



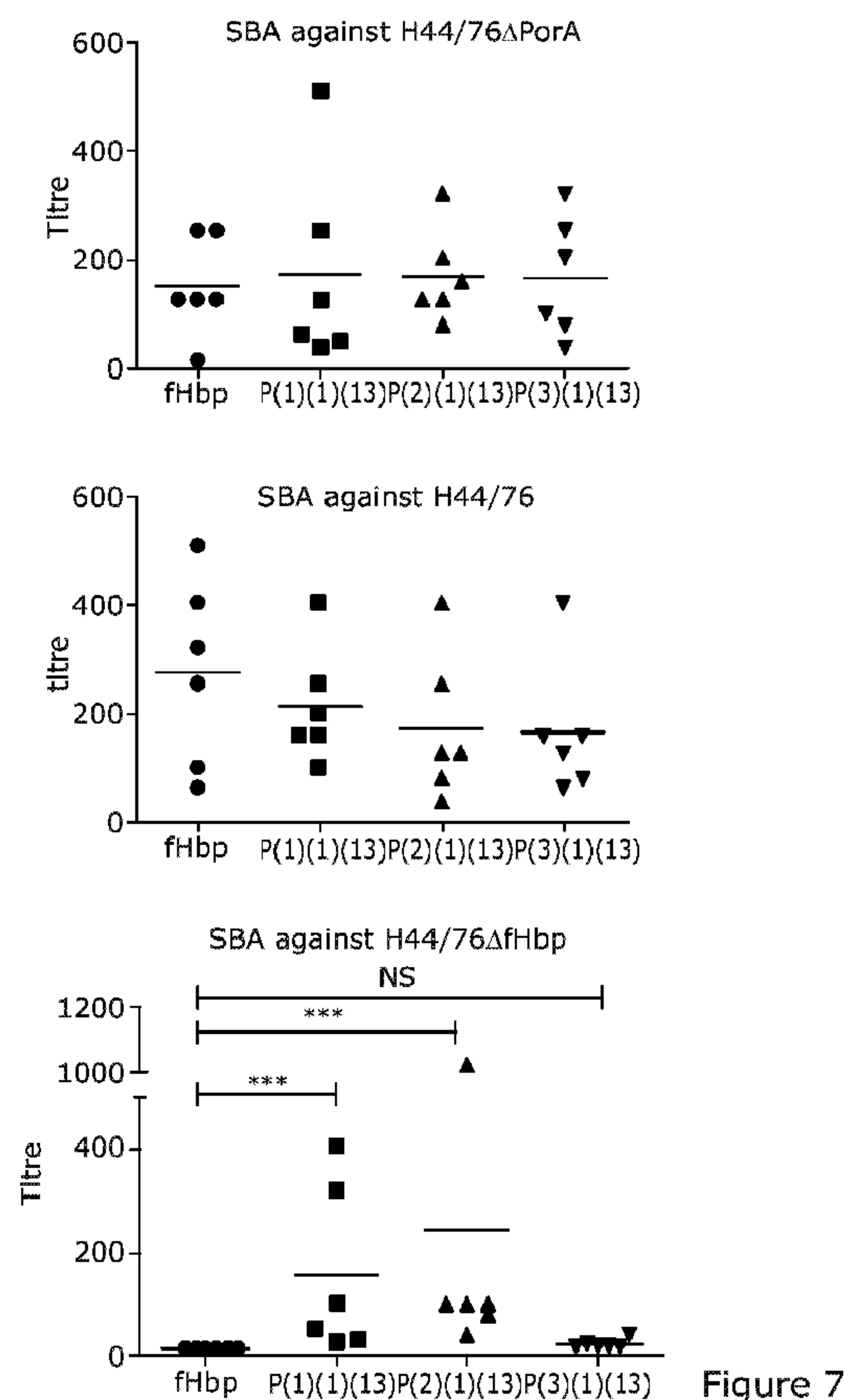
(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2017/08/31
(87) Date publication PCT/PCT Publication Date: 2018/03/08
(85) Entrée phase nationale/National Entry: 2019/02/26
(86) N° demande PCT/PCT Application No.: GB 2017/052535
(87) N° publication PCT/PCT Publication No.: 2018/042178
(30) Priorité/Priority: 2016/08/31 (GB1614687.0)

(51) Cl.Int./Int.Cl. *A61K 39/095* (2006.01),
A61P 31/04 (2006.01), *A61P 37/04* (2006.01)
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(54) Titre : PROTEINE DE LIAISON AU FACTEUR H MODIFIEE
(54) Title: MODIFIED FACTOR H BINDING PROTEIN



(57) **Abrégé/Abstract:**

The invention relates to a modified factor H binding protein (fHbp), comprising fHbp, or a variant thereof, modified with the addition of at least one exogenous peptide loop; and associated nucleic acid, compositions, and uses. The invention further relates to treatment or prevention of a pathogenic infection or colonisation of a subject using the modified factor H binding protein (fHbp).

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
08 March 2018 (08.03.2018)



(10) International Publication Number
WO 2018/042178 A1

(51) International Patent Classification:
A61K 39/095 (2006.01)

(21) International Application Number:
PCT/GB2017/052535

(22) International Filing Date:
31 August 2017 (31.08.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
1614687.0 31 August 2016 (31.08.2016) GB

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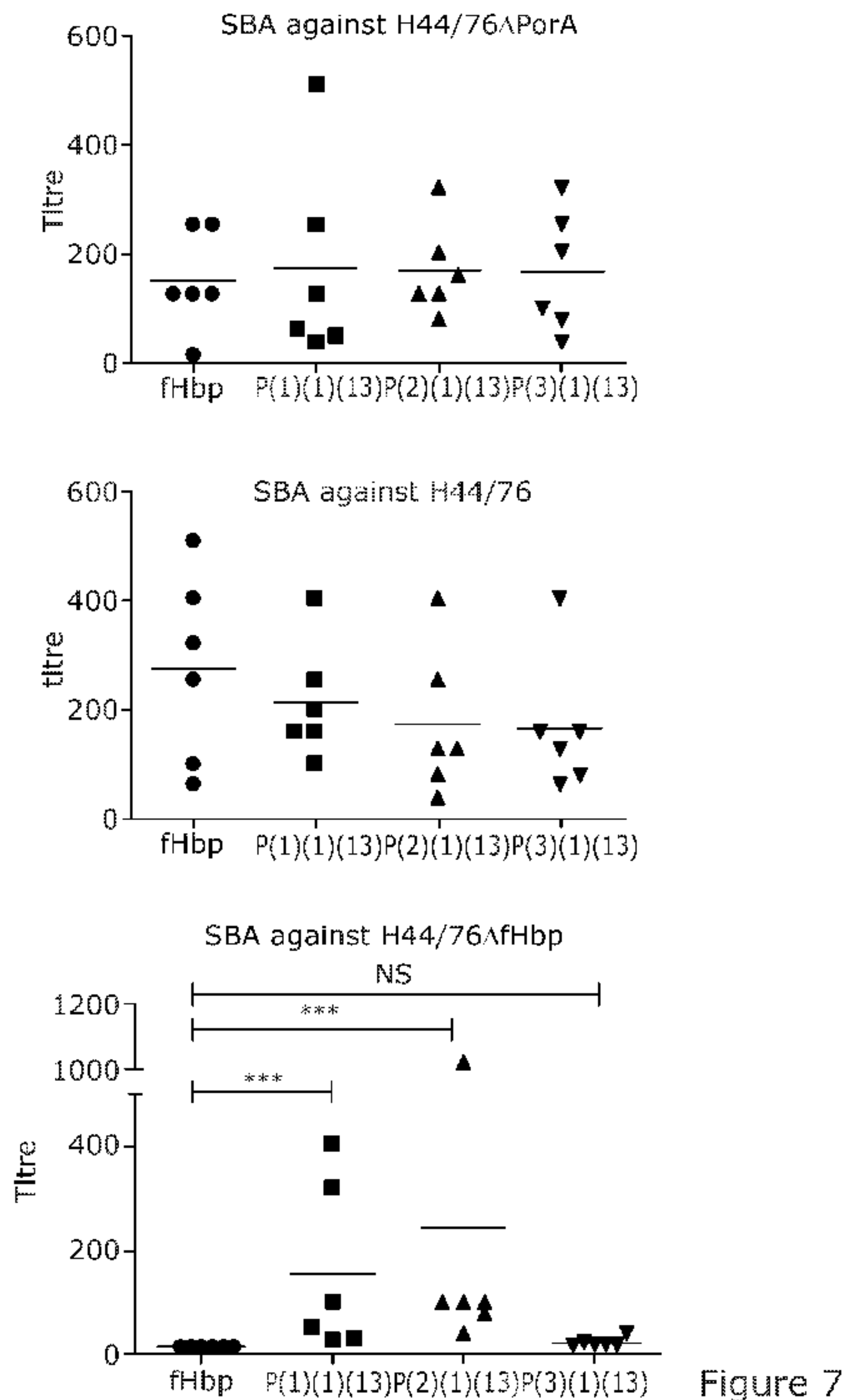
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: MODIFIED FACTOR H BINDING PROTEIN



(57) Abstract: The invention relates to a modified factor H binding protein (fHbp), comprising fHbp, or a variant thereof, modified with the addition of at least one exogenous peptide loop; and associated nucleic acid, compositions, and uses. The invention further relates to treatment or prevention of a pathogenic infection or colonisation of a subject using the modified factor H binding protein (fHbp).

WO 2018/042178 A1



Published:

— *with international search report (Art. 21(3))*

MODIFIED FACTOR H BINDING PROTEIN

This invention relates to a modified factor H binding protein (fHbp) and its use to elicit an immune response against pathogenic infection or colonisation, such as against *Neisseria meningitidis* or *Neisseria gonorrhoeae*.

Neisseria meningitidis (*Nm*) remains a leading cause of sepsis and bacterial meningitis in children and young adults. The onset of disease can be extremely rapid, with fatality rates of around 10% for septicaemic disease¹, while those that survive can suffer significant disabilities including loss of limbs and neurological deficits¹. Therefore prophylactic immunisation is the best way to protect individuals from meningococcal infection. Vaccines are available based on the bacterial capsule², but there are only partially effective vaccines available for endemic serogroup B infection, which causes over 80% of cases in the UK currently³; the polysaccharide of serogroup B capsule *Nm* is poorly immunogenic as it has structural identity with a human glycoprotein in neural tissue and could induce autoimmunity if used as a vaccine⁴. Therefore there is an urgent need to generate novel vaccines, and there are intense efforts in academia and industry to achieve this important goal.

20

The major target of the immune response elicited against meningococcal outer membrane vesicles (OMVs) is PorA^{16,17,18}, an integral outer membrane protein (OMP) in the meningococcus¹⁹. However, the sequence of this protein is diverse and the prevalence of particular variants differs by geographic region, and OMV vaccines are largely PorA-specific. Variants of PorA are identified by sequences in the variable-regions (VR) of the protein, which are located in the surface-exposed loops of the protein and are the target of immune responses¹⁷. PorA has seven extracellular loops; the fourth loop is variable region 2 (VR2) and is the target of most serum bactericidal activity (SBA) generated by PorA following natural infection and after immunisation with OMVs^{16,18}. SBA is a known correlate of protection against meningococcal disease. Despite sequence

30

diversity, around 70 % of UK isolates would be covered by vaccines containing six PorA proteins (<http://pubmlst.org/neisseria/PorA/>).

A major obstacle for bacterial vaccine development is the difficulty in
5 producing quantities of integral OMPs, such as PorA, in their native conformation. This is because OMPs contain hydrophobic domains which span the membrane and do not fold correctly when expressed as soluble recombinant proteins. Correct folding is critical for PorA as SBA is elicited by conformational, and not linear, epitopes of the protein²⁰. Previous attempts to
10 use PorA peptides as vaccines have not been successful because they have not been sufficiently immunogenic and do not present the immunogenic portion of PorA in its correct conformation. Consequently, the only PorA-based vaccines under development are OMVs, which have limited efficacy in infants², are reactogenic²¹, and poorly defined providing regulatory issues. OMVs as
15 immunogens are not favoured because consistency and toxicity can be problematic during manufacture. For example, OMVs may contain toxic lipopolysaccharide (LPS).

Meningococcal factor H binding protein (fHbp) is a surface-exposed lipoprotein
20 that consists of two β -barrels⁵ (Fig. 1A). The N-terminal β -barrel of fHbp has a relatively open structure, while the C-terminal β -barrel is stabilised by extensive hydrogen bonding between the seven β -strands, which form this barrel⁵.

25 Importantly, fHbp is a key antigen in vaccines against serogroup B *Nm* under development by pharmaceutical companies such as Pfizer, GSK, and others, and is included in next generation OMV vaccines^{6,7,8}. The Pfizer vaccine consists of two fHbps, while the GSK vaccine has a single fHbp in a cocktail of other antigens which includes an OMV. fHbp binds human, but not murine, factor H
30 (fH)^{9,10}, an abundant plasma protein that down regulates the complement system¹¹, a critical aspect of immunity against *Nm*¹². Immunisation of adolescents and adults with fHbp elicits SBA¹³.

fHbp has been categorised into different schemes based on its predicted amino acid sequence. In the present application, three variant groups (v1, v2 and v3⁷) and peptide numbers (www.mlst.org) are recognised. Importantly, serum raised
5 against v1 fHbp does not mediate SBA against *Nm* expressing v2 and v3 proteins, and *vice versa*. The GSK vaccine contains a single v1 protein (v1.1), while the Pfizer formulation includes a v1 and v3 fHbp^{13,14}. Therefore no current vaccine includes a v2 fHbp even though between a significant proportion of disease in the UK is currently caused by strains expressing fHbp
10 from this variant group^{3,15}.

WO2011024072 is a patent application that describes the use of fHbp which is selected or engineered to have a sequence which can elicit broad-spectrum bactericidal anti-meningococcal antibodies after administration to a host
15 animal. This document teaches that additional meningococcal antigens may be provided with the engineered fHbp in the form of a N- or C-terminal fusion protein. However, such a proposal is unlikely to produce a protein that would present the immunogenic portion of many meningococcal antigens, such as PorA, in correct conformation and they would not be sufficiently immunogenic.

20

An aim of the present invention is to provide an alternative and improved immunogenic molecules for vaccination against pathogenic organisms, particularly to prevent or reduce meningococcal or gonococcal infection or colonisation.

25

According to a first aspect of the invention, there is provided a modified factor H binding protein (fHbp), comprising fHbp, or a variant thereof, to act as a molecular scaffold by modification with the addition of at least one exogenous peptide loop from a different antigen.

30

It has been shown herein that immunogenic peptides, such as those from PorA, can be introduced into factor H binding protein (fHbp), which acts as a

molecular scaffold. The peptides that are introduced into fHbp are presented to the immune system and are able to elicit protective responses such as SBA. Advantageously, the fHbp molecule provides an ideal molecular scaffold for stable inclusion of peptide loops for the display of epitopes, particularly for epitopes that are difficult to stabilise and display in their native conformation, for example loops from integral OMPs such as PorA. This is in contrast to teachings such as in WO2011024072, where simple N- or C- terminal fusions of fHbp and additional antigen would not solve inherent stability and solubility difficulties with some antigens. In particular, many OMPs, such as PorA, are difficult to express because of the insolubility of their membrane spanning domains. PorA has a 16-beta stranded barrel structure with the surface-exposed loops between strands 1 and 2 (loop 1), strands 7 and 8 (loop 4), strands 9 and 10 (loop 5) and strands 11 and 12 (loop 7) demonstrated to be the most effective antigens. fHbp contains two beta barrels, therefore the peptide loop sequences from OMPs can be inserted into the tips of the loops between beta-strands of fHbp to present the extra-cellular loop fragments from integral OMPs, in their native conformations for immunisation. Therefore, the modified fHbp scaffold molecule of the invention may be used as a prophylactic or a therapeutic vaccine directed to *Nm* or the gonococcus in which a single protein presents key epitopes from two different antigens.

In one embodiment, the fHbp is meningococcal fHbp. In another embodiment, the fHbp is gonococcal fHbp. The fHbp may comprise any one of variants v1, v2 and v3. In one embodiment, the fHbp may comprise fHbp v1. In another embodiment, the fHbp may comprise fHbp v2. In another embodiment, the fHbp may comprise fHbp v3.

In one embodiment, the fHbp variant v1 may be variant v1.1, v1.13, v1.14, v1.15, v1.4, or v1.55. In one embodiment, the fHbp variant v1 may not be v1.1. In one embodiment, the fHbp variant v1 may not be v1.55. In one embodiment, the fHbp variant v1 may not be v1.1 or v1.55. In one embodiment, the fHbp variant v2 may be variant v2.16, v2.19, v2.22, or v2.25. In one embodiment, the

fHbp variant v3 may be variant v3.45. In one embodiment, the fHbp comprises any one of fHbp variants v1.4, v2.25 or v3.45.

A variant of fHbp may comprise an orthologue of fHbp. For example, a variant
5 of fHbp may comprise Ghfp, the Gonococcal homologue of fHbp. Ghfp is non-functional and closely related to V3 fHbps (>95% aa identity, dissociation constant $KD > 100 \mu M$ with factor H).

In one embodiment, the fHbp which is to be further modified with the at least
10 one exogenous peptide loop may comprise or consist of the sequence of
CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LQSLTLDQSV RKNEKLKLAA
QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQVYK
QSHSALTAFQ TEQIQDSEHS GKMVAKRQFR IGDIAGEHTS FDKLPEGGRA
TYRGTAFGSD DAGGKLTYTI DFAAKQGNGK IEHLKSPELN VDLAAADIKP
15 DGKRHAVISG SVLYNQAIEKG SYSLGIFGGK AQEVAGSAEV KTVNGIRHIG
LAAKQ (SEQ ID NO: 1, fHbp V1.1 GI:316985482).

In another embodiment, the fHbp which is to be further modified with the at least one exogenous peptide loop may comprise or consist of the sequence of
20 CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKLAA
QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQVYK
QSHSALTALQ TEQVQDSEHS GKMVAKRQFR IGDIAGEHTS FDKLPEGGRA
TYRGTAFGSD DASGKLTYTI DFAAKQGHGK IEHLKSPELN VDLAASDIKP
DKKRHAVISG SVLYNQAIEKG SYSLGIFGGQ AQEVAGSAEV ETANGIRHIG
25 LAAKQ (SEQ ID NO: 2, fHbp V1.4 GI:989557230).

In another embodiment, the fHbp which is to be further modified with the at least one exogenous peptide loop may comprise or consist of the sequence of
30 CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LQSLTLDQSV RKNEKLKLAA
QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGKLIT LESGEFQVYK
QSHSALTALQ TEQVQDSEDS GKMVAKRQFR IGDIAGEHTS FDKLPKGGSA
TYRGTAFGSD DAGGKLTYTI DFAAKQGHGK IEHLKSPELN VELATAYIKP
DEKRHAVISG SVLYNQDEKG SYSLGIFGGQ AQEVAGSAEV ETANGIHHIG
LAAKQ (SEQ ID NO: 3, fHbp V1.13 GI:752774533).

In another embodiment, the fHbp which is to be further modified with the at least one exogenous peptide loop may comprise or consist of the sequence of

CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKLAA
 5 QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQVYK
 QSHSALTALQ TEQEQDPEHS GKMVAKRFRK IGDIAGEHTS FDKLPKDVMA
 TYRGTAFGSD DAGGKLTYTI DFAAKQGHGK IEHLKSPELN VELATAYIKP
 DEKHHAVISG SVLYNQDEKG SYSLGIFGGQ AQEVAGSAEV ETANGIHHIG
 LAAKQ (SEQ ID NO: 4, fHbp V1.14 GI:630057376).

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In another embodiment, the fHbp which is to be further modified with the at least one exogenous peptide loop may comprise or consist of the sequence of

CSSGGGGSGG GGVAADIGAG LADALTAPLD HKDKGLKSLT LEDSISQNGT
 LTLSAQGAER TFKAGDKDNS LNTGKLKNDK ISRFD FIRQI EVDGQLITLE
 15 SGEFQVYKQS HSALTALQTE QVQDSEHSGK MVAKRQFRIG DIVGEHTSFG
 KLPKDV MATY RGTAFGSDDA GGKLTYTIDF AAKQGHGKIE HLKSPELNVD
 LAAADIKPDE KHHAVISGSV LYNQAEKGSY SLGIFGGQAQ EVAGSAEVET
 ANGIRHIGLA AKQ (SEQ ID NO: 5, fHbp V1.15 GI:504394462).

