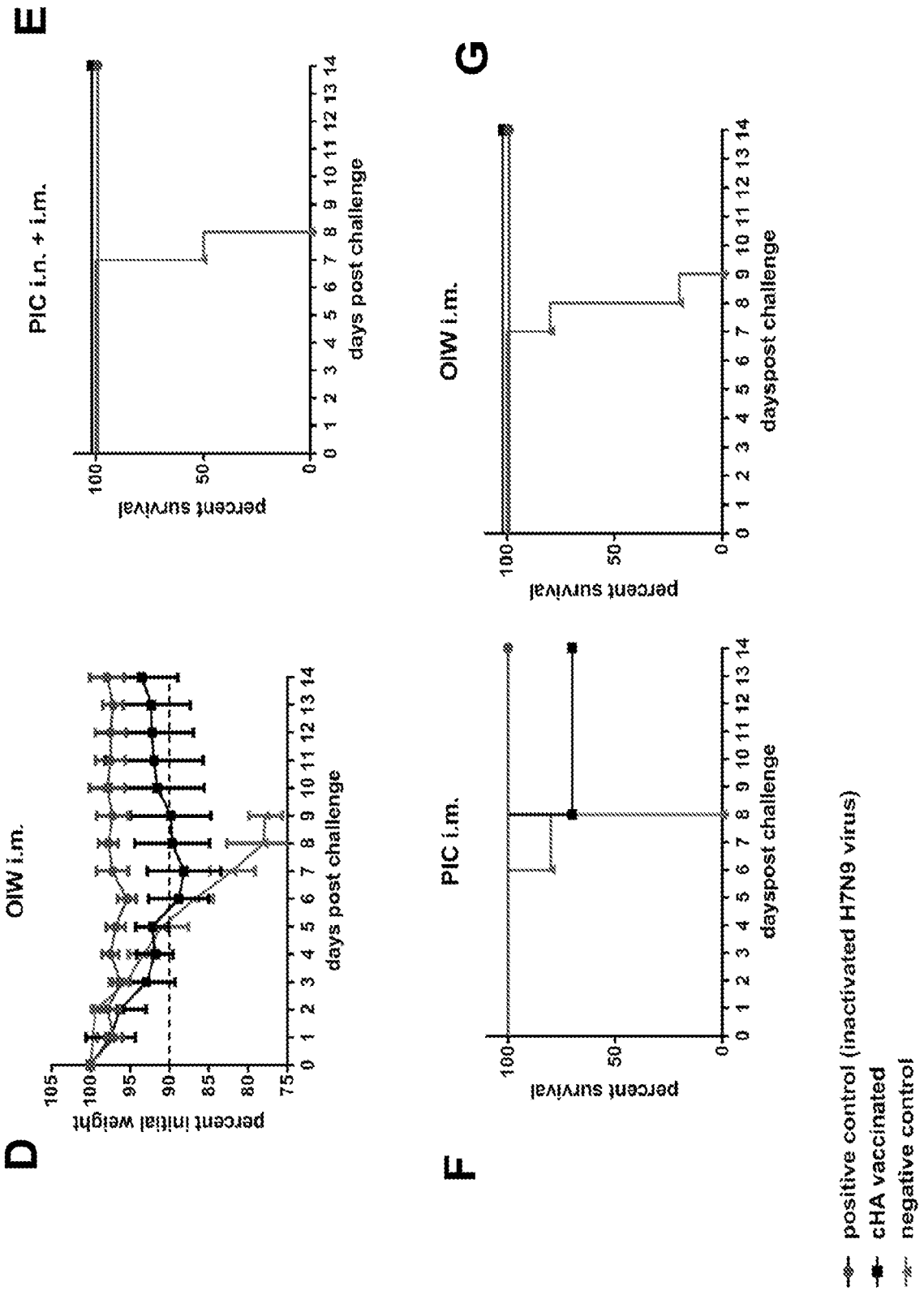

D
OIW i.m.
percent initial weight
105
100
95
90
85
80
75
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
days post challenge
E
PIC i.n. + i.m.
percent survival
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
days post challenge
F
PIC i.m.
percent survival
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
dayspost challenge
G
OIW i.m.
percent survival
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
dayspost challenge
positive control (inactivated H7N9 virus)
cHA vaccinated
negative control