20 In another embodiment, the fHbp which is to be further modified with the at least one exogenous peptide loop may comprise or consist of the sequence of

CSSGGGGSGG GGV TADIGTG LADALTAPLD HKDKGLKSLT LEDSISQNGT
 LTLSAQGA EK TYGNGDSLNT GK LKNDK VSR FDFIRQIEVD GQLITLESGE
 FQVYKQSHSA LTALQTEQEQ DPEHSEKMVA KRRFRIGDIA GEHTSFDKLP
 25 KDVMATYRGT AFGSDDAGGK LTYTIDFAAK QGHGKIEHLK SPELNVDLAV
 AYIKPDEKHH AVISGSVLYN QDEKGSYSLG IFGEKAQ EVA GSAEVETANG
 IHHIGLAAKQ (SEQ ID NO: 6, fHbp V 1.55 GI:40353481).

30 In another embodiment, the fHbp which is to be further modified with the at least one exogenous peptide loop may comprise or consist of the sequence of

CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKLAA
 QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQIYK
 QDHS AVVALQ IEKINNPDKI DSLINQRSFL VSGLGGEHTA FNQLPDGKAE
 YHGKAFSSDD AGGKLTYTID FAAKQGHGKI EHLKTPEQNV ELAAAE LKAD

EKSHAVILGD TRYGSEEKGT YHLALFGDRA QEIAGSATVK IGEKVHEIGI
AGKQ (SEQ ID NO: 7, fHbp V2.16 GI:488155511).

In another embodiment, the fHbp which is to be further modified with the at
5 least one exogenous peptide loop may comprise or consist of the sequence of
CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKLAA
QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQIYK
QDHS AVVALQ IEKINNPDKI DSLINQRSFL VSGLGGEHTA FNQLPSGKAE
YHGKAFSSDD AGGKLTYTID FAAKQGHGKI EHLKTPEQNV ELASAELKAD
10 EKSHAVILGD TRYGGEEKGT YHLALFGDRA QEIAGSATVK IREKVHEIGI
AGKQ (SEQ ID NO: 8, fHbp V2.19 GI:488148626).

In another embodiment, the fHbp which is to be further modified with the at
least one exogenous peptide loop may comprise or consist of the sequence of
15 CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKLAA
QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQIYK
QDHS AVVALQ IEKINNPDKI DSLINQRSFL VSGLGGEHTA FNQLPSGKAE
YHGKAFSSDD PNGRLHYSID FTKKQGYGRI EHLKTPEQNV ELASAELKAD
EKSHAVILGD TRYGGEEKGT YHLALFGDRA QEIAGSATVK IREKVHEIGI
20 AGKQ (SEQ ID NO: 9, fHbp V2.22 GI:120865922).

In another embodiment, the fHbp which is to be further modified with the at
least one exogenous peptide loop may comprise or consist of the sequence of
CSSGGGGVAA DIGAGLADAL TTPLDHKDKS LQSLTLDQSV RKNEKLKLAA
25 QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQTIT LASGEFQIYK
QNHS AVVALQ IEKINNPDKI DSLINQRSFL VSGLGGEHTA FNQLPDGKAE
YHGKAFSSDD PNGRLHYSID FTKKQGYGRI EHLKTPEQNV ELASAELKAD
EKSHAVILGD TRYGGEEKGT YHLALFGDRA QEIAGSATVK IREKVHEIGI
AGKQ (SEQ ID NO: 10, fHbp 2.25 GI:488158712).

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In another embodiment, the fHbp which is to be further modified with the at
least one exogenous peptide loop may comprise or consist of the sequence of
CSSGSGSGGG GVAADIGTGL ADALTAPLDH KDKGLKSLTL EDSISQNGTL
TLSAQGAECT FKVGDKDNSL NTGKLKNDKI SRFDFVQKIE VDGQTITLAS
35 GEFQIYKQDH SAVVALQIEK INNPDKIDSL INQRSFLVSG LGGEHTAFNQ

LPSGKAEYHG KAFSSDDAGG KLTYTIDFAA KQGHGKIEHL KTPEQNVELA
SAELKADEKS HAVILGDTRY GSEEKGTYHL ALFGDRAQEI AGSATVKIRE
KVHEIGIAGK Q (SEQ ID NO: 11, fHbp V3.45 GI:284466869).

- 5 In another embodiment, the fHbp which is to be further modified with the at
least one exogenous peptide loop may comprise or consist of the sequence of
CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LKSLTLEDSI SQNGTLTLISA
QGAEKTFKVG DKDNSLNTGK LKNDKISRFD FVQKIEVDGQ TITLASGEFQ
IYKQNHSVV ALQIEKINNP DKIDSLINQR SFLVSGLGGE HTAFNQLPGG
10 KAEYHGKAFFS SDDAGGKLTY TIDFAAKQGH GKIEHLKTPE QNVELAAAEL
KADEKSHAVI LGDTRYGSEE KGTYHLALFG DRAQEIAGSA TVKIGIEKVHE
ISIAGKQ (SEQ ID NO: 12, fHbp V3.47 GI:284466897).

- In another embodiment the Ghfp which is to be further modified with the at
15 least one exogenous peptide loop, may comprise or consist of the sequence of
MTRSKPVNRT TFCCLSLTAG PDSRLQQRG GGGGGVAADI GTGLADALTA
PLDHKDKGLK SLTLEASIPQ NGTLTLISAQG AEKTFKAGGK DNSLNTGKGLK
NDKISRFDV QKIEVDGQTI TLASGEFQIY KQDHSAVVAL RIEKINNPDK
IDSLINQRSF LVSDLGGEHT AFNQLPDGKA EYHGKAFSSD DADGKLTITI
20 DFAAKQGHGK IEHLKTPEQN VELASAELKA DEKSHAVILG DTRYGGEEKG
TYRLALFGDR AQEIAGSATV KIGIEKVHEIG IADKQ (SEQ ID NO: 13, GHFP).

- A variant of fHbp may comprise one or more amino acid residue mutations,
including additions, deletions or substitutions, relative to wild type fHbp in
25 addition to the exogenous peptide loop(s) provided on the modified fHbp. For
example, the fHbp may act as a scaffold upon which exogenous peptide loops
are provided and the variants relative to wild-type may comprise amino acid
mutations in the scaffold framework in regions outside of the exogenous
peptide loop(s) attachment points. Reference to wild-type fHbp may refer to
30 any one of the variants of fHbp discussed herein, for example any one of SEQ
ID NOs: 1 to 13).

- A variant of fHbp may comprise at least one amino acid change compared to the
amino acid in the wild type protein. A variant of fHbp may comprise no more
35 than one amino acid change compared to the wild type protein. A variant of

fHbp may comprise no more than three amino acid changes compared to the wild type protein. A variant of fHbp may comprise no more than four amino acid changes compared to the wild type protein. A variant of fHbp may comprise no more than five amino acid changes compared to the wild type protein. A variant of fHbp may comprise no more than six amino acid changes compared to the wild type protein. In one embodiment, a variant of fHbp is provided which comprises six amino acid mutations compared to the wild type protein.

10 Amino acid substitutions may be conservative substitutions. For example, a mutated residue may comprise substantially similar properties as the wild-type substituted residue. For example, a substituted residue may comprise substantially similar or equal charge or hydrophobicity as the wild-type substituted residue. For example, a substituted residue may comprise
15 substantially similar molecular weight or steric bulk as the wild-type substituted residue.

In one embodiment a variant fHbp may have at least 75% identity with wild-type. In another embodiment a variant fHbp may have at least 80% identity with
20 wild-type. In another embodiment a variant fHbp may have at least 85% identity with wild-type. In another embodiment a variant fHbp may have at least 90% identity with wild-type. In another embodiment a variant fHbp may have at least 95% identity with wild-type. In another embodiment a variant fHbp may have at least 98% identity with wild-type. In another embodiment a
25 variant fHbp may have at least 99% identity with wild-type. In another embodiment a variant fHbp may have at least 99.5% identity with wild-type. The above percentage variation is not intended to include percentage identity variation with addition of the exogenous peptide loop(s) (i.e. it is the percentage identity of the fHbp component alone relative to the wild-type), and
30 does not include deletion of fHbp sequence at the site where loops from other proteins are inserted.

The modified fHbp may be modified such that it is not capable of binding factor H, or at least has reduced factor H binding activity. The modified fHbp may be non-functional relative to the function of wild-type fHbp. In one embodiment, the modified fHbp has an impaired capacity to bind CFH with a $K_D > 2$ orders
5 of magnitude compared with the wild-type protein. Non-functional fHbps may be provided by mutation of the fHbp sequence. In one embodiment, non-functional fHbps may be provided by one or more of the exogenous peptide loops preventing the binding site of factor H.

10 The amino acid residue mutation(s) may prevent or reduce complement factor H binding of the modified fHbp. In another embodiment, the amino acid residue mutation(s) may not substantially affect the fHbp function. In one embodiment, the amino acid residue mutation(s) in the fHbp, or variants thereof, may be selected from the group consisting of the amino acid at position 85, 133, 134,
15 135, 136, 204, 206, 211, 212, 213, 222, 225, 227, 231, and 252 on v1.1 fHbp or corresponding position in other fHbps.

In one embodiment, the amino acid residue mutation(s) may comprise or consist of a substitution to alanine instead of the wild type residue. In one embodiment,
20 the amino acid residue change(s) may comprise or consist of a substitution to any other amino acid instead of the wild type residue.

Advantageously, providing a non-functional fHbp (i.e. non- or less- binding of factor H) can eliminate or reduce any adverse effects of factor H recruitment on
25 the success of the vaccine.

In one embodiment, the amino acid residue mutation(s) may enhance the stability of the modified fHbp in particular, in an embodiment wherein fHbp V2 is provided, the fHbp V2 may be stabilised by mutations in the fHbp V2
30 sequence. Details of the mutations for V2 stability may be found in WO2014030003, which is herein incorporated by reference. For example, the amino acid substitution for stabilisation may be at one or more of the amino

acids at position 35, 36, 42, 43, 46, 107, 112, 114, 137 and 138 in fHbp V2. The substitution for stabilisation may be at one or more of Ser35, Leu36, Asp42, Glu43, Arg46, Asp107, Val112, Leu114, Ser137, and Gly138.

- 5 In one embodiment, the exogenous peptide loop(s) is immunogenic. The exogenous peptide loop(s) may be derived from an outer membrane/surface exposed protein.

The exogenous peptide loop(s) may be prokaryotic in origin. The exogenous
10 peptide loop(s) may be derived from a protein on the bacterium, such as an outer membrane protein (OMP) of a pathogen. The OMP may be an integral OMP or a lipoprotein. The exogenous peptide loop(s) may be derived from a meningococcal protein, such as a meningococcal outer membrane protein. The exogenous peptide loop(s) may be derived from an outer membrane protein of
15 another pathogen such as *N. gonorrhoeae*.

The exogenous peptide loop may comprise a fragment of a transmembrane beta barrel protein. The exogenous peptide loop may comprise a fragment of a beta barrel porin protein. The exogenous peptide loop may comprise a fragment of
20 PorA. In another embodiment, the exogenous peptide loop may comprise a fragment of FetA.

The exogenous peptide loop(s), such as PorA fragments, may be 16 amino acids in length. In one embodiment, the exogenous peptide loop(s), such as PorA
25 fragments, may be between 8 and 20 amino acids in length. In another embodiment, the exogenous peptide loop(s), such as PorA fragments, may be between 8 and 16 amino acids in length. In another embodiment, the exogenous peptide loop(s), such as PorA fragments, may be between 10 and 16 amino acids in length. In another embodiment, the exogenous peptide loop(s), such as
30 PorA fragments, may be between 12 and 16 amino acids in length. In another embodiment, the exogenous peptide loop(s), such as PorA fragments, may be between 14 and 18 amino acids in length. In another embodiment, the

exogenous peptide loop(s), such as PorA fragment, may be any length sufficient to provide an immunogenic epitope. In another embodiment, the exogenous peptide loop(s), such as PorA fragments, may be any length sufficient to provide an immunogenic epitope and maintain native conformation relative to the fragment in wild-type.

The exogenous peptide loop(s) may be selected from any one of the PorA loops 1 to 7, or fragments thereof; and/or combinations thereof. The exogenous peptide loop(s) may be selected from any one of the PorA loops of loop 1 (from between beta-strands 1 and 2), loop 4 (from between beta-strands 7 and 8), loop 5 (from between beta-strands 9 and 10) and loop 7 (from between beta-strands 11 and 12; or fragments thereof; and/or combinations thereof.

The exogenous peptide loop may comprise any one peptide selected from PorA loop 1 (between beta-strands 1 and 2); loop 4 (between beta-barrels 7 and 8); and loop 5 (between beta-strands 9 and 10); or fragments thereof; and/or combinations thereof.

The exogenous peptide loop may comprise PorA loop 1 (between beta-barrels 1 and 2), or a fragment thereof. The exogenous peptide loop may comprise PorA loop 4 (between beta-strands 7 and 8), or a fragment thereof. The exogenous peptide loop may comprise PorA loop 5 (between beta-strands 9 and 10), or a fragment thereof.

The skilled person will understand that variant sequences of PorA loops may be provided with minor mutations relative to wild-type and may still function as an epitope. Therefore, the exogenous peptide loop(s) may comprise PorA loop variants. Variants may include one or more amino acid additions, deletions or substitutions relative to the wild-type sequence. In another embodiment, variants may include no more than one amino acid addition, deletion or substitution relative to the wild-type sequence. In another embodiment, variants may include no more than 2, 3, 4 or 5 amino acid additions, deletions or

substitutions relative to the wild-type sequence. The substitutions may be conservative substitutions. For example, providing an alternative amino acid residue having substantially similar properties, such as charge, hydrophobicity, steric size or molecular weight. Variants may include sequences having at least
5 85% sequence identity with wild-type PorA loop sequence. In another embodiment, variants may include sequences having at least 90%, 95%, 98%, 99%, or 99.5% sequence identity with wild-type PorA loop sequence.

In one embodiment, the modified fHbp may comprise two or more exogenous
10 peptide loops. In one embodiment, the modified fHbp may comprise three or more exogenous peptide loops. In one embodiment, the modified fHbp may comprise between 1 and 7 exogenous peptide loops. In one embodiment, the modified fHbp may comprise between 1 and 5 exogenous peptide loops. In one
15 embodiment, the modified fHbp may comprise between 1 and 3 exogenous peptide loops. In one embodiment, the modified fHbp may comprise between 2 and 7 exogenous peptide loops. In one embodiment, the modified fHbp may comprise between 3 and 7 exogenous peptide loops. In one embodiment, the modified fHbp may comprise between 2 and 5 exogenous peptide loops. In one
20 embodiment, the modified fHbp may comprise between 3 and 5 exogenous peptide loops. The modified fHbp, or variants thereof, may be modified with an exogenous peptide loop in at least one position. The modified fHbp, or variant thereof, may be modified with an exogenous peptide loop in at least two positions. The modified fHbp, or variant thereof, may be modified with an
25 exogenous peptide loop in at least three positions. The modified fHbp, or variant thereof, may be modified with an exogenous peptide loop in at least four positions. The modified fHbp, or variant thereof, may be modified with an exogenous peptide loop in at least five positions. The modified fHbp, or variant thereof, may be modified with an exogenous peptide loop in at least six positions. The modified fHbp, or variant thereof, may be modified with an
30 exogenous peptide loop in at least seven positions.

A peptide loop may be provided (for example using fHbp as a scaffold) between two beta sheets of the factor H binding protein. The peptide loop may be inserted into fHbp, or a variant thereof, at one or more of amino acid positions selected from positions where exogenous peptide loops, such as PorA loops, can be inserted (range is inclusive of all residues in loop) between amino acids 49-54, 83-88, 114-124, 199-206, 227-233, and 240-246 of v1.1 fHbp or corresponding positions in other fHbps.

The at least one exogenous peptide loop may not be provided as an N- or C-terminal fusion.

Sequences of fHbp into which the exogenous peptide loop(s) can be inserted may be at any of those positions underlined with regards to fHbp V1.1 primary amino acid sequence below. In an embodiment using an alternative variant fHBP, the insert sites may be at equivalent positions.