Fig. 43 D-G

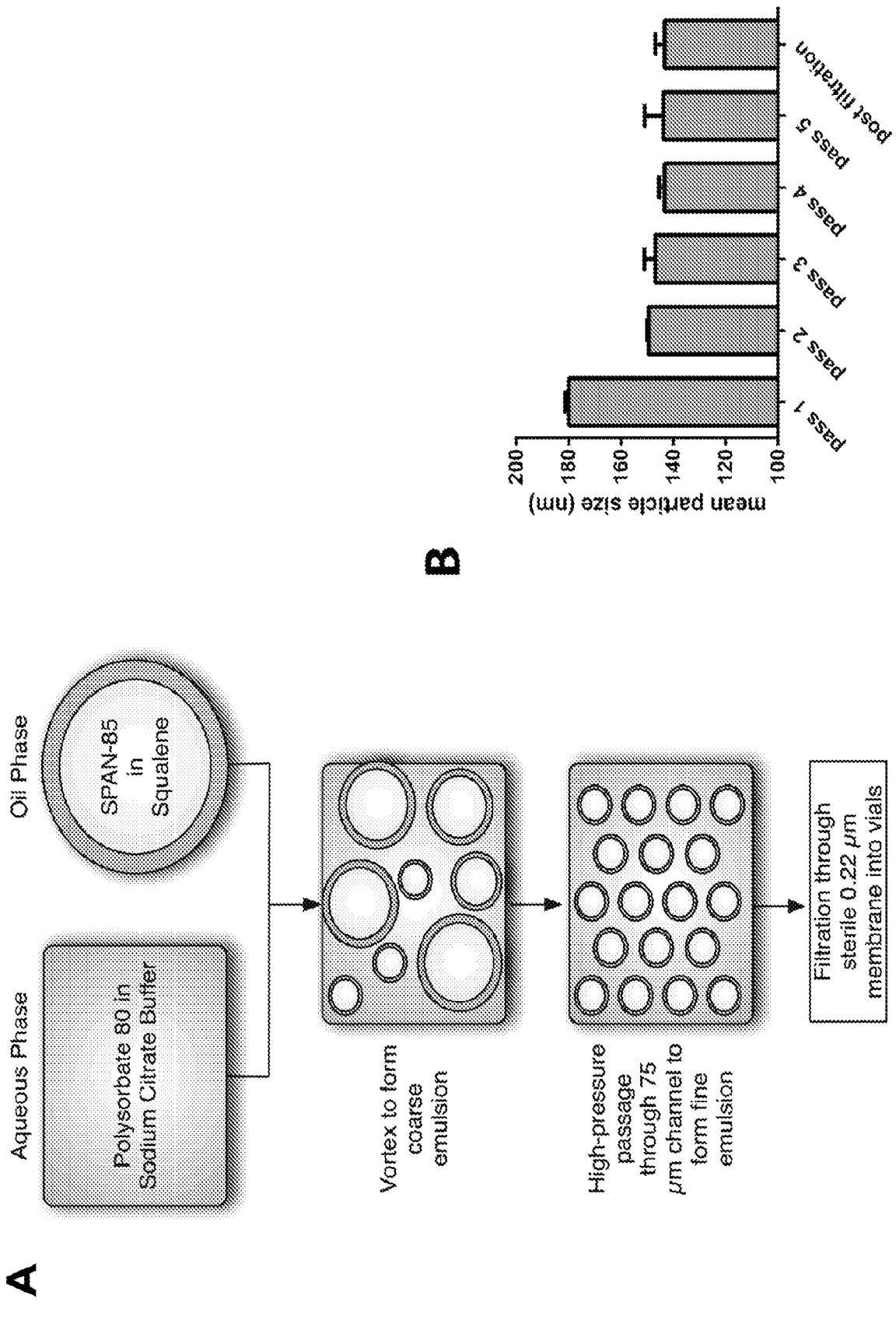

A
Aqueous Phase
Polysorbate 80 in Sodium Citrate Buffer
Oil Phase
SPAN-85 in Squalene
Vortex to form coarse emulsion
High-pressure passage through 75 μm channel to form fine emulsion
Filtration through sterile 0.22 μm membrane into vials
B
mean particle size (nm)
200
180
160
140
120
100
pass 1
pass 2
pass 3
pass 4
pass 5
post filtration