Position 1 in fHbp (P1), residues 83-88

P2, residues 199-206

P3, residues 227-233

P4, residues 49-54

P5, residues 114-124

P7, residues 240-246

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1  CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LQSLTLDQSV RKNEKLKLAA
25                                     P4
51  QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLLIT LESGEFQVYK
    P4                               P1
101 QSHSALTAFQ TEQIQDSEHS GKMVAKRQFR IGDIAGEHTS FDKLPEGGRA
    P5
30 151 TYRGTAFGSD DAGGKLTYTI DFAAKQGNGK IEHLKSPELN VDLAAADIKP
    P2
201 DGKRHAVISG SVLYNQAEKG SYSLGIFGGK AQEVAGSAEV KTVNGIRHIG
    P2           P3           P7

```

251 LAAKQ (SEQ ID NO: 14)

In one embodiment, the insert site of an exogenous peptide loop may be at position 1 in fHbp (P1), residues 83-88 (Sequence EVDGQL (SEQ ID NO: 15)).
 5 In another embodiment, the insert site of an exogenous peptide loop may be at position P2, residues 199-206 (Sequence KPDGKRHA (SEQ ID NO: 16)). In another embodiment, the insert site of an exogenous peptide loop may be at position P3, residues 227-233 (Sequence FGGKAQE (SEQ ID NO: 17)). In another embodiment, the insert site of an exogenous peptide loop may be at
 10 position P4, residues 49-54 (Sequence AAQGAE (SEQ ID NO: 18)). In another embodiment, the insert site of an exogenous peptide loop may be at position P5, residues 114-124 (Sequence IQDSEHSGKM (SEQ ID NO: 19)). In another embodiment, the insert site of an exogenous peptide loop may be at position P7, residues 240-246 (Sequence KTVNGI (SEQ ID NO: 20)).

15

The exogenous peptide loop insert site at any given position 1-7 may be between any of the residues identified at positions 1 to 7 of fHbp. Alternatively, The exogenous peptide loop insert site at any given position 1-7 may be before the first residue or after the last residue identified at positions 1 to 7 of fHbp.
 20 For example if the exogenous peptide loop is inserted at position 4, the insert may be *AAQGAE (SEQ ID NO: 21), A*AQGAE (SEQ ID NO: 22), AA*QGAE (SEQ ID NO: 23), AAQ*GAE (SEQ ID NO: 24), AAQG*AE (SEQ ID NO: 25), AAQGA*E (SEQ ID NO: 26), or AAQGAE* (SEQ ID NO: 27), where * denotes the insert site.

25

In another embodiment, the skilled person will understand that the exogenous peptide loop insertion site may be variable such that between 1 and 5 residues in the region of the insertion site may be removed from fHbp (e.g. replaced by the loop) without significantly affecting the fHbp structure. In one embodiment,
 30 one or more of the amino acid residues at the identified positions are replaced/substituted by an exogenous peptide loop. In an alternative embodiment, two, three, four, five or more of the amino acid residues at the

identified positions are replaced/substituted by an exogenous peptide loop. In another embodiment, the exogenous peptide loop insertion site may be variable such that between 1 and 3 residues in the region of the insertion site may be removed from fHbp (e.g. replaced by the loop) without significantly affecting the fHbp structure. In another embodiment, the exogenous peptide loop insertion site may be variable such that 1 or 2 residues in the region of the insertion site may be removed from fHbp (e.g. replaced by the loop) without significantly affecting the fHbp structure. In another embodiment, the exogenous peptide loop insertion site may be variable such that 1 residue in the region of the insertion site may be removed from fHbp (e.g. replaced by the loop) without significantly affecting the fHbp structure. For example, where an insertion site is amino acid residue 86 at one end of the loop and residue 87 at the other end of the loop, a variant can include a 5, 4, 3, 2, or 1 residue replacement with the loop in the region of the stated insertion site. In an alternative embodiment, six or more of the amino acid residues at the identified positions are replaced/substituted by an exogenous peptide loop. In an alternative embodiment, seven or more (where applicable) of the amino acid residues at the identified positions are replaced/substituted by an exogenous peptide loop. In an alternative embodiment, eight or more (where applicable) of the amino acid residues at the identified positions are replaced/substituted by an exogenous peptide loop. In an alternative embodiment, nine or more (where applicable) of the amino acid residues at the identified positions are replaced/substituted by an exogenous peptide loop. The entire amino acid residues of any of insert positions 1 to 7 may be substituted with an exogenous peptide loop.

For example if the exogenous peptide loop is inserted at position 4 and substitutes one or more residues, the insert may be A*QGAE (SEQ ID NO: 28), AA*GAE (SEQ ID NO: 29), AA*AE (SEQ ID NO: 30), AA*E (SEQ ID NO: 31), AA*, A*GAE (SEQ ID NO: 32), A*AE (SEQ ID NO: 33), AA*E (SEQ ID NO: 34), A*E, A*, *AQGAE (SEQ ID NO: 35), *QGAE (SEQ ID NO: 36), *GAE (SEQ ID NO: 37), *AE, *E, AAQ*AE (SEQ ID NO: 38), AAQ*E (SEQ

ID NO: 39), AAQ* (SEQ ID NO: 40), AAQG*E (SEQ ID NO: 41), AAQG* (SEQ ID NO: 42), AAQGA* (SEQ ID NO: 43), where * denotes the insert site, and one or more residues are removed from the original sequence.

- 5 The skilled person will understand that equivalent combinations of insertion sites and/or substitutions with the exogenous peptide loop may be made with alternative sequences of the other identified insert positions 1 to 7.

10 In one embodiment, the region of the insertion site may be shifted +/- 5 residues up or downstream of any the positions 1 to 7. Alternatively, the region of the insertion site may be shifted +/- 4 residues up or downstream of any the positions 1 to 7. Alternatively, the region of the insertion site may be shifted +/- 3 residues up or downstream of any the positions 1 to 7. Alternatively, the region of the insertion site may be shifted +/- 2 residues up or downstream of
15 any the positions 1 to 7. Alternatively, the region of the insertion site may be shifted +/- 1 residue up or downstream of any the positions 1 to 7.

Additionally or alternatively, the peptide loop(s) may be provided in a position that sterically prevents fHbp:CFH interaction. These include an exogenous
20 peptide loop inserted into any one or more site between residues 114-124, 199-206, or 240-246 (e.g. Positions 2, 5 and 7), underlined on V1 primary sequence below:

1 CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LQSLTLDQSV RKNEKLKLAA
25 51 QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQVYK
101 QSHSALTAFQ TEQIQDSEHS GKMVAKRQFR IGDIAGEHTS FDKLPEGGRA
151 TYRGTAFGSD DAGGKLTYTI DFAAKQGNGK IEHLKSPELN VDLAAADIKP
201 DGKRHAVISG SVLYNQAEKG SYSLGIFGGK AQEVAGSAEV KTVNGIRHIG
251 LAAKQ (SEQ ID NO: 44)

30

In one embodiment of the invention, the modified fHbp is immunogenic. The modified fHbp may be a recombinant protein. The modified fHbp may be a fusion protein, such as a recombinant fusion protein. The modified fHbp may be

an isolated modified fHbp molecule. The modified fHbp molecule of the invention may be described as a single protein, multi-valent vaccine. The modified fHbp could be included in an OMV vaccine.

- 5 In embodiments where more than one exogenous peptide loop is inserted into fHbp, or variants thereof, the exogenous peptide loops may be the same, e.g. the same sequence, or substantially similar. For example, some epitopes such as PorA epitopes, may not elicit sufficient functional responses when displayed singly on fHbp. In this instance, the present invention may be used to provide
10 the same epitope at multiple sites on the same modified fHbp molecule, thereby enhancing the immunogenic recognition of the epitope.

Alternatively, the exogenous peptide loops may be different relative to each other. For example, where the exogenous peptide loops are derived from a
15 single protein, such as PorA, the different exogenous peptide loops may be from distinct regions of the protein, such as PorA. In one embodiment, the different exogenous peptide loops may be derived from overlapping and distinct regions of the protein, such as PorA.

- 20 In embodiments where more than one exogenous peptide loop is inserted into fHbp, or variants thereof, the exogenous peptide loops may be derived from different species or strains. For example, when a multivalent vaccine is desired for multiple different antigens including different organisms.

- 25 The PorA peptide loop sequence may be selected from any of the sequences provided in Table 1 (e.g. any of SEQ ID NOs: 45 to 79). Combinations of different PorA sequences of Table 1 may be provided (one loop per site) in any of insertion sites P1 to P7 described herein. Additionally or alternatively, two or more of the same PorA sequences of Table 1 may be provided (one loop per
30 site) in any of insertion sites P1 to P7 described herein.

Table 1: sequences of PorA VR2 loops, any of which may be inserted into any variant of fHbp to make a chimeric fHbp-PorA protein.

PorA VR2 Loop	Primary sequence
P1.1	YVAVENGVAKKVA (SEQ ID NO: 45)
P1.2	HFVQQTPKSQPTLVP (SEQ ID NO: 46)
P1.2_2	HFVQQTPQSQPTLVP (SEQ ID NO: 47)
P1.3	TLANGANNTIIRVP (SEQ ID NO: 48)
P1.3_5	TLAKGANNTIIRVP (SEQ ID NO: 49)
P1.4	HVVVNNKVATHVP (SEQ ID NO: 50)
P1.9	YVDEQSKYHA (SEQ ID NO: 51)
P1.10	HFVQNKQNQRPTLVP (SEQ ID NO: 52)
P1.10_1	HFVQNKQNPPTLVP (SEQ ID NO: 53)
P1.10_2	HFVQDKKGQPPTLVP (SEQ ID NO: 54)
P1.10_8	HFVQNKQNQQNPPTLVP (SEQ ID NO: 55)
P1.10_4	HFVQNKQNKQNPPTLVP (SEQ ID NO: 56)
P1.10_7	HFVQNKQNKPPTLVP (SEQ ID NO: 57)
P1.13	YWTTVNTGSATTTTFVP (SEQ ID NO: 58)
P1.13_1	YWTTVNTGSATTTTFVP

	(SEQ ID NO: 59)
P1.13_2	YWTTVTNTGSATTTTFVP (SEQ ID NO: 60)
P1.13_4	YYTTVTQGSATTTTFVP (SEQ ID NO: 61)
P1.14	YVDEKKMVHA (SEQ ID NO: 62)
P1.14_6	YVDEKQVSHA (SEQ ID NO: 63)
P1.14_26	YVDEKKVVHA (SEQ ID NO: 64)
P1.15	HYTRQNNADVFP (SEQ ID NO: 65)
P1.15_1	HYTRQNNTDVP (SEQ ID NO: 66)
P1.15_11	HYTRQNNIDVP (SEQ ID NO: 67)
P1.16	YYTKDTNNLTLVP (SEQ ID NO: 68)
P1.16_3	YYTKDKNDLTLVP (SEQ ID NO: 69)
P1.16_4	YYTKDKNDKLTLP (SEQ ID NO: 70)
P1.16_26	YYTNTNNLTLVP (SEQ ID NO: 71)
P1.23	HWNTVYNTNGTTTTFVP (SEQ ID NO: 72)
P1.23_2	HWNTVYNTNGTTTTTFVP (SEQ ID NO: 73)
P1.25	TYTVDSSGVVTPVP (SEQ ID NO: 74)
P1.26	HFVADSQGKITRVP (SEQ ID NO: 75)

P1.28	YYTATNSSTSTTFVP (SEQ ID NO: 76)
P1.30	HYTTVYNATTTTTTFVP (SEQ ID NO: 77)
P1.30_2	HYTTVYNATTTTTTFVP (SEQ ID NO: 78)
P1.34	YVDDQGKVKGP (SEQ ID NO: 79)

The skilled person will understand that variants involving one or two or more amino acid substitutions, additions or deletions may be provided for the PorA sequences of Table 1 without substantially removing the immunogenic function.

5 Substitutions may be to similar amino acid residues, for example having similar MW, charge, hydrophobicity or moieties, or synthetic analogues. The skilled person will further understand that variants may be truncations of the PorA sequences of Table 1, wherein the truncated variants provide sufficient amino acid residues to form a recognisable epitope. In one embodiment the PorA

10 sequence has at least 80% identity to any one of the sequences in Table 1. In another embodiment the PorA sequence has at least 85% identity to any one of the sequences in Table 1. In another embodiment the PorA sequence has at least 90% identity to any one of the sequences in Table 1. In another embodiment the PorA sequence has at least 95% identity to any one of the sequences in Table 1.

15 In another embodiment the PorA sequence has at least 98% identity to any one of the sequences in Table 1.

In one embodiment, the fHbp which is to be further modified with an exogenous peptide loop may comprise or consist of the sequence:

20 CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LQSLTLDQSV RKNEKLKLAA
QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQVYK
QSHSALTAFQ TEQIQDSEHS GKMVAKRQFR IGDIAGEHTS FDKLPEGGRA
TYRGTAFGSD DAGGKLTYTI DFAAKQGNGK IEHLKSPELN VDLAAADIKP
DGKRHAVISG SVLYNQAEKG SYSLGIFGGK AQEVAGSAEV KTVNGIRHIG
25 LAAKQ (SEQ ID NO: 80, fHbp V1.1 GI:316985482), wherein the sequence of

any PorA VR2 loop in Table 1 may replace any amino acid or groups of amino acids highlighted by a box.

In one embodiment, the fHbp which is to be further modified with an exogenous peptide loop may comprise or consist of the sequence:

5 CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKL[AA]
 [QGAEK]TYGNG DSLNTGKLKN DKVSRFDFIR QI[EVDGQL]IT LESGEFQVYK
 QSHSALTALQ TEQ[VQDSEHS GKMV]AKRQFR IGDIAGEHTS FDKLPEGGRA
 TYRGTAFGSD DASGKLTYTI DFAAKQGHGK IEHLKSPELN VDLAASDI[KP]
 10 [DKKRHA]VISG SVLYNQAEKG SYSLGI[FGGQ AQE]VAGSAEV [ETANGI]RHIG
 LAAKQ (SEQ ID NO: 81, fHbp V1.4 GI:989557230), wherein the sequence of
 any PorA VR2 loop in Table 1 may replace any amino acid or groups of amino
 acids highlighted by a box.

15 In one embodiment, the fHbp which is to be further modified with an exogenous peptide loop may comprise or consist of the sequence:

CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LQSLTLDQSV RKNEKLKL[AA]
 [QGAEK]TYGNG DSLNTGKLKN DKVSRFDFIR QI[EVDGKL]IT LESGEFQVYK
 QSHSALTALQ TEQ[VQDSEDS GKMV]AKRQFR IGDIAGEHTS FDKLPKGGSA
 20 TYRGTAFGSD DAGGKLTYTI DFAAKQGHGK IEHLKSPELN VELATAYI[KP]
 [DEKRHA]VISG SVLYNQDEKG SYSLGI[FGGQ AQE]VAGSAEV [ETANGI]HHIG
 LAAKQ (SEQ ID NO: 82, fHbp V1.13 GI:752774533), wherein the sequence of
 any PorA VR2 loop in Table 1 may replace any amino acid or groups of amino
 acids highlighted by a box.

25

In one embodiment, the fHbp which is to be further modified with an exogenous peptide loop may comprise or consist of the sequence:

CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKL[AA]
 [QGAEK]TYGNG DSLNTGKLKN DKVSRFDFIR QI[EVDGQL]IT LESGEFQVYK
 30 QSHSALTALQ TEQ[EQDPEHS GKMV]AKRRFK IGDIAGEHTS FDKLPKDVMA
 TYRGTAFGSD DAGGKLTYTI DFAAKQGHGK IEHLKSPELN VELATAYI[KP]
 [DEKHHA]VISG SVLYNQDEKG SYSLGI[FGGQ AQE]VAGSAEV [ETANGI]HHIG
 LAAKQ (SEQ ID NO: 83, fHbp V1.14 GI:630057376), wherein the sequence of

any PorA VR2 loop in Table 1 may replace any amino acid or groups of amino acids highlighted by a box.

- 5 In one embodiment, the fHbp which is to be further modified with an exogenous peptide loop may comprise or consist of the sequence:
 CSSGGGGSGG GGVAADIGAG LADALTAPLD HKDKGLKSLT LEDSISQNGT
 LTL[SAQGAER] TFKAGDKDNS LNTGKLKNDK ISRFD FIRQI [EVDGQL]ITLE
 SGEFQVYKQS HSALTALQTE Q[VQDSEHSGK MV]AKRQFRIG DIVGEHTSFG
 KLPKDV MATY RGTAFGSDDA GGKLTYTIDF AAKQGHGKIE HLKSP ELNVD
 10 LAAADI[KPDE KHHA]VISGSV LYNQAEKGSY SLGI[FGGQAQ E]VAGSAEV[ET]
 [ANGI]RHIGLA AKQ (SEQ ID NO: 84, fHbp V1.15 GI:504394462), wherein the
 sequence of any PorA VR2 loop in Table 1 may replace any amino acid or
 groups of amino acids highlighted by a box.

- 15 In one embodiment, the fHbp which is to be further modified with an exogenous peptide loop may comprise or consist of the sequence:
 CSSGGGGSGG GGV TADIGTG LADALTAPLD HKDKGLKSLT LEDSISQNGT
 LTL[SAQGAEK] TYGNGDSLNT GK LKNDK VSR FDFIRQI[EVD GQL]ITLES GE
 FQVYKQSHSA LTALQTEQ[EQ DPEHSEKMVA] KRRFRIGDIA GEHTSFDKLP
 20 KDVMATYRGT AFGSDDAGGK LTYTIDFAAK QGHGKIEHLK SPELNVDLAV
 AYIKP[DEKHH A]VISGSVLYN QDEKGSYSLG I[FG EKAQE]VA GSAEV[ETANG]
 [I]HHIGLAAKQ (SEQ ID NO: 85, fHbpV 1.55 GI:40353481), wherein the sequence
 of any PorA VR2 loop in Table 1 may replace any amino acid or groups of
 amino acids highlighted by a box.

25

- In one embodiment, the fHbp which is to be further modified with an exogenous peptide loop may comprise or consist of the sequence:
 CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKL[AA]
 [QGAEK]TYGNG DSLNTGKLKN DKVSRFD FIR QI[EVDGQL]IT LESGEFQIYK
 30 QDHS AVVALQ IEK[INNPDKI DSLI]NQRSFL VSGLGGEHTA FNQLPDGKAE
 YHGKAFSSDD AGGKLTYTID FAAKQGHGKI EHLKTPEQNV ELAAAELKAD[
 [EKSHA]VILGD TRYGSEEKGT YHLAL[FGDRA Q]EIAGSATV[K IGEKV]HEIGI
 AGKQ (SEQ ID NO: 86, fHbp V2.16 GI:488155511), wherein the sequence of any

PorA VR2 loop in Table 1 may replace any amino acid or groups of amino acids highlighted by a box.

In one embodiment, the fHbp which is to be further modified with an exogenous peptide loop may comprise or consist of the sequence:

5 CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKL^{AA}
¹⁰ ^{QGAEK}TYGNG DSLNTGKLKN DKVSRFDFIR QI^{EVDGQL}IT LESGEFQIYK
 QDHS^{AVVALQ} IEK^{INNPDKI} ^{DSL}INQRSFL VSGLGGEHTA FNQLPSGKAE
 YHGKAFSSDD AGGKLTYTID FAAKQGHGKI EHLKTPEQNV ELASAELKA^D
¹⁰ ^{EKSHA}VILGD TRYGGEEKGT YHLAL^{FGDRA} Q^{EIAGSATV}^K ^{IREKV}HEIGI
 AGKQ (SEQ ID NO: 87, fHbp V2.19 GI:488148626), wherein the sequence of any
 PorA VR2 loop in Table 1 may replace any amino acid or groups of amino acids
 highlighted by a box.

15 In one embodiment, the fHbp which is to be further modified with an exogenous peptide loop may comprise or consist of the sequence:

CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKL^{AA}
²⁰ ^{QGAEK}TYGNG DSLNTGKLKN DKVSRFDFIR QI^{EVDGQL}IT LESGEFQIYK
 QDHS^{AVVALQ} IEK^{INNPDKI} ^{DSL}INQRSFL VSGLGGEHTA FNQLPSGKAE
²⁰ YHGKAFSSDD PNGRLHYSID FTKKQGYGRI EHLKTPEQNV ELASAELKA^D
^{EKSHA}VILGD TRYGGEEKGT YHLAL^{FGDRA} Q^{EIAGSATV}^K ^{IREKV}HEIGI
 AGKQ (SEQ ID NO: 88, fHbp V2.22 GI:120865922), wherein the sequence of any
 PorA VR2 loop in Table 1 may replace any amino acid or groups of amino acids
 highlighted by a box.

25

In one embodiment, the fHbp which is to be further modified with an exogenous peptide loop may comprise or consist of the sequence:

CSSGGGGVAA DIGAGLADAL T^TPLDHKDKS LQSLTLDQSV RKNEKLKL^{AA}
³⁰ ^{QGAEK}TYGNG DSLNTGKLKN DKVSRFDFIR QI^{EVDGQT}IT LASGEFQIYK
 QNHS^{AVVALQ} IEK^{INNPDKI} ^{DSL}INQRSFL VSGLGGEHTA FNQLPDGKAE
 YHGKAFSSDD PNGRLHYSID FTKKQGYGRI EHLKTPEQNV ELASAELKA^D
^{EKSHA}VILGD TRYGGEEKGT YHLAL^{FGDRA} Q^{EIAGSATV}^K ^{IREKV}HEIGI
 AGKQ (SEQ ID NO: 89, fHbp 2.25 GI:488158712), wherein the sequence of any

PorA VR2 loop in Table 1 may replace any amino acid or groups of amino acids highlighted by a box.

In one embodiment, the fHbp which is to be further modified with an exogenous peptide

5 loop may comprise or consist of the sequence:

CSSGSGSGGG GVAADIGTGL ADALTAPLDH KDKGLKSLTL EDSISQNGTL
 TL[SAQGAEK]T FKVGDKDNSL NTGKLKNDKI SRFDFVQKI[E VDGQT]ITLAS
 GEFQIYKQDH SAVVALQIEK[INNPDKIDSL]INQRSFLVSG LGGEHTAFNQ
 LPSGKAEYHG KAFSSDDAGG KLTYTIDFAA KQGHGKIEHL KTPEQNVELA
 10 SAELKA[DEKS HA]VILGDTRY GSEEKGTYHL AL[FGDRAQ]EI AGSATV[KIRE]
 [KV]HEIGIAGK Q (SEQ ID NO: 90, fHbp V3.45 GI:284466869), wherein the
 sequence of any PorA VR2 loop in Table 1 may replace any amino acid or
 groups of amino acids highlighted by a box.

15 In one embodiment, the fHbp which is to be further modified with an exogenous peptide
 loop may comprise or consist of the sequence:

CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LKSLTLEDSI SQNGTLTL[SA]
 [QGAEK]TFKVG DKDNSLNTGK LKNDKISRFD FVQKI[EVDGQ]TITLASGEFQ
 IYKQNHSVV ALQIEK[INNP DKIDSL]INQR SFLVSGLGGE HTAFNQLPGG
 20 KAEYHGKAFFS SDDAGGKLTY TIDFAAKQGH GKIEHLKTPE QNVELAAAEL
 KA[DEKSHA]VI LGDTRYGSEE KGTYHLAL[FG DRAQ]EIAGSA TV[KIGEKV]HE
 ISIAGKQ (SEQ ID NO: 91, fHbp V3.47 GI:284466897), wherein the sequence of
 any PorA VR2 loop in Table 1 may replace any amino acid or groups of amino
 acids highlighted by a box.

25

The modified fHbp may comprise the sequence of any one of SEQ ID NOs: 92
 to 109.

The modified fHbp may comprise the sequence of SEQ ID NO: 92 to 109, wherein the
 30 sequence YYTKDTNNLTLVP (SEQ ID NO: 68) is replaced by any PorA VR2 loop
 sequence, for example any VR2 loop sequence provided in Table 1.

In one embodiment, the modified fHbp may comprise or consist of the sequence:

CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LQSLTLDQSV RKNEKLKLAA
 QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDYYTKDT NNNLTLVPQL
 ITLESGEFQV YKQSHSALTA FQTEQIQDSE HSGKMVAKRQ FRIGDIAGEH
 TSFDKLPEGG RATYRGTAFG SDDAGGKLT Y TIDFAAKQGN GKIEHLKSPE
 5 LNVDLAAADI KPDGKRHAVI SGSVLYNQAE KGSYSLGIFG GKAQEVAGSA

EVKTVNGIRH IGLAAKQ (SEQ ID NO: 92, fHbp V1.1 GI:316985482, PorA VR2
 P1.16 in P1), or the same sequence whereby the sequence YYTKDTNNNLTLVP
 (SEQ ID NO: 68) is replaced by any PorA VR2 loop sequence, for example any
 PorA loop sequence as provided in Table 1.

10

In one embodiment, the modified fHbp may comprise or consist of the sequence:

CSSGSGSGGG GVAADIGTGL ADALTAPLDH KDKGLKSLTL EDSISQNGTL
 TLSAQGAEKT FKVGDKDNSL NTGKLKNDKI SRFDFVQKIE VDYITKDTNN
 NLTLVPQTIT LASGEFQIYK QDHSAVVALQ IEKINNPDKI DSLINQRSFL
 15 VSGLGGEHTA FNQLPSGKAE YHGKAFSSDD AGGKLT Y TID FAAKQGHGKI
 EHLKTPEQNV ELASAE LKAD EKSHAVILGD TRYGSEEKGT YHLALFGDRA
 QEIAGSATVK IREKVHEIGI AGKQ (SEQ ID NO: 93, fHbp V3.45

GI:284466869, PorA VR2 P1.16 in P1), or the same sequence whereby the
 sequence YYTKDTNNNLTLVP (SEQ ID NO: 68) is replaced by any PorA VR2
 20 loop sequence, for example any PorA loop sequence as provided in Table 1.

In one embodiment, the modified fHbp may comprise or consist of the sequence:

CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKLAA
 QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDYYTKD TNNNLTLVPQ
 25 LITLESGEFQ IYKQDHSAVV ALQIEKINNP DKIDSLINQR SFLVSGLGGE
 HTAFNQLPSG KAEYHGKA FS SDDAGGKLT Y TIDFAAKQGH GKIEHLKTPE
 QNVELASAE L KADEKSHAVI LGDTRYGGEE KGT YHLALFG DRAQEIAGSA
 TVKIREKVHE IGIAGKQ (SEQ ID NO: 94, fHbp V2.19 GI:488148626, PorA VR2 P1.16 in

P1), or the same sequence whereby the sequence YYTKDTNNNLTLVP (SEQ ID NO:
 30 68) is replaced by any PorA VR2 loop sequence, for example any PorA loop sequence
 as provided in Table 1.

In one embodiment, the modified fHbp may comprise or consist of the sequence:

CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LQSLTLDQSV RKNEKLKLAA
 35 QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQVYK

QSHSALTAFQ TEQIQDSEHS GKMVAKRQFR IGDIAGEHTS FDKLPEGGRA
 TYRGTAFGSD DAGGKLTYTI DFAAKQGNGK IEHLKSPELN VDLAAADIKP
 DYYTKDTNNN LTLVPKRHAV ISGSVLYNQA EKGSYSLGIF GGKAQEVAGS

- AEVKTVNGIR HIGLAAKQ (SEQ ID NO: 95, fHbp V1.1 GI:316985482, PorA VR2 P1.16
 5 in P2), or the same sequence whereby the sequence YYTKDTNNNLTLVP (SEQ ID
 NO: 68) is replaced by any PorA VR2 loop sequence, for example any PorA loop
 sequence as provided in Table 1.

In one embodiment, the modified fHbp may comprise or consist of the sequence:

10 CSSGSGSGGG GVAADIGTGL ADALTAPLDH KDKGLKSLTL EDSISQNGTL
 TLSAQGAECT FKVGDKDNSL NTGKLKNDKI SRFDFVQKIE VDGQTITLAS
 GEFQIYKQDH SAVVALQIEK INNPDKIDSL INQRSFLVSG LGGEHTAFNQ
 LPSGKAEYHG KAFSSDDAGG KLTYTIDFAA KQGHGKIEHL KTPEQNVELA
 SAELKADYYT KDTNNNLTLV PKSHAVILGD TRYGSEEKGT YHLALFGDRA

- 15 QEIAGSATVK IREKVHEIGI AGKQ (SEQ ID NO: 96, fHbp V3.45 GI:284466869, PorA
 VR2 P1.16 in P2), or the same sequence whereby the sequence YYTKDTNNNLTLVP
 (SEQ ID NO: 68) is replaced by any PorA VR2 loop sequence, for example any PorA
 loop sequence as provided in Table 1.

- 20 In one embodiment, the modified fHbp may comprise or consist of the sequence:

CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKLAA
 QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQIYK
 QDHS AVVALQ IEKINNPDKI DSLINQRSFL VSGLGGEHTA FNQLPSGKAE
 YHGKAFSSDD AGGKLTYTID FAAKQGHGKI EHLKTPEQNV ELASAELKAD
 25 YYTKDTNNNL TLVPKSHAVI LGDTRYGGEE KGTYHLALFG DRAQEIAGSA

TVKIREKVHE IGIAGKQ (SEQ ID NO: 97, fHbp V2.19 GI:488148626, PorA VR2 P1.16 in
 P2), or the same sequence whereby the sequence YYTKDTNNNLTLVP (SEQ ID NO:
 68) is replaced by any PorA VR2 loop sequence, for example any PorA loop sequence
 as provided in Table 1.

30

In one embodiment, the modified fHbp may comprise or consist of the sequence:

CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LQSLTLDQSV RKNEKLKLAA
 QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQVYK
 QSHSALTAFQ TEQIQDSEHS GKMVAKRQFR IGDIAGEHTS FDKLPEGGRA
 35 TYRGTAFGSD DAGGKLTYTI DFAAKQGNGK IEHLKSPELN VDLAAADIKP
 DGKRHAVISG SVLYNQAIEG SYSLGIFGYG TKDTNNNLTL VPKAQEVAGS
 AEVKTVNGIR HIGLAAKQ (SEQ ID NO: 98, fHbp V1.1 GI:316985482, PorA VR2

P1.16 in P3), or the same sequence whereby the sequence YYTKDTNNNLTLVP (SEQ ID NO: 68) is replaced by any PorA VR2 loop sequence, for example any PorA loop sequence as provided in Table 1.

5 In one embodiment, the modified fHbp may comprise or consist of the sequence:

CSSGSGSGGG	GVAADIGTGL	ADALTAPLDH	KDKGLKSLTL	EDSISQNGTL
TLAQGAECT	FKVGDKDNSL	NTGKLKNDKI	SRFDFVQKIE	VDGQTITLAS
GEFQIYKQDH	SAVVALQIEK	INNPDKIDSL	INQRSFLVSG	LGGEHTAFNQ
LPSGKAEYHG	KAFSSDDAGG	KLTYTIDFAA	KQGHGKIEHL	KTPEQNVELA
10 SAELKADEKS	HAVILGDTRY	GSEEKGTYHL	ALFGYYTKDT	NNNLTLVPRA

QEIAGSATVK IREKVHEIGI AGKQ (SEQ ID NO: 99, fHbp V3.45 GI:284466869, PorA VR2 P1.16 in P3), or the same sequence whereby the sequence YYTKDTNNNLTLVP (SEQ ID NO: 68) is replaced by any PorA VR2 loop sequence, for example any PorA loop sequence as provided in Table 1.

15

In one embodiment, the modified fHbp may comprise or consist of the sequence:

CSSGGGGVAA	DIGAGLADAL	TAPLDHKDKS	LQSLTLDQSV	RKNEKLKLAA
QGAEKTYGNG	DSLNTGKLKN	DKVSRFDFIR	QIEVDGQLIT	LESGEFQIYK
QDHSVVALQ	IEKINNPDKI	DSLINQRSFL	VSGLGGEHTA	FNQLPSGKAE
20 YHGKAFSSDD	AGGKLTYTID	FAAKQGHGKI	EHLKTPEQNV	ELASAELKAD
EKSHAVILGD	TRYGGEEKGT	YHLALFGYYT	KDTNNNLTLV	PRAQEIAGSA

TVKIREKVHE IGIAGKQ (SEQ ID NO: 100, fHbp V2.19 GI:488148626, PorA VR2 P1.16 in P3), or the same sequence whereby the sequence YYTKDTNNNLTLVP (SEQ ID NO: 68) is replaced by any PorA VR2 loop sequence, for example any PorA loop sequence as provided in Table 1.

25

In one embodiment, the modified fHbp may comprise or consist of the sequence:

CSSGGGGVAA	DIGAGLADAL	TAPLDHKDKG	LQSLTLDQSV	RKNEKLKLAA
QYYTKDTNNN	LTLPVPAEKTY	GNGDSLNTGK	LKNDKVSFRD	FIRQIEVDGQ
30 LITLESGEFQ	VYKQSHSALT	AFQTEQIQDS	EHSKGMVAKR	QFRIGDIAGE
HTSFDKLPEG	GRATYRGTA	GSDDAGGKLT	YTIDFAAKQG	NGKIEHLKSP
ELNVDLAAAD	IKPDGKRHAV	ISGSVLYNQA	EKGSYSLGIF	GGKAQEVAGS

AEVKTVNGIR HIGLAAKQ (SEQ ID NO: 101, fHbp V1.1 GI:316985482, PorA VR2 P1.16 in P4), or the same sequence whereby the sequence YYTKDTNNNLTLVP (SEQ ID NO: 68) is replaced by any PorA VR2 loop sequence, for example any PorA loop sequence as provided in Table 1.

35

In one embodiment, the modified fHbp may comprise or consist of the sequence:

CSSGSGSGGG GVAADIGTGL ADALTAPLDH KDKGLKSLTL EDSISQNGTL
 TLSAQYYTKD TNNNLTLVPA EKTFKVGDKD NSLNTGKLKN DKISRFDFVQ
 5 KIEVDGQTIT LASGEFQIYK QDHS AVVALQ IEKINNPDKI DSLINQRSFL
 VSGLGGEHTA FNQLPSGKAE YHGKAFSSDD AGGKLTYTID FAAKQGHGKI
 EHLKTPEQNV ELASAE LKAD EKSHAVILGD TRYGSEEKGT YHLALFGDRA
 QEIAGSATVK IREKVHEIGI AGKQ (SEQ ID NO: 102, fHbp V3.45 GI:284466869, PorA
 VR2 P1.16 in P4), or the same sequence whereby the sequence YYTKDTNNNLTLVP
 10 (SEQ ID NO: 68) is replaced by any PorA VR2 loop sequence, for example any PorA
 loop sequence as provided in Table 1.

In one embodiment, the modified fHbp may comprise or consist of the sequence:

CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKLAA
 15 QYYTKDTNNN LTLVPAEKTY GNGDSLNTGK LKNDKVS RFD FIRQIEVDGQ
 LITLESGEFQ IYKQDHS AVV ALQIEKINNP DKIDSLINQR SFLVSGLGGE
 HTAFNQLPSG KAEYHGKA FS SDDAGGKLT Y TIDFAAKQGH GKIEHLKTPE
 QNVELASAE L KADEKSHAVI LGDTRYGGEE KGT YHLALFG DRAQEIAGSA
 TVKIREKVHE IGIAGKQ (SEQ ID NO: 103, fHbp V2.19 GI:488148626, PorA VR2 P1.16 in
 20 P4), or the same sequence whereby the sequence YYTKDTNNNLTLVP (SEQ ID NO:
 68) is replaced by any PorA VR2 loop sequence, for example any PorA loop sequence
 as provided in Table 1.

In one embodiment, the modified fHbp may comprise or consist of the sequence:

25 CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LQSLTLDQSV RKNEKLKLAA
 QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQVYK
 QSHSALTAFQ TEQIQDSYYT KDTNNNLTLV PHSGKMVAKR QFRIGDIAGE
 HTSFDKLPEG GRATYRGTA F GSDDAGGKLT YTIDFAAKQG NGKIEHLKSP
 ELNVDLAAAD IKPDGKRHAV ISGSVLYNQA EKGSYSLGIF GGKAQEVAGS
 30 AEVKTVNGIR HIGLAAKQ (SEQ ID NO: 104, fHbp V1.1 GI:316985482, PorA VR2 P1.16
 in P5), or the same sequence whereby the sequence YYTKDTNNNLTLVP (SEQ ID
 NO: 68) is replaced by any PorA VR2 loop sequence, for example any PorA loop
 sequence as provided in Table 1.

35 In one embodiment, the modified fHbp may comprise or consist of the sequence:

CSSGSGSGGG GVAADIGTGL ADALTAPLDH KDKGLKSLTL EDSISQNGTL
 TLSAQGA EKT FKVGDKDNSL NTGKLKNDKI SRFDFVQKIE VDGQTITLAS

GEFQIYKQDH SAVVALQIEK INNPYYTKDT NNNLTLVPKI DSLINQRSFL
 VSGLGGEHTA FNQLPSGKAE YHGKAFSSDD AGGKLTYTID FAAKQGHGKI
 EHLKTPEQNV ELASAEKAD EKSHAVILGD TRYGSEEKGT YHLALFGDRA
 QEIAGSATVK IREKVHEIGI AGKQ (SEQ ID NO: 105, fHbp V3.45 GI:284466869, PorA
 5 VR2 P1.16 in P5), or the same sequence whereby the sequence YYTKDTNNNLTLVP
 (SEQ ID NO: 68) is replaced by any PorA VR2 loop sequence, for example any PorA
 loop sequence as provided in Table 1.

In one embodiment, the modified fHbp may comprise or consist of the sequence:

10 CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKLAA
 QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQIYK
 QDHS AVVALQ IEKINNPYYT KDTNNNLTLV PKIDSLINQR SFLVVSGLGGE
 HTAFNQLPSG KAEYHGKAFS SDDAGGKLTY TIDFAAKQGH GKIEHLKTPE
 QNVELASAEK KADEKSHAVI LGDTRYGGEE KGT YHLALFG DRAQEIAGSA
 15 TVKIREKVHE IGIAGKQ (SEQ ID NO: 106, fHbp V2.19 GI:488148626, PorA VR2 P1.16 in
 P5), or the same sequence whereby the sequence YYTKDTNNNLTLVP (SEQ ID NO:
 68) is replaced by any PorA VR2 loop sequence, for example any PorA loop sequence
 as provided in Table 1.

20 In one embodiment, the modified fHbp may comprise or consist of the sequence:
 CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LQSLTLDQSV RKNEKLKLAA
 QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQVYK
 QSHSALTAFQ TEQIQDSEHS GKMVAKRQFR IGDIAGEHTS FDKLPEGGRA
 TYRGTAFGSD DAGGKLTYTI DFAAKQGNGK IEHLKSPELN VDLAAADIKP
 25 DGKRHAVISG SVLYNQAEKG SYSLGIFGGK AQEVAGSAEV KTVYYTKDTN
 NNLTLVPGIR HIGLAAKQ (SEQ ID NO: 107, fHbp V1.1 GI:316985482, PorA VR2
 P1.16 in P7), or the same sequence whereby the sequence YYTKDTNNNLTLVP
 (SEQ ID NO: 68) is replaced by any PorA VR2 loop sequence, for example any
 PorA loop sequence as provided in Table 1.

30

In one embodiment, the modified fHbp may comprise or consist of the sequence:

CSSGSGSGGG GVAADIGTGL ADALTAPLDH KDKGLKSLTL EDSISQNGTL
 TLSAQGAECT FKVGDKDNSL NTGKLKNDKI SRFDFVQKIE VDGQTITLAS
 GEFQIYKQDH SAVVALQIEK INNPDKIDSL INQRSFLVSG LGGEHTAFNQ
 35 LPSGKA EYHG KAFSSDDAGG KLT YTIDFAA KQGHGKIEHL KTPEQNVELA
 SAELKADEKS HAVILGDTRY GSEEKGT YHL ALFGDRAQEI AGSATVKIRY

YTKDTNNNLT LVPKVHEIGI AGKQ (SEQ ID NO: 108, fHbp V3.45 GI:284466869, PorA VR2 P1.16 in P7), or the same sequence whereby the sequence YYTKDTNNNLTLPV (SEQ ID NO: 68) is replaced by any PorA VR2 loop sequence, for example any PorA loop sequence as provided in Table 1.

5

In one embodiment, the modified fHbp may comprise or consist of the sequence:

CSSGGGGVAA	DIGAGLADAL	TAPLDHKDKS	LQSLTLDQSV	RKNEKLKLAA
QGAEKTYGNG	DSLNTGKLKN	DKVSRFDFIR	QIEVDGQLIT	LESGEFQIYK
QDHSVVALQ	IEKINNPDKI	DSLINQRSFL	VSGLGGEHTA	FNQLPSGKAE
10 YHGKAFSSDD	AGGKLTYTID	FAAKQGHGKI	EHLKTPEQNV	ELASAEKAD
EKSHAVILGD	TRYGGEEKGT	YHLALFGDRA	QEIAGSATVK	IRYYTKDTNN

NLTLVPKVHE IGIAGKQ (SEQ ID NO: 109, fHbp V2.19 GI:488148626, PorA VR2 P1.16 in P7), or the same sequence whereby the sequence YYTKDTNNNLTLPV (SEQ ID NO: 68) is replaced by any PorA VR2 loop sequence, for example any PorA loop sequence

15 as provided in Table 1.

The skilled person will understand that one, two, three or four or more amino acid substitutions, deletions or additions may be made to the modified fHbp of the invention herein without substantially removing its immunogenic function

20 or affecting stability. Substitutions may be to similar amino acid residues, for example having similar MW, charge, hydrophobicity or moieties, or synthetic analogues. Such modifications are envisaged as part of the invention. In one embodiment the modified fHbp may have at least 75% identity with any one of the modified fHbp described herein. In one embodiment the modified fHbp may

25 have at least 80% identity with any one of the modified fHbp described herein. In one embodiment the modified fHbp may have at least 85% identity with any one of the modified fHbp described herein. In one embodiment the modified fHbp may have at least 90% identity with any one of the modified fHbp described herein. In one embodiment the modified fHbp may have at least 95%