Fig. 44 A-B

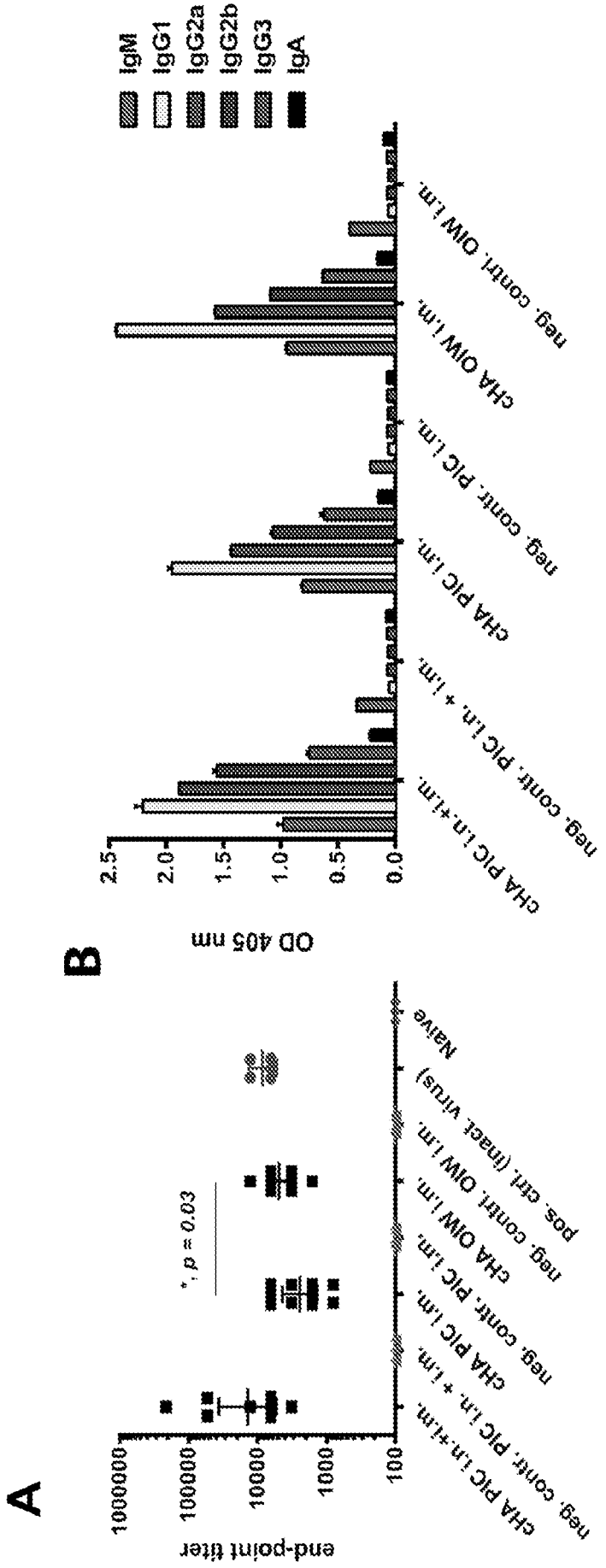

A
end-point titer
1000000
100000
10000
1000
100
*; p = 0.03
CHA PIC i.n.+i.m.
neg. contr. PIC i.n. + i.m.
CHA PIC i.m.
neg. contr. PIC i.m.
CHA OIW i.m.
neg. contr. OIW i.m.
pos. ctrl. (inact. virus)
Naive
B
OD 405 nm
2.5
2.0
1.5
1.0
0.5
0.0
IgM
IgG1
IgG2a
IgG2b
IgG3
IgA
CHA PIC i.n.+i.m.
neg. contr. PIC i.n. + i.m.
CHA PIC i.m.
neg. contr. PIC i.m.
CHA OIW i.m.
neg. contr. OIW i.m.

Fig. 45 A-B