30 identity with any one of the modified fHbp described herein. In one embodiment the modified fHbp may have at least 98% identity with any one of the modified fHbp described herein. In one embodiment the modified fHbp may have at least 99% identity with any one of the modified fHbp described herein.

In one embodiment the modified fHbp may have at least 99.5% identity with any one of the modified fHbp described herein.

According to another aspect of the invention, there is provided a nucleic acid
5 encoding essentially or at least the modified fHbp according to the invention herein.

The nucleic acid may be in a vector, such as a viral vector.

10 According to another aspect of the invention, there is provided a composition comprising the modified fHbp according the invention herein.

The composition may comprise two or more different modified fHbp molecules (e.g. different forms/species thereof) according to the invention herein. For
15 example, the composition may comprise two or more different variants of the modified fHbp according to the invention. For example, the composition may comprise fHbp variants v1 and v2. The composition may comprise fHbp variants v2 and v3. In another embodiment, the composition comprises at least fHbp variant v2.

20

According to another aspect of the invention, there is provided a composition comprising the nucleic acid according the invention herein.

The composition may comprise a pharmaceutically acceptable carrier. The
25 composition may further comprise an adjuvant.

According to a further aspect of the invention, there is provided a modified fHbp, nucleic acid, or composition according to the invention, for use as a medicament.

30

According to a further aspect of the invention, there is provided a modified fHbp, nucleic acid, or composition according to the invention, for use in the treatment or prevention of a pathogenic infection or colonisation of a subject.

- 5 According to a further aspect of the invention, there is provided a method of treatment or prevention of a pathogenic infection or colonisation of a subject, comprising the administration of a modified fHbp, nucleic acid, or composition according to the invention to the subject.
- 10 According to a further aspect of the invention, there is provided a method of vaccination, comprising the administration of a modified fHbp, nucleic acid, or composition according to the invention to a subject.

The administration may be provided in a therapeutically effective amount. A
15 skilled person will be capable of determining an appropriate dosage and repetitions for administration.

The subject may be mammalian, such as human.

- 20 The infection may be a bacterial infection. For example, the infection may be meningitis, such as *Neisseria meningitidis*, or *Neisseria gonorrhoeae*.

According to a further aspect of the invention, there is provided a single protein, multi-valent vaccine comprising the modified factor H binding protein
25 (fHbp) according to the invention.

The vaccine may be used as a prophylactic or a therapeutic vaccine directed to *Nm*.

- 30 The use may be with a pharmaceutically acceptable carrier. Additionally or alternatively, the use may be with an adjuvant. Suitable pharmaceutically acceptable carriers and adjuvants are well known to the skilled person.

According to a further aspect of the invention, there is provided a combination of the modified fHbp according to the invention and at least one other prophylactically or therapeutically active molecule.

5

The at least one other prophylactically or therapeutically active molecule may comprise a vaccine or antigen different to the modified fHbp according to the invention herein. The combination may be used in a combination vaccine or therapy. For example, the combination may be used in a combination vaccine or
10 therapy where another meningococcal antigen is provided.

In one embodiment, the at least one other prophylactically or therapeutically active molecule comprises a monovalent protein:capsule polysaccharide vaccine. The monovalent protein:capsule polysaccharide vaccine may comprise
15 any of serogroup C or A capsule with bacterial toxoids, bi-valent vaccines (with serogroup C and A capsular polysaccharide conjugated to bacterial toxoids), quadri- (serogroups A, C, Y, W) or penta- (A, C, Y, W, X) valent conjugate vaccines.

20 Alternatively, the at least one other prophylactically or therapeutically active molecule may comprise a conjugate vaccine, wherein antigen(s) comprising the fHbp scaffold bearing exogenous peptide loops (such as PorA loops) may be incorporated as the protein carrier molecule in the conjugate vaccine. The conjugate vaccine may comprise any of serogroup capsular polysaccharides
25 from A, C, Y, W, or X strains individually or in combination.

According to another aspect of the invention, there is provided the use of factor H binding protein (fHbp) as an epitope display scaffold.

30 The use as an epitope display scaffold may comprise the use of a factor H binding protein (fHbp) comprising any of the modifications described herein.

In addition to their potential use as vaccines, compositions or modified fHbps according to the invention may be useful as diagnostic reagents and as a measure of the immune competence of a vaccine.

- 5 The term “immunogenic” means that the molecule is capable of eliciting an immune response in a human or animal body. The immune response may be protective.

The immune response elicited by the modified fHbp of the invention may affect
10 the ability of *Neisseria meningitidis* (*Nm*) to infect a subject immunised with the modified fHbp of the invention. Preferably, the ability of *Nm* to infect a subject immunised with the modified fHbp of the invention is impeded or prevented. The immune response elicited may recognise and destroy *Nm*. Alternatively or additionally, the immune response elicited may impede or
15 prevent replication of *Nm*. Alternatively or additionally, the immune response elicited may impede or prevent *Nm* causing disease in the human or non-human animal.

The term “peptide loop” used herein is intended to refer to a single chain
20 polypeptide sequence anchored at both ends (e.g. anchored to a scaffold such as fHbp). The term “loop” does not infer or require any particular secondary structure adopted by the polypeptide.

The term “exogenous” used in the context of “exogenous peptide loop” is
25 understood to mean that the peptide loop is derived from a different source relative to the fHbp protein (i.e. it is not fHbp or a fragment thereof). However, it may be from the same organism as the fHbp. For example a modified fHbp may include an *N. meningitidis* fHbp modified with (exogenous) peptide loop(s) derived from *N. meningitidis* PorA.

30

The term “fusion protein” used herein is understood to mean a polypeptide comprising a combination of sequences from different gene products or sources.

Term “fusion protein” may be used interchangeably with the term “chimeric molecule”.

Reference to sequence “identity” used herein may refer to the percentage
5 identity between two aligned sequences using standard NCBI BLASTp parameters (<http://blast.ncbi.nlm.nih.gov>).

The term “isolated”, when applied to the modified fHbp of the present invention means a protein: (i) encoded by nucleic acids using recombinant DNA methods
10 or a viral vector; or (ii) synthesized by, for example, chemical synthetic methods; or (iii) separated from biological materials, and then purified. An isolated polypeptide of the invention includes a protein expressed from a nucleotide sequence encoding the protein, or from a recombinant vector containing a nucleotide sequence encoding the protein.

15

The term “protective” means prevention of a disease, a reduced risk of disease infection, transmission and/or progression, reduced severity of disease, a cure of a condition or disease, an alleviation of symptoms, or a reduction in severity of a disease or disease symptoms.

20

The term “prophylaxis” means prevention of or protective treatment for a disease. The prophylaxis may include a reduced risk of infection, transmission and/or progression, or reduced severity of disease.

25 The term “treatment”, means a cure of a condition or disease, an alleviation of symptoms, or a reduction in severity of a disease or disease symptoms.

The skilled person will understand that optional features of one embodiment or aspect of the invention may be applicable, where appropriate, to other
30 embodiments or aspects of the invention.

Embodiments of the invention will now be described in more detail, by way of example only, with reference to the accompanying drawings.

Figure 1 Design of chimeric fHbp:PorAs

- 5 (A) Schematic of the surface of *N. meningitidis*, showing the pre-dominant outer membrane features, lipo-oligosaccharide and type 4 pili, and the important antigens, fHbp and PorA. The immunogenic PorA VR2 loop is highlighted and fHbp is shown interacting with domains 6 and 7 of human CFH.
- 10 (B) Structure of V1 fHbp with CFH domains 6-7 showing the six positions (P1-5, and P7) used to generate chimeric fHbp:PorAs into which we have inserted PorA loops. N.B. position 5 is in the fHbp:CFH interface.

Figure 2: Use of fHbp as a molecular scaffold

- 15 A) Protein structure of fHbp V1.1 (grey, ribbon representation) with the six positions (P1-5, and P7) used to generate chimeric fHbp:PorAs. B) Secondary structure of fHbp V1.1 (grey, arrows represent β -sheets, rectangles represent α -helices). Locations of the VR2 P1.16 PorA insertion sites are indicated by solid black lines, numbers indicate the residue range that the PorA VR2 loops may be inserted (corresponds with residue numbers in 1D). C) Analysis of
- 20 purified fHbp:PorAs with the PorA P1.16 VR2 loop in positions 1-5 or 7 of fHbp, the wild-type (WT V1.1) by SDS-PAGE and Western blot. Blots were probed with α -V1 fHbp pAb and an α -P1.16 mAb. D) Primary sequence (SEQ ID NO: 1) of V1.1 fHbp indicating the locations (underlined) of positions 1-5
- 25 and 7 into which loops from other proteins can be inserted.

Figure 3 Characterisation of chimeric fHbp:PorAs

- 30 (A) Structure of fHbp:PorAs overlaid with the P1.16 loop (black, PDBID: 2mpa) with the Fab of the α -P1.16 mAb and fHbp:PorAs with the loop in position 1, position 3 and position 7, demonstrating that the epitope is in a conformation recognised by a bactericidal antibody.
- (B) Stability of fHbp N-terminal (NT) and C-terminal (CT) beta-barrels of fHbps by Differential Scanning Calorimetry analysis performed using a 20-120°C temperature gradient. Melting temperature is shown for the fHbp
- 35 N-terminal (NT_{TM}) and C-terminal (CT_{TM}) barrels. Binding fHbp:PorAs to

Complement Factor H (CFH) and mAbs SPR analysis of fHbp and chimeric fHbp:PorAs coupled to a BIAcore CM5 chip. CFH (fH67) was flowed over at a dilution range of 0.5–32 nM, and the dissociation constant (K_D) calculated; dissociation constants (K_{DS}) for fHbp:PorAs confirms lack of CFH binding of fHbp with a loop in position 5 which impinges on the fHbp:CFH interface (Fig. 2A). NB = Non-binding.

Figure 4 Repertoire of antigens selected for chimeric fHbp:PorAs

(A, B) Frequency of fHbp variants and PorA VR2 subtypes, respectively, in *N. meningitidis* disease isolates in the UK between 2010-2015 from the Meningitis Research Foundation Genome Library (<http://www.meningitis.org/research/genome>), shown as Pie Charts (above) and Tables (below) with the frequency of specific fHbps and PorAs. N.B. V2 fHbp accounts for 38.9% of isolates.

Table 2. Exact sequence matches for all *N. meningitidis* UK isolates between 2009-2015:

Antigen	Variant	% MenB coverage
fHbp	1.4	21.5
	2.19	9.1
	3.45	4.8
PorA	P1.4	19.3
	P1.9	19.1
	P1.14	16.3
	P1.16	7.0
	P1.15	5.5
	P1.15_11	5.3
% coverage minus antigen overlap in ALL UK strains (isolated between 2009-2015)		70.6
% coverage minus antigen overlap in UK MenB strains (isolated between 2009-2015)		79.2

Exact sequence matches for:

- Pfizer vaccine, 3.02%
- Bexsero fHbp (V1.1) or PorA (P1.16), 15.86%
- Chimeric fHbp:PorAs, fHbp or PorA, 72%
(23.5% with fHbp AND PorA)

5

Figure 5 Recognition of *Neisseria meningitidis* fHbp (A) and PorA (B) antigens by mouse immune sera. Whole cell lysates from *Neisseria meningitidis* strains H44/76 (WT), H44/67 Δ fHbp and H44/67 Δ PorA, were separated by SDS-PAGE, transferred to a PVDF membrane and probed with mouse immune sera. Mouse immune sera were obtained by immunising BalbC mice three times with 20 μ g of purified fHbp-PorA chimeras with VR2 loops in P1, P2, P4, P5 or P7.

Figure 6 Stabilisation of V2 fHbp: construction and immunogenicity of fHbp:PorAs

(A) Stabilisation of V2 fHbp V2.22 and V2.25 with six (M6) or two (M2) a.a. substitutions. DSC analysis was performed with 20 μ M of protein, using a 20-120°C temperature gradient. Melting temperature is recorded for the fHbp N-terminal (NT_{TM}) and C-terminal barrels (CT_{TM}).

(B) Chimeric fHbp:PorAs PorA loops are recognised by corresponding mAbs. PorA VR2 loops from P1.2, P1.4, P1.9, P1.14 and P1.15 were inserted into position 1 of V2.25 fHbp. SDS-PAGE and Western blot analysis of purified wild type V2.25 fHbp and V3.45 fHbp-PorA chimeras. Western blots were probed with fHbp V2.25 pAbs and loop specific PorA mAbs (NIBSC). CFH binding was detected by far Western blot analysis with normal human sera and CFH pAbs.

Figure 7 Chimeric fHbp:PorAs elicit protective immunity. Mice were immunised with chimeric fHbp:PorA i.e. P(1)(1)(13), P(2)(1)(13), and P(3)(1)(13) on three occasions, and SBAs measured against the strains indicated; an SBA > 8 is considered protective. The lack of PorA-directed SBA (i.e. SBA 0, against the fHbp mutant) with VR2 loop in position 3 i.e. P(3)(1)(13) is likely because the loop does not protrude from fHbp barrel as far as in pos. 1.

35

Figure 8. Frequency of PorA VR2 (A) and fHbp variants (B) in *N. meningitidis* serogroup B strains (n=243) isolated in 2016 in the UK. Data downloaded from the Meningococcal Research Foundation, 27 June 2017. Other: remaining alleles that occur in <4 isolates. (C) Analysis of recombinant Chimeric antigens by SDS-PAGE and Western blot. Immunoblots are probed with α -PorA VR2 mAbs: P1.4, P1.9, P1.14 and P1.15. (D) Detection of PorA in a panel of *N. meningitidis* serogroup B isolates by mouse polyclonal antisera from Chimeric Antigens fHbp^{V1.4}:PorA^{151/P1.1.10_1}, fHbp^{V1.4}:PorA^{151/P1.14} and fHbp^{V1.4}:PorA^{151/P1.15}, fHbp^{V3.45}:PorA^{158/P1.4} and fHbp^{V3.45}:PorA^{158/P1.9}.

Example 1

It has been shown that immunogenic peptides can be introduced into factor H binding protein (fHbp), and the peptides are presented to the immune system and are able to elicit protective responses (Fig. 5 and 7). Peptides have been used from the integral membrane protein PorA for proof-in principle of this approach. PorA is difficult to express because of the insolubility of its membrane spanning domains. The immunogenic portions of the molecule reside in extracellular loops which are exposed to the immune system. However effective immune responses are only generated against the loops in their right conformation; linear peptide sequences do not elicit functional immune responses. Through knowledge of the structure of fHbp, it is possible to introduce PorA loops into fHbp and generate relevant responses against PorA. This results in a chimeric molecule, based on fHbp and PorA sequences in specific sites to generate a chimeric molecule. This approach can be used for any other immunogenic integral outer membrane protein.

It has been shown that the likely reason for the exclusion of v2 fHbp from vaccines is the inherent instability of its N-terminal β -barrel: i) it was not possible to determine the atomic structure of this portion of v2 fHbp¹⁰, ii) v2 fHbp is sensitive to protease digestion (mass spectrometry demonstrates that the cleaved sites reside in the N-terminal β -barrel, not shown), and iii) differential

scanning calorimetry confirms that the instability lies in this region of v2 fHbp¹⁰.

Stable v2 fHbps have been successfully generated. Mutagenesis affecting the N-terminal barrel has been undertaken, substituting amino acids (a.a.s) singly or
5 in combination. Substitution of six amino acids in M6 fHbp stabilises v2 fHbp (*i.e.* 6 changes in c 130 a.a.s of this β -barrel, < 0.5%) (see WO2014030003 for details of the mutations). This is evident from differential scanning calorimetry (DSC) and protease sensitivity (see WO2014030003 for details). The side
10 chains of the altered residues promote interactions between the β -sheets of the N-terminal barrel, so are orientated towards the centre of the molecule; the changes do not affect the immunogenicity of the protein as expected (no difference in SBA, or α -fHbp IgG levels not shown).

15 Chimeric v1.1 fHbp has been generated incorporating the 13 amino acid VR2 from P1.16 PorA which elicits SBA in recipients of OMV vaccines¹⁶. While integral membrane proteins contain hydrophobic (thence insoluble) β -barrels, fHbp contains two barrels which can be expressed and purified to high levels. The VR2 sequence has been introduced into six different positions of fHbp
20 (Fig. 2B); these sites were selected on the basis of similar spacing of flanking β -sheets in PorA²² and fHbp to reduce the likelihood of the insertion disrupting the overall structure of fHbp (Fig. 2B for predicted effect of the insertions). The immunogenicity of three fHbps with insertion of VR2 into a different of fHbp (Fig. 2B) has been assessed. All proteins elicit antibody responses that
25 recognise against fHbp and PorA (Fig. 5 and 7), and importantly both proteins tested so far (with the VR2 in site 1 or 2) elicit SBA against fHbp and PorA independently (SBA for *Nm* H44/76, 512; and for *Nm* H44/76 α fHbp, 256 for both fHbps). This provides proof of principle for this approach.

30 **fHbp as a scaffold for multi-valent vaccines**

Non-functional fHbps as vaccines- The function of fHbp was not known when clinical trials of fHbp-containing vaccines began; fHbp displays high affinity interactions with fH (dissociation constant <5 nM) irrespective of variant group, with fH engaging a large area of fHbp⁵. This interaction could impair the use of fHbp as a vaccine by i) blocking immunogenic epitopes and preventing the generation of antibodies that could compete with fH, and ii) reducing complement activation (through fH recruitment) and thence B cell activation at the site of immune induction. The use of non-functional fHbps circumvents these problems. Key amino acids of v1, 2 and 3 fHbps have been identified that are necessary for fH interactions¹⁰, and modification of single a.a.s of v1, v2 and v3 fHbps which prevent fH binding have been shown to retain or even enhance immunogenicity of this important vaccine antigen^{10,23}.

To generate and evaluate single protein, multi-valent vaccine candidates:

Protective PorA epitopes from prevalent serosubtypes (which are defined by their PorA sequence) of *Nm*¹⁵ have been introduced into v1, stable v2 and v3 proteins; their stability and recognition by PorA and fHbp mAbs has been determined. PorA sequences have been selected to cover the diversity of isolates in the UK but data from any collection of meningococcal strains can be used.

Methods of research

Generation and characterisation of vaccine candidates - Recombinant fHbps were constructed and expressed in *E. coli* using standard plasmid vectors; proteins were affinity purified using the polyHis tag in the protein, anion exchange and gel filtration¹⁰ of chimeric v1, V2 and V3 fHbps as these are either in existing vaccines (v1.1 and 3.45) or because of experience with the protein (v2), or because of their prevalence in *Nm* strains. PorA loops have been introduced into fHbp by standard methods, and we have demonstrated that the fusion proteins bind mAbs against common serosubtypes of PorA. This strategy has been used to compare native and designed sequences, and perform fine

mapping of antigenic and fH-binding of the candidates. DSC has been carried out using a VP Capillary DSC (GEHealthcare) and SPR with a Biacore 3000 (GE Healthcare) or ProteOn XPR36 (BioRad) as previously¹⁰.

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All references are herein incorporated by reference.

Example 2 – Chimeric Antigens containing an expanded range of PorA VR2 loops generate immune responses

To test the adaptability of the fHbp:PorA Chimeric antigens, several Chimeric antigens composed from different combinations of fHbp and PorA VR2 were generated. The comprehensive meningococcal genome data available for strains isolated in the UK (Meningitis Research Foundation Meningococcus Genome Library developed by Public health England, the Wellcome Trust Sanger Institute and the University of Oxford as a collaboration.) enables construction of Chimeric antigens that have exact sequence matches to the most common antigens in a given region. In 2016, the most prevalent PorA VR2s in serogroup B *N. meningitidis* isolates were P1.4 (15.2%), P1.14 (15.2%), P1.9 (12.8%), P1.16 (11.1%) and P1.15 (5.8%, **Figure 8B**). VR2 P1.10_1 was present in 1.6% serogroup B isolates. The most prevalent variant 1, variant 2 and variant 3 fHbps were V1.4, V2.19 and V3.45, present in 21.8%, 5.3% and 4.9% of serogroup B *N. meningitidis* isolates respectively (**Figure 8C**). Five different Chimeric antigens were constructed, in which a PorA VR2 was inserted position 151 (V1.4) or position 158 (V3.45, **Figure 8A**). Following Chimeric antigen expression and purification, Western blot analyses confirmed these Chimeric antigens all retained epitopes recognised by their cognate α -VR2 mAb and α -fHbp pAbs (**Figure 8D**). The thermal stability of wild type fHbps V1.1, V1.4 and V3.45 and the Chimeric antigens was determined by differential scanning calorimetry (DSC, **Table 3**).

To examine the ability of these fHbp:PorA Chimeric antigens to elicit immune responses, groups of CD1 mice were immunized with each Chimeric antigen/alum; antisera obtained post immunisation were pooled. To assess the resulting PorA immune responses, Western blot were conducted with pooled antisera and a panel of serogroup B *N. meningitidis* disease isolates. **Figure 8E** demonstrates that all Chimeric antigens elicited α -PorA antibodies that recognised their cognate PorA VR2. To evaluate α -PorA SBA responses, Serum Bactericidal Assays were performed with pooled Chimeric antigen/alum antisera and serogroup B *N. meningitidis* strains with mismatched fHbp variants, to negate fHbp cross-protection. Titres range between ≥ 20 to ≥ 1280 and are above the ≥ 8 threshold for an accepted correlate of protective immunity

against *N. meningitidis* (Andrews, N. et al. *Clin Diagn Lab Immunol* 10, 780-786 (2003)) (Table 4).

Table 3: Stability of wild type fHbp and Chimeric Antigens

5

Protein	Cp (kcal mole ⁻¹ °C ⁻¹)	
	N-terminal T _m	C-Terminal T _m
fHbp V1.1	69.8	87.9
fHbp V1.4	64.0	89.0
fHbp V3.45	41.0	83.0
fHbp ^{V1.4} :PorA ^{151/P1.1.10_1}	54.0	89.0
fHbp ^{V1.4} :PorA ^{151/P1.14}	55.0	88.0
fHbp ^{V1.4} :PorA ^{151/P1.15}	55.0	89.0
fHbp ^{V3.45} :PorA ^{158/P1.4}	40.0	81.0
fHbp ^{V3.45} :PorA ^{158/P1.9}	39.0	80.0

Melting temperature, T_m

Table 4: Serum bactericidal assay titres

Pooled antisera	Serogroup isolate	B fHbp variant	PorA VR2	SBA titre
fHbp ^{V3.45} :PorA ^{158/P1.4}	M10240123	V1.92*	P1.4	1/160
fHbp ^{V3.45} :PorA ^{158/P1.9}	M11240431	V2.19	P1.9	1/1280
fHbp ^{V1.4} :PorA ^{151/P1.1.10_1}	M11240189	V3.84	P1.10_1	1/20
fHbp ^{V1.4} :PorA ^{151/P1.14}	M15240853	V3.45	P1.14	1/640

10

α-PorA SBA titres generated using pooled Chimeric Antigen/alum antisera and serogroup B *N. meningitidis* isolates with mismatched fHbp variants. * fHbp truncated at residue 242.

fHbp^{V1.4}:PorA^{151/P1.15} not tested, as it was needed to generate Δ*fHbp* strains, as the fHbp in strains with PorA VR2 P1.15 is not mismatched.

15

CLAIMS

1. A modified factor H binding protein (fHbp), comprising fHbp, or a variant thereof, modified with the addition of at least one exogenous peptide loop.
5
2. The modified factor H binding protein according to claim 1, wherein the fHbp is meningococcal or gonococcal fHbp, or a variant thereof.
3. The modified factor H binding protein according to claim 1 or claim 2,
10 wherein the modified fHbp is modified such that it is not capable of binding factor H, or at least has reduced factor H binding activity.
4. The modified factor H binding protein according to any preceding claim, wherein the exogenous peptide loop(s) is immunogenic.
15
5. The modified factor H binding protein according to any preceding claim, wherein the exogenous peptide loop(s) is derived from an integral outer membrane protein.
- 20 6. The modified factor H binding protein according to any preceding claim, wherein the exogenous peptide loop(s) is prokaryotic in origin.
7. The modified factor H binding protein according to any preceding claim, wherein the exogenous peptide loop(s) is derived from a membrane protein.
25
8. The modified factor H binding protein according to any preceding claim, wherein the exogenous peptide loop(s) is derived from a meningococcal protein.
- 30 9. The modified factor H binding protein according to any preceding claim, wherein the exogenous peptide loop(s) is derived from a gonococcal protein.

10. The modified factor H binding protein according to any preceding claim, wherein the exogenous peptide loop(s) comprise a fragment of PorA, or a variant thereof.

5

11. The modified factor H binding protein according to any preceding claim, wherein the exogenous peptide loop(s) are between 8 and 20 amino acids in length.

10 12. The modified factor H binding protein according to any preceding claim, wherein the exogenous peptide loop(s) are about 16 amino acids in length.

13. The modified factor H binding protein according to any preceding claim, wherein the exogenous peptide loop(s) are selected from any one of the PorA
15 loops 1 to 7, or fragments thereof; and/or variants thereof; and/or combinations thereof; or

wherein the exogenous peptide loop(s) are selected from any one of the PorA loops provided in Table 1 herein (e.g. any of SEQ ID NOs: 45 to 79), or variant thereof.

20

14. The modified factor H binding protein according to any preceding claim, wherein the modified fHbp comprises two or more exogenous peptide loops.

15. The modified factor H binding protein according to any preceding claim,
25 wherein the modified fHbp comprises between 1 and 7 exogenous peptide loops.

16. The modified factor H binding protein according to any preceding claim, wherein the modified fHbp, or variant thereof, is modified with an exogenous
30 peptide loop in at least two positions.

17. The modified factor H binding protein according to any preceding claim, wherein the peptide loop is inserted into fHbp, or a variant thereof, at one or more of amino acid positions selected from 49-54, 83-88, 114-124, 199-206, 227-233, and 240-246.

5

18. The modified factor H binding protein according to any preceding claim, wherein the modified fHbp is immunogenic.

19. The modified factor H binding protein according to any preceding claim,
10 wherein the modified fHbp is a fusion protein.

20. The modified factor H binding protein according to any preceding claim, wherein the modified fHbp comprises the sequence of any one of SEQ ID NOs: 1 to 13; or a variant thereof.

15

21. A nucleic acid encoding the modified fHbp according to any of claims 1 to 20.

22. The nucleic acid according to claim 21, wherein the nucleic acid is a vector,
20 optionally a viral vector.

23. A composition comprising the modified fHbp according to any of claims 1 to 10 or the nucleic acid according to claims 21 or 22.

24. The composition according to claim 23, wherein the composition comprises
25 two or more different modified fHbp molecules.

25. The composition according to claim 23 or claim 24, wherein the composition comprises a pharmaceutically acceptable carrier.

30

26. The composition according to any of claims 23 to 25, wherein the composition further comprises an adjuvant.

27. The composition according to any of claims 23 to 26, wherein the composition further comprises at least one other prophylactically or therapeutically active molecule; and optionally

wherein the at least one other prophylactically or therapeutically active molecule comprises:

a monovalent protein:capsule polysaccharide vaccine; or

a conjugate vaccine, wherein antigen(s) comprising the fHbp scaffold bearing exogenous peptide loops is incorporated as the protein carrier molecule in the conjugate vaccine.

10

28. A modified fHbp according to any of claims 1 to 20, a nucleic acid according to claim 20 or 21, or a composition according to any of claims 23 to 27, for use as a medicament.

15 29. A modified fHbp according to any of claims 1 to 20, a nucleic acid according to claim 21 or 22, or a composition according to any of claims 23 to 27, for use in the treatment or prevention of a pathogenic infection or colonisation of a subject.

20 30. A method of treatment or prevention of a pathogenic infection or colonisation of a subject, comprising the administration of a modified fHbp according to any of claims 1 to 20, a nucleic acid according to claim 21 or 22, or a composition according to any of claims 23 to 27, to the subject.

25 31. A method of vaccination, comprising the administration of a modified fHbp according to any of claims 1 to 20, a nucleic acid according to claim 21 or 22, or a composition according to any of claims 23 to 27, to a subject.

30 32. A combination of the modified fHbp according to any of claims 1 to 20, a nucleic acid according to claim 21 or 22, or a composition according to any of claims 23 to 27 and at least one other prophylactically or therapeutically active molecule.

33. The combination according to claim 32, wherein at least one other prophylactically or therapeutically active molecule comprises a monovalent protein:capsule polysaccharide vaccine.

5

34. The combination according to claim 33 or composition according to claim 26, wherein the monovalent protein:capsule polysaccharide vaccine comprises any of serogroup C or A capsule with bacterial toxoids, bi-valent vaccines (with serogroup C and A capsular polysaccharide conjugated to bacterial toxoids),
10 quadri- (serogroups A, C, Y, W) or penta- (A, C, Y, W, X) valent conjugate vaccines.

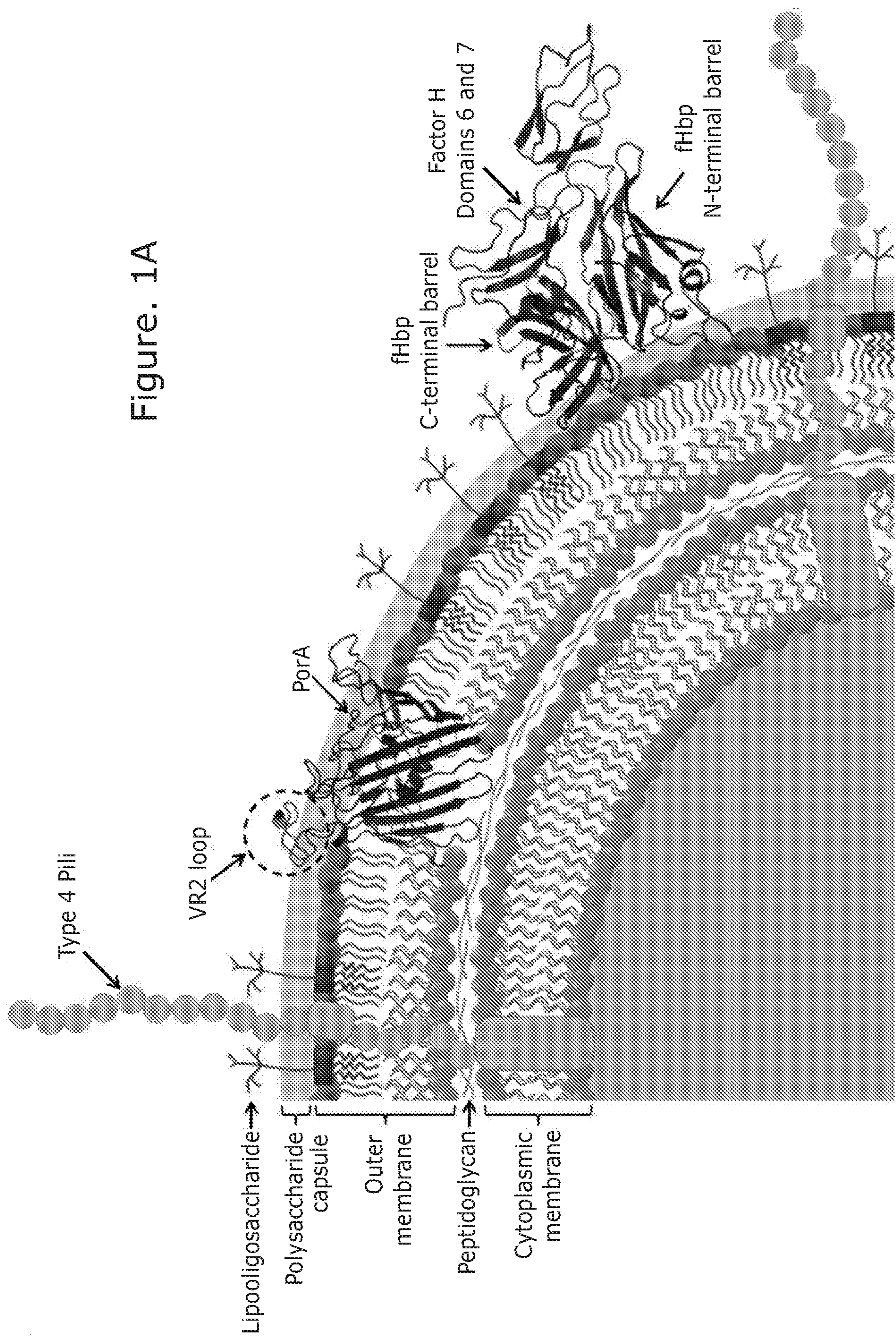
35. The combination according to claim 32 or composition according to claim 26, wherein the at least one other prophylactically or therapeutically active
15 molecule comprises a conjugate vaccine, wherein antigen(s) comprising the fHbp scaffold bearing exogenous peptide loops is incorporated as the protein carrier molecule in the conjugate vaccine, optionally wherein the conjugate vaccine comprises any of serogroup capsular polysaccharides selected from A, C, Y, W, or X strains, or combinations thereof.

20

36. Use of factor H binding protein (fHbp) as an epitope display scaffold.

37. The modified fHbp, nucleic acid, composition, method or use essentially as described herein, optionally with reference to the accompanying figures.

25



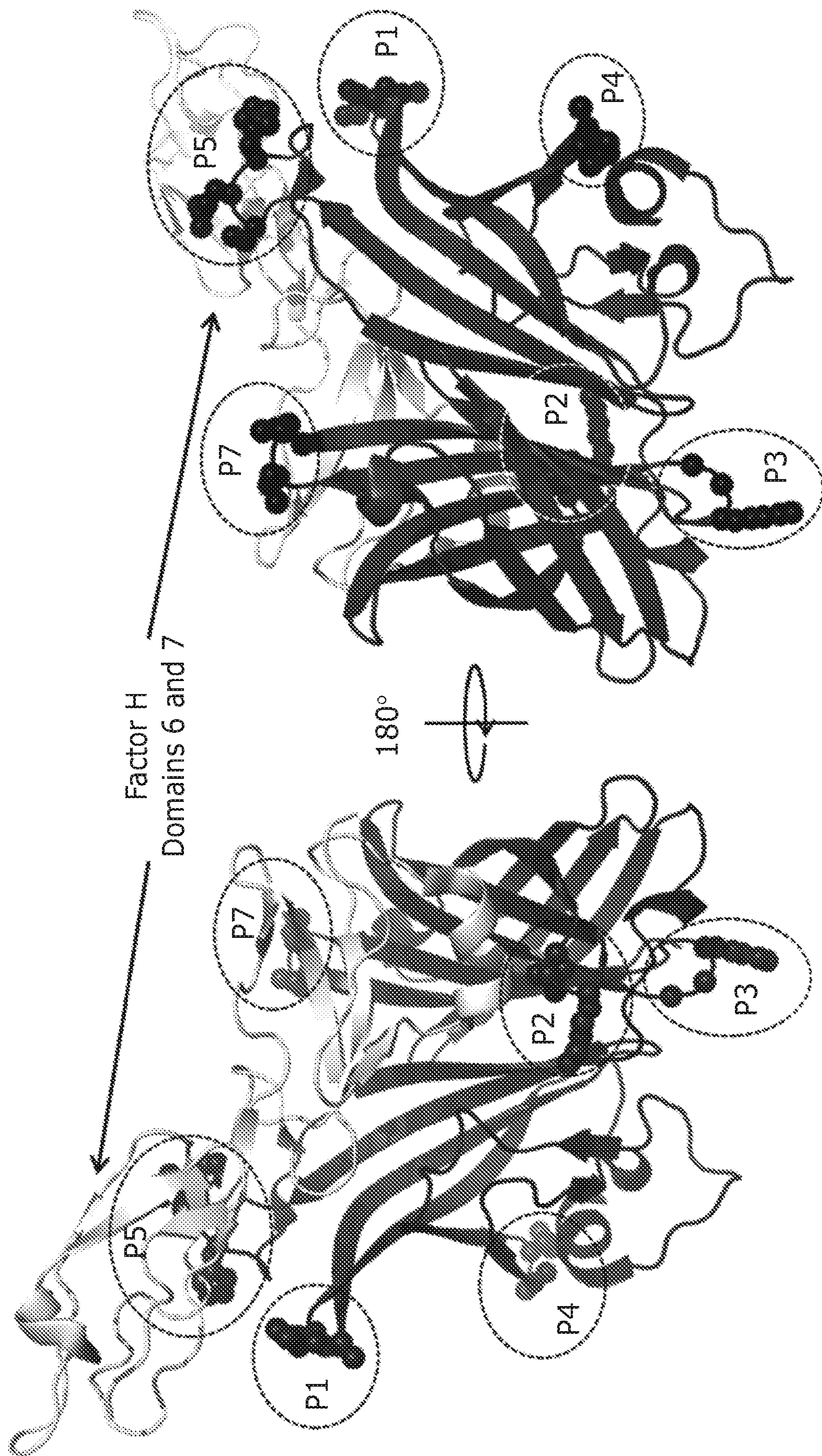


Figure 1B

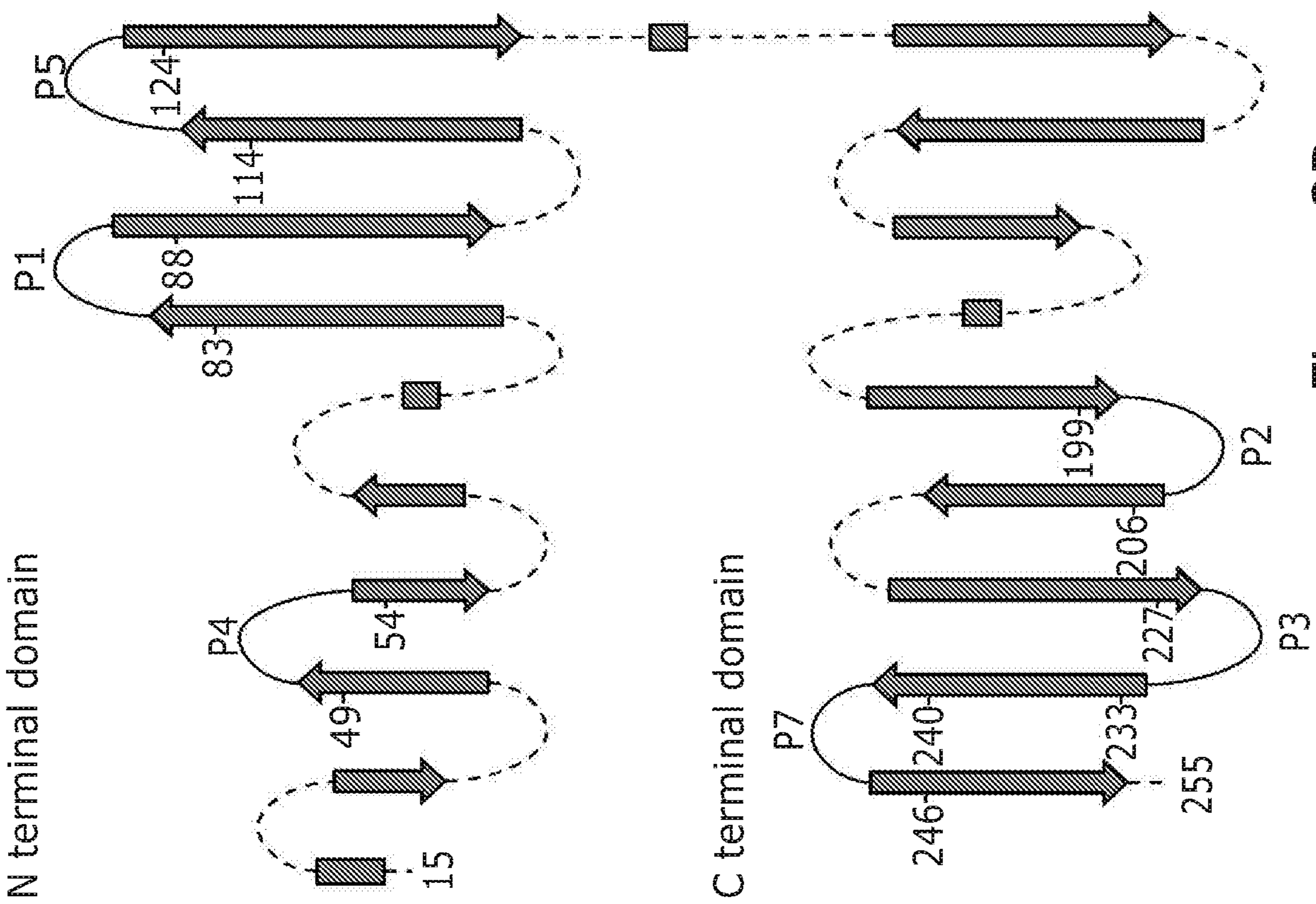


Figure 2B

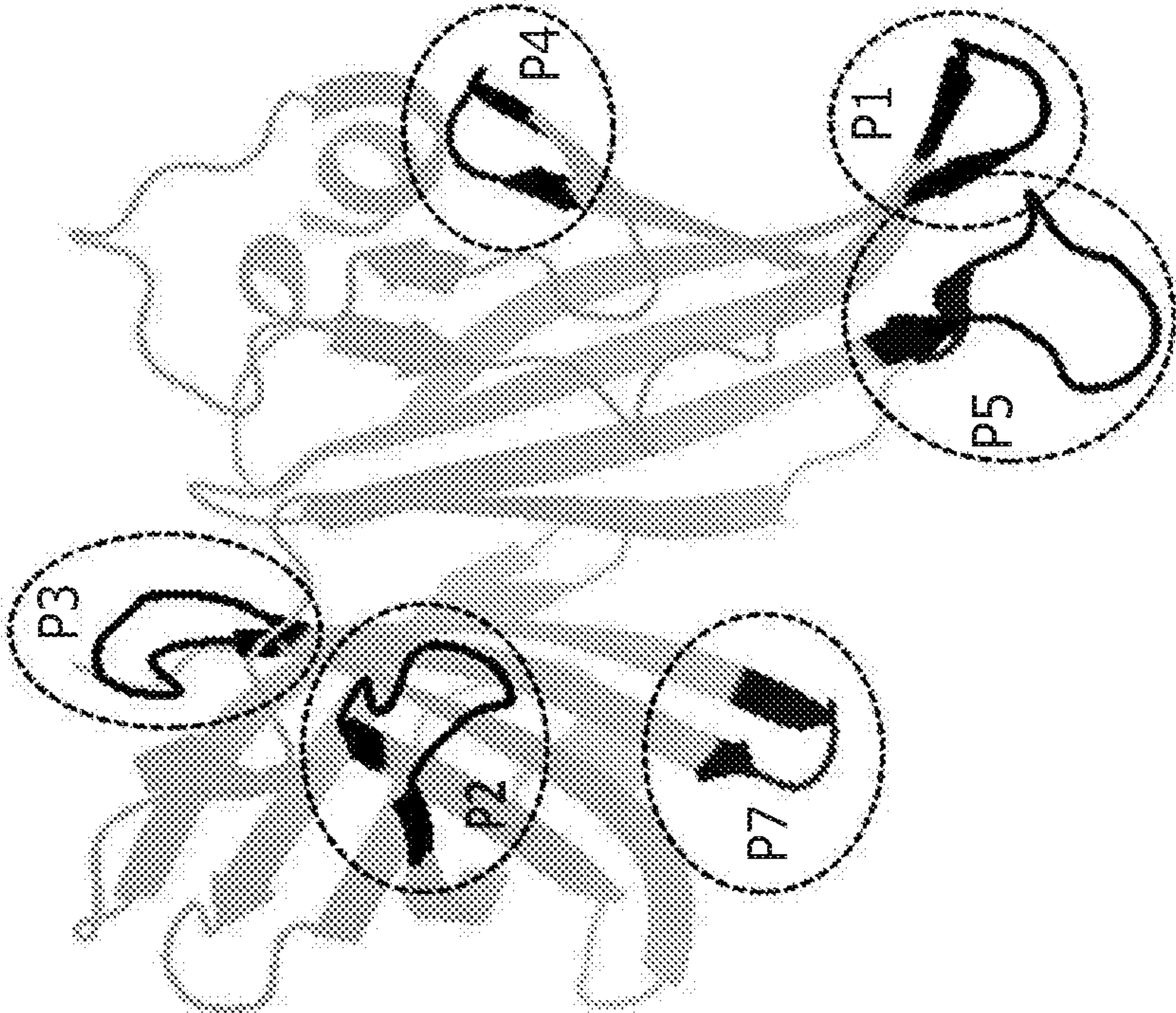


Figure 2A

4/13

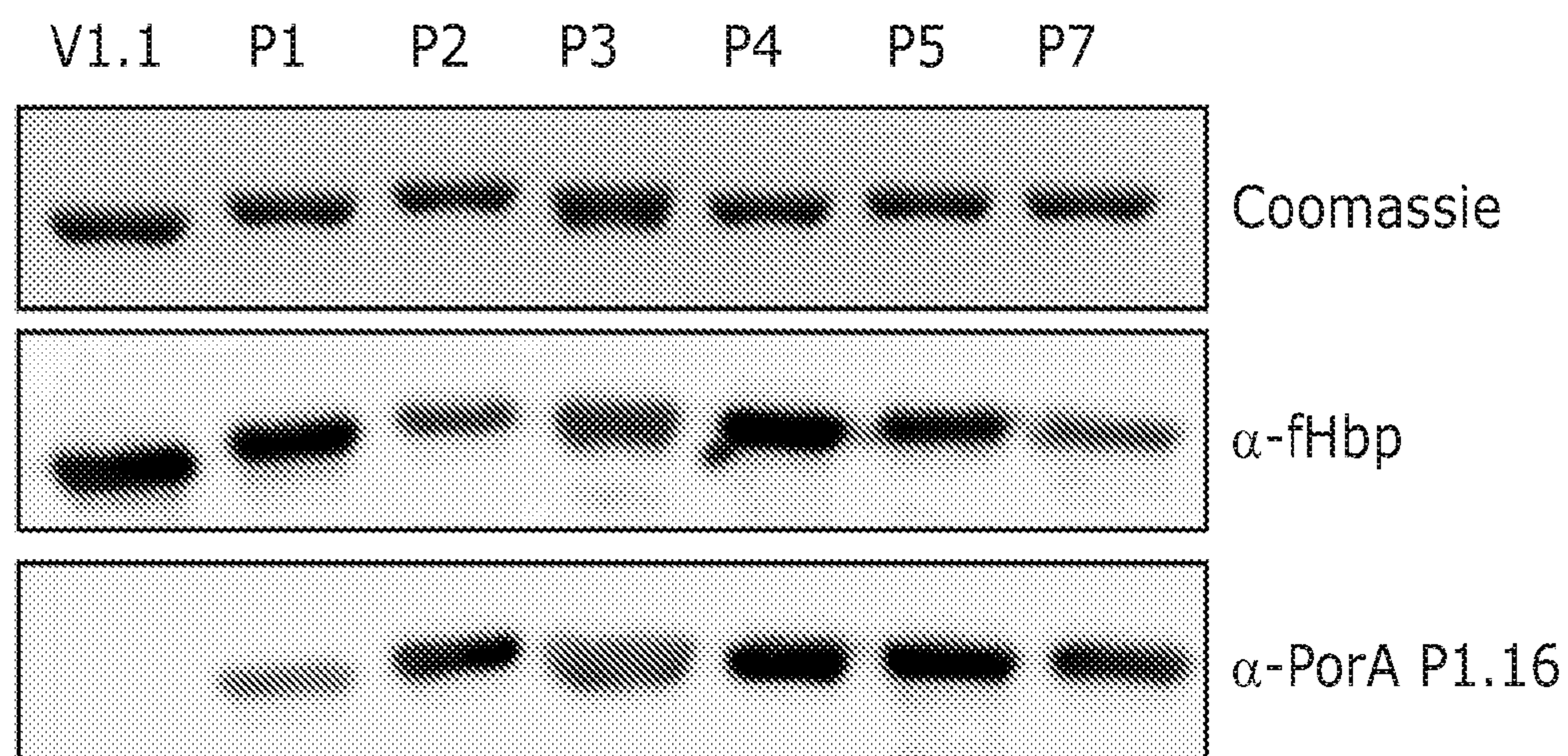


Figure 2C

```
1  CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LQSLTLDQSV

41  RKNEKLKLAA QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR
      ..P4...

81  QIEVDGQLIT LESGEFQVYK QSHSALTAFQ TEQIQDSEHS
      ..P1...                               ...P5...

121 GKMVAKRQFR IGDIAGEHTS FDKLPEGGRA TYRGTAFGSD
      ....

161 DAGGKLTYTI DFAAKQGNGK IEHLKSPELN VDLAAADIKP

201 DGKRHAVISG SVLYNQAEKG SYSLGIFGGK AQEVAGSAEV
      .P2...                               ...P3...

241 KTVNGIRHIG LAAKQ
      ..P7..
```

Figure 2D

5/13

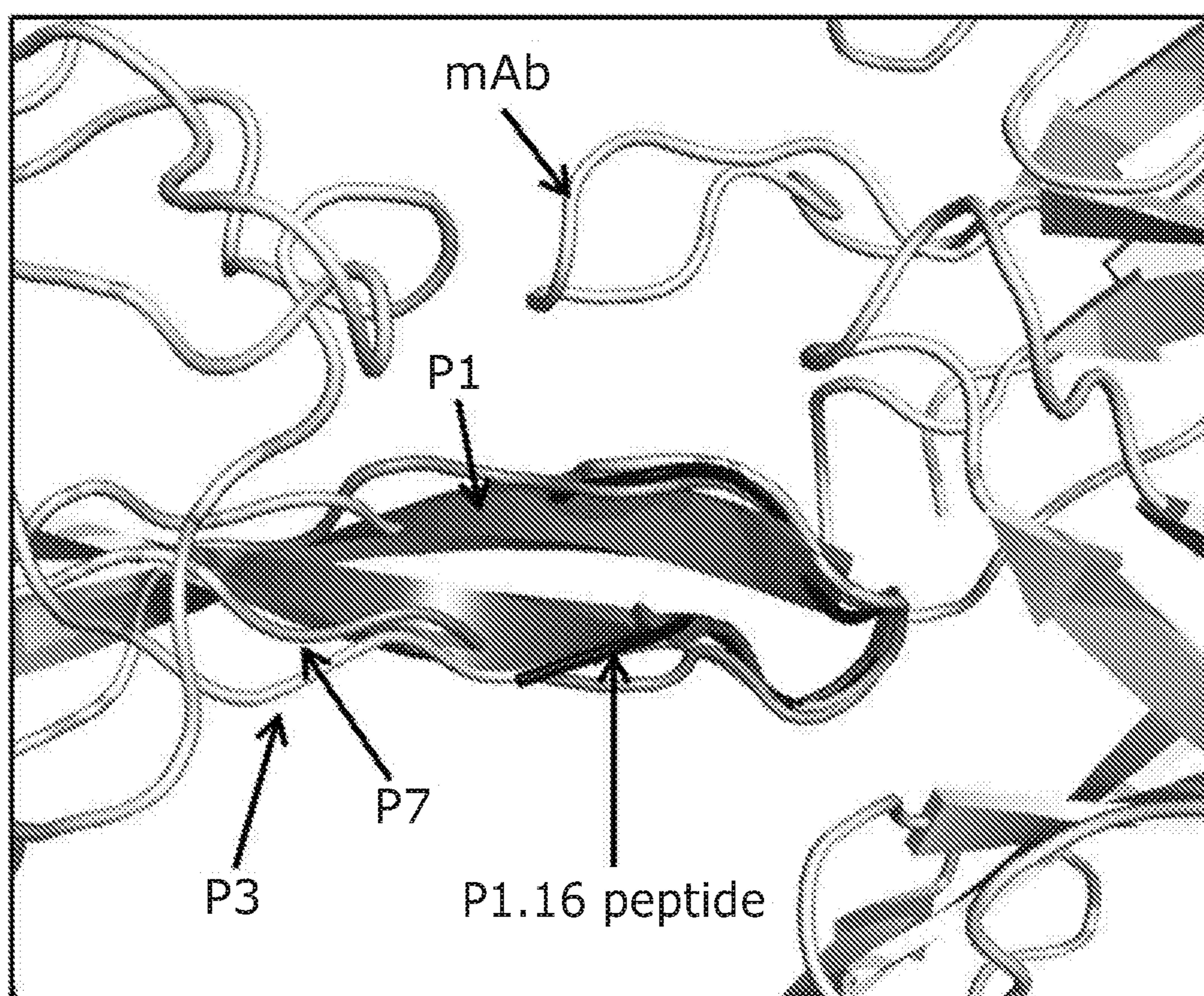
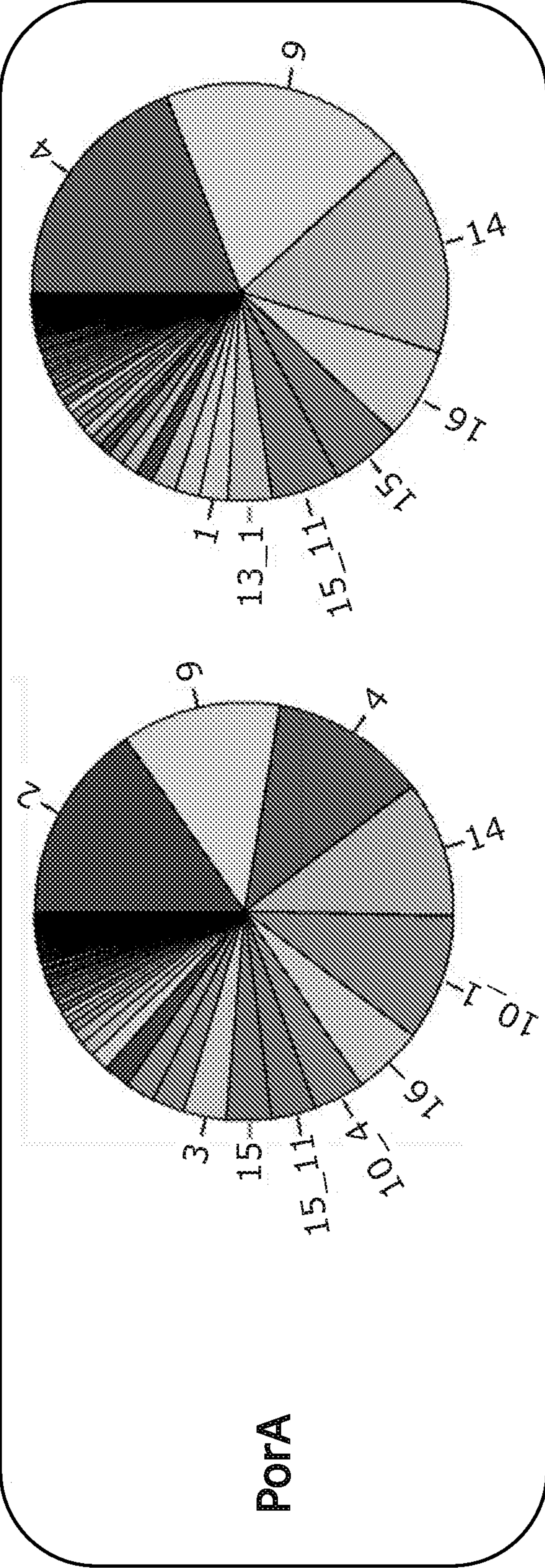
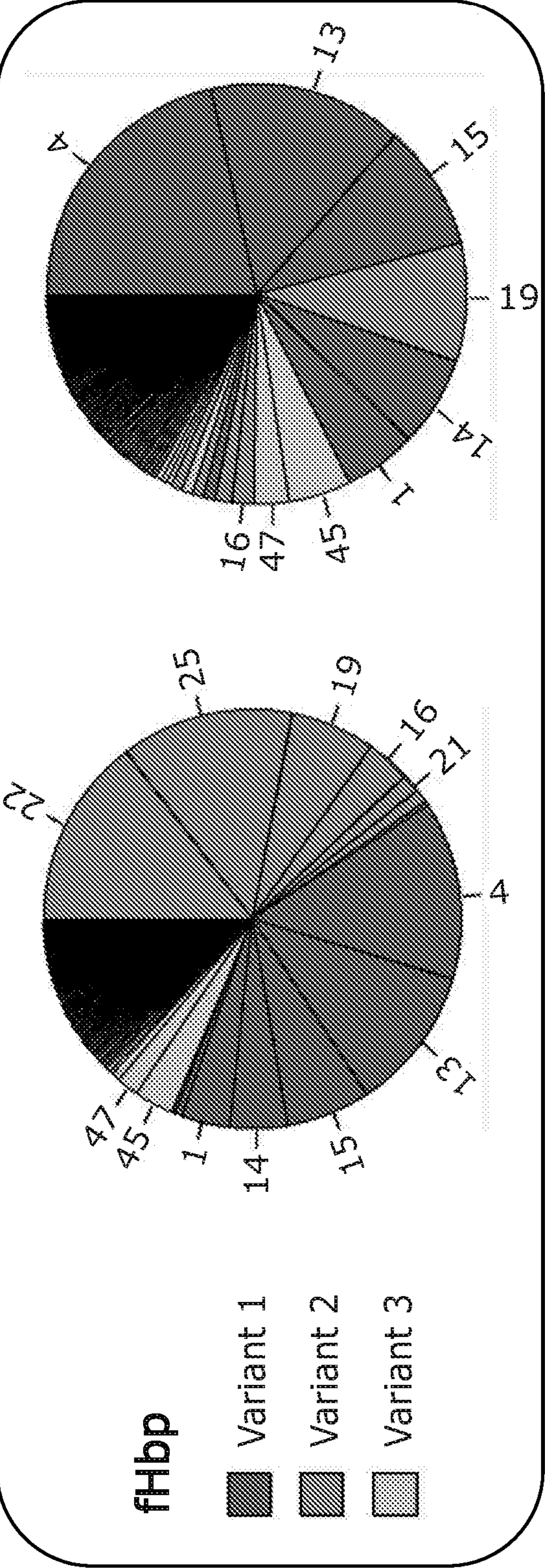


Figure 3A

Protein	Cp (kcal mole ⁻¹ °C ⁻¹)		Kd (nm)
	NT	CT	
V1.1	69.8	87.9	3.6
P1	60.5	87.3	2.3
P2	68.4	75.7	28
P3	61.7	72.4	2.7
P4	65	87.9	2.3
P5	68.8	88	NB
P7	69	76.9	5.5

Figure 3B

MenABCWXY MenB
(2616 cases) (1645 cases)



	Variant	% MenABCWXY coverage (2009-2015 UK)	% MenB coverage (2009-2015 UK)
fHbp	1.4	13.8	21.5
	2.22	14.8	0.2
	2.19	6.5	9.1
	3.45	3.0	4.8
PorA	P1.2	15.4	1.3
	P1.9	12.3	19.1
	P1.4	12.1	19.3
	P1.14	10.5	16.3
	P1.10_1	9.7	1.3
	P1.16	5.4	7.0
	P1.15	3.4	5.5

Figure 4
Continued

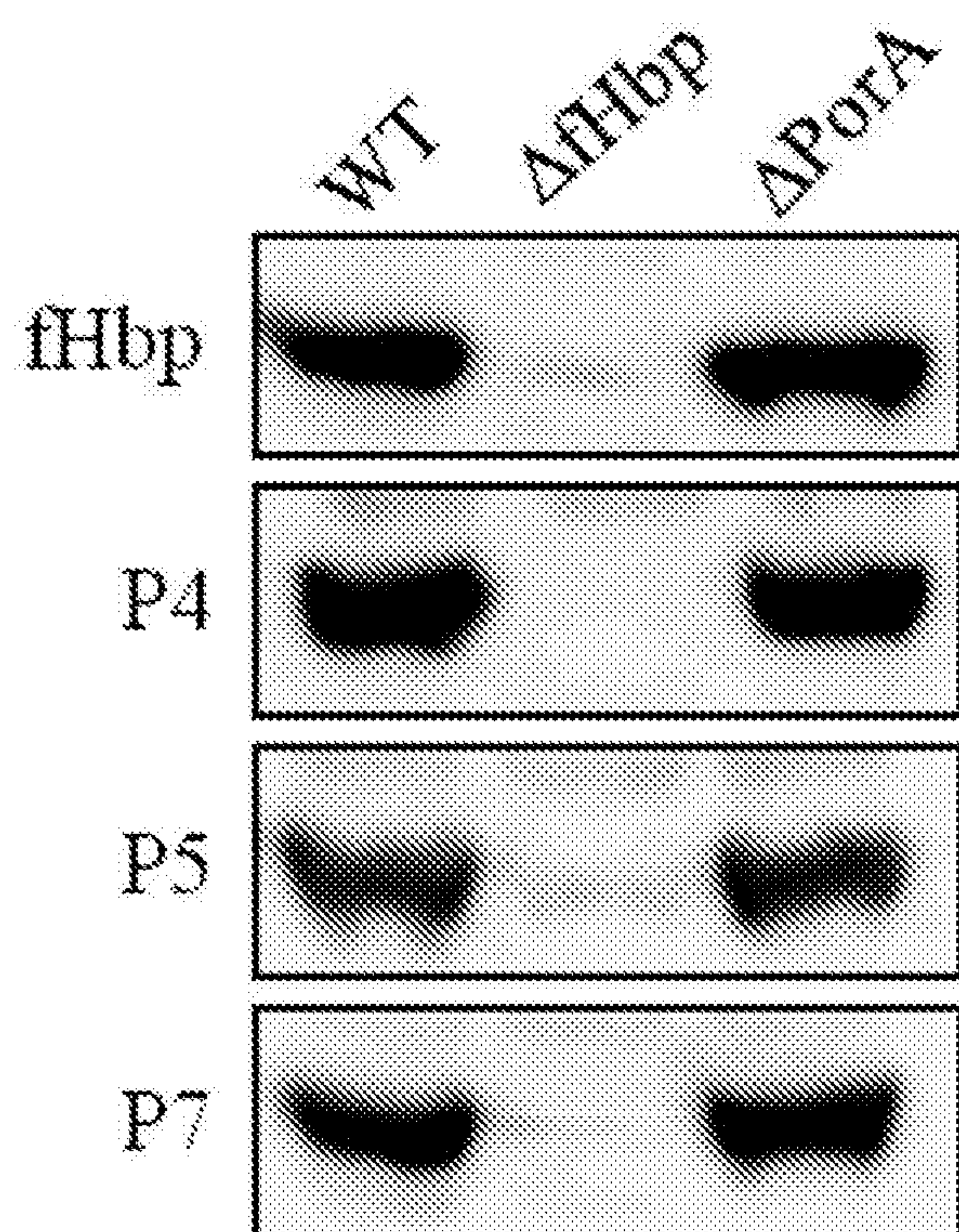
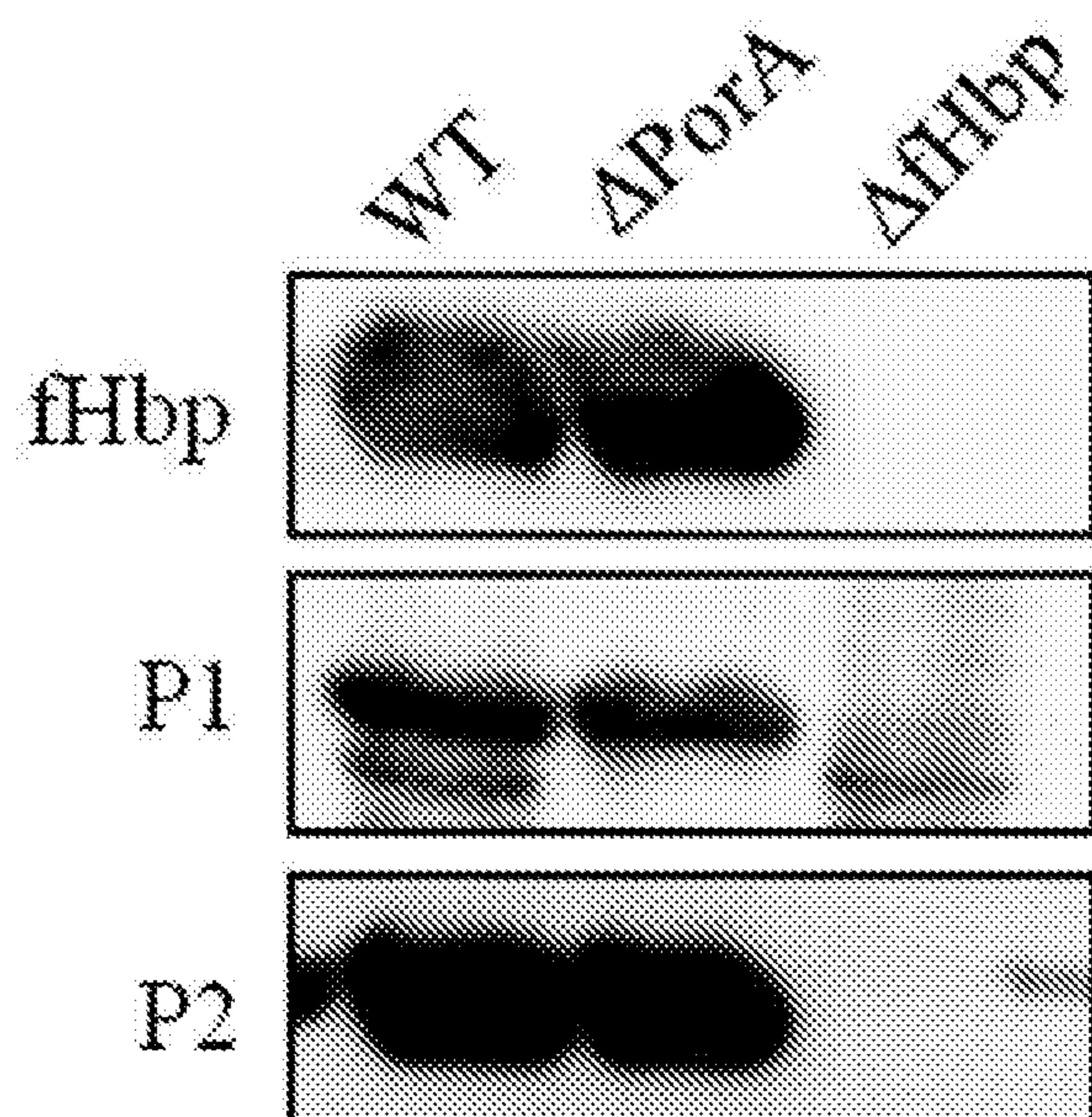


Figure 5A

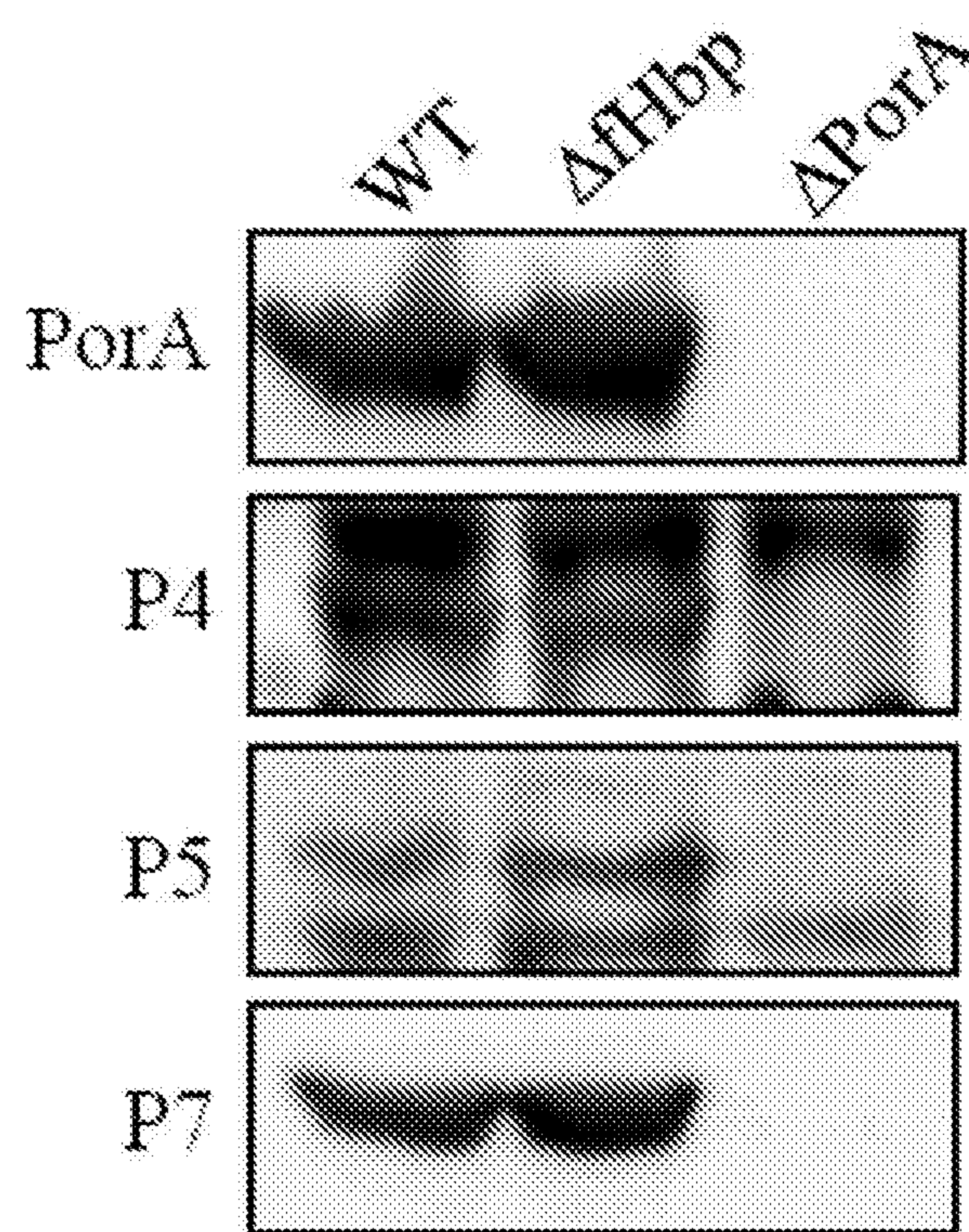
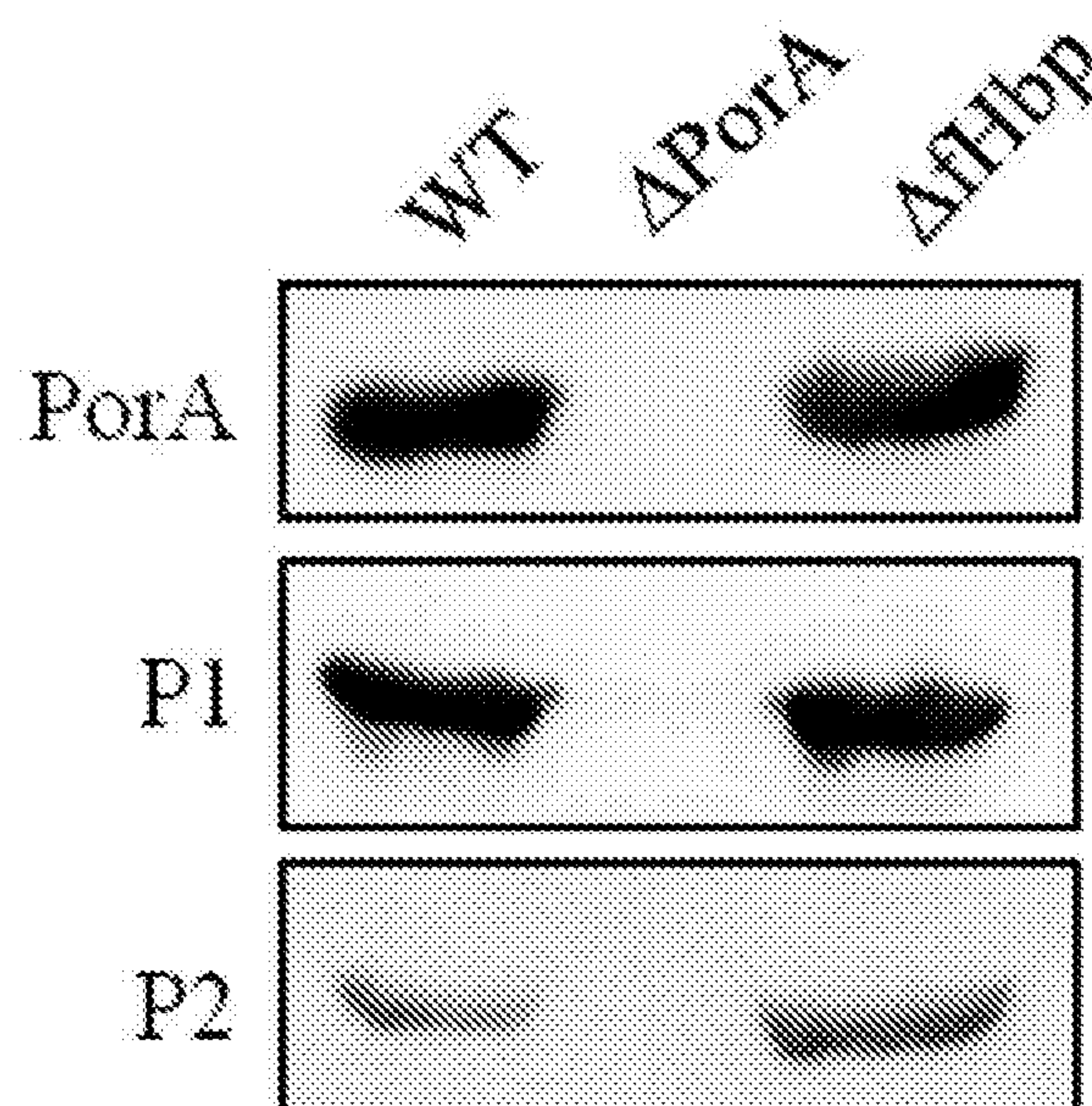


Figure 5B

Chimera	NT _{TM} (°C)	CT _{TM} (°C)
V1.1 WT	71	90
V2.25 WT	33?	87
V2.25 MT	67	87
V2.25 M6 PorA (P1) P1.16	40	86

Figure 6A

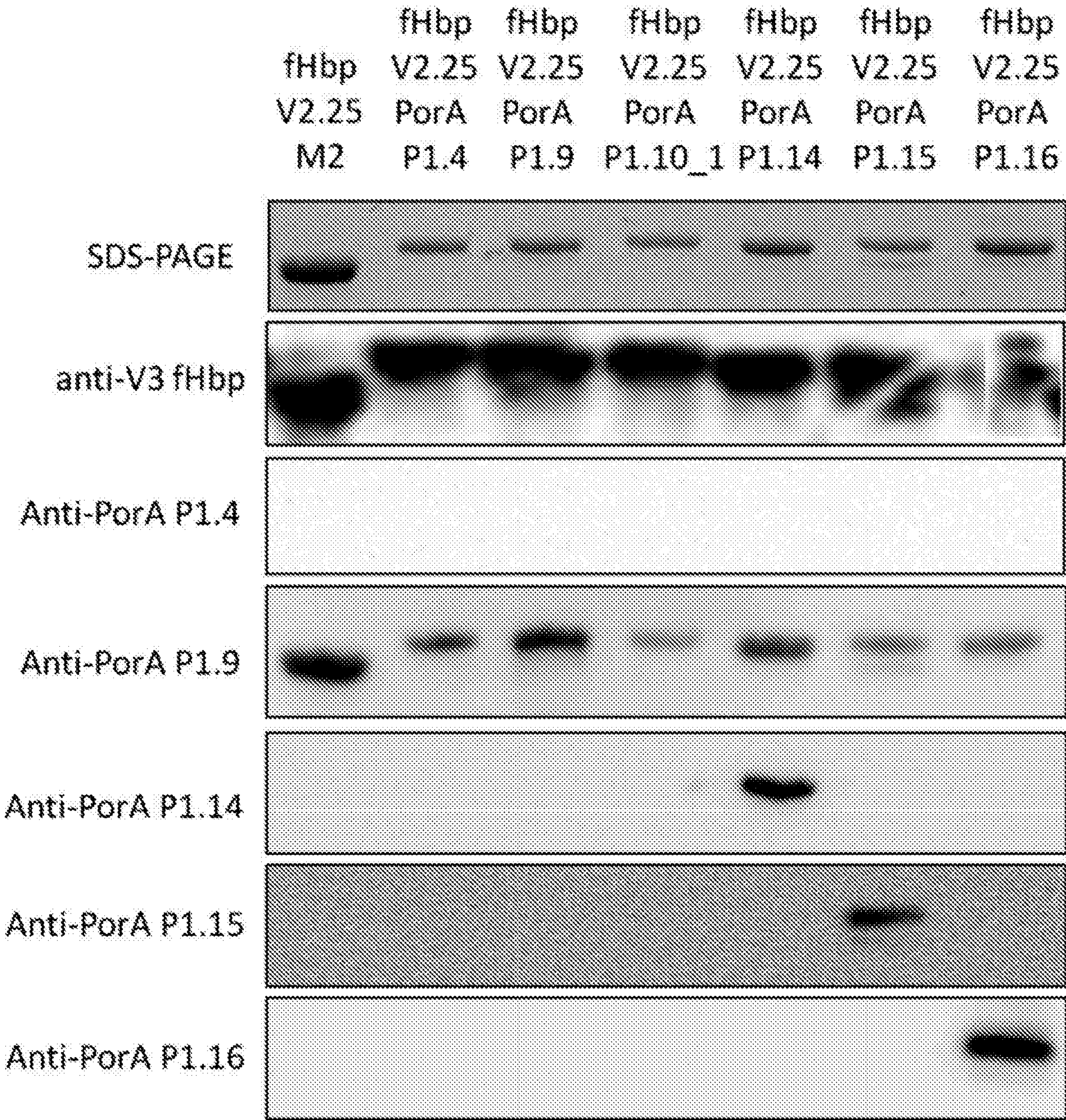
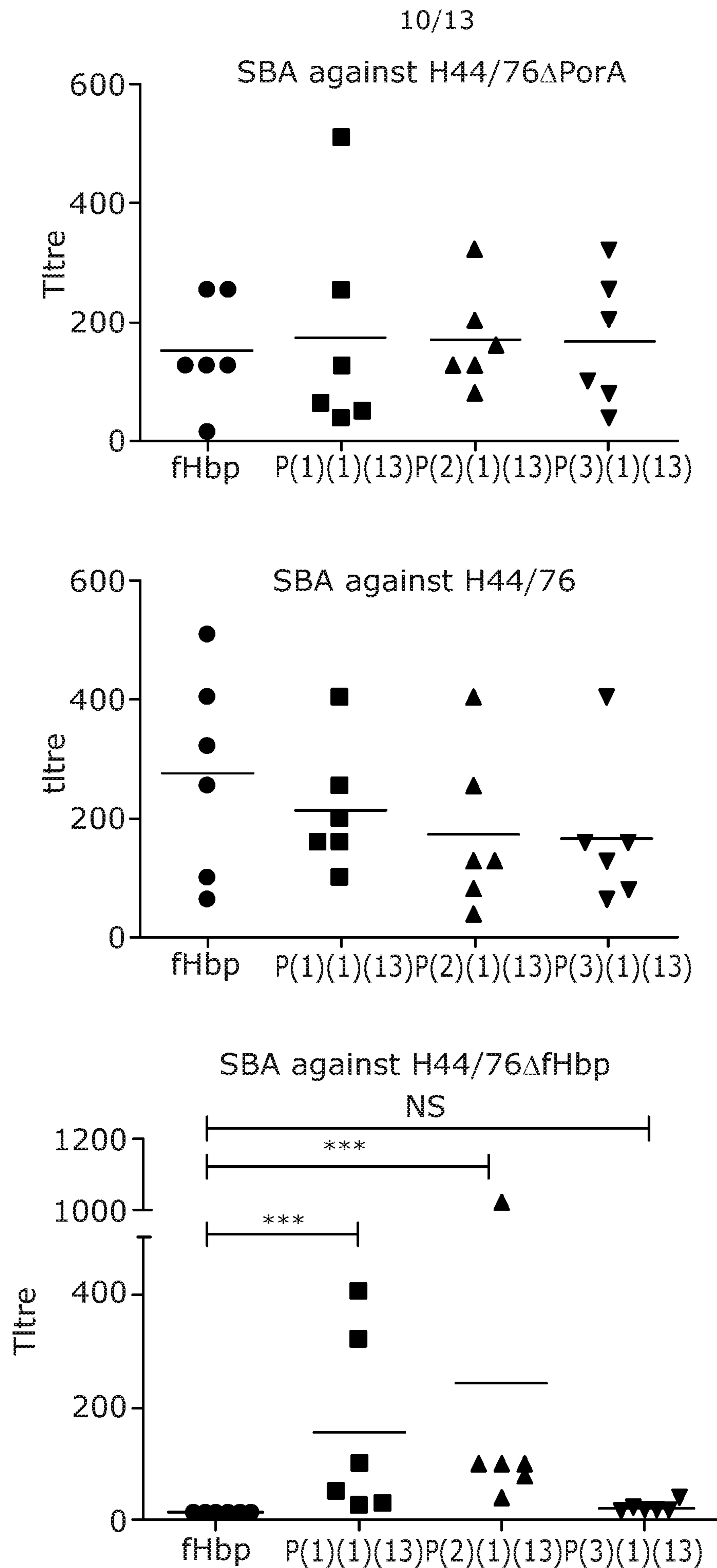


Figure 6B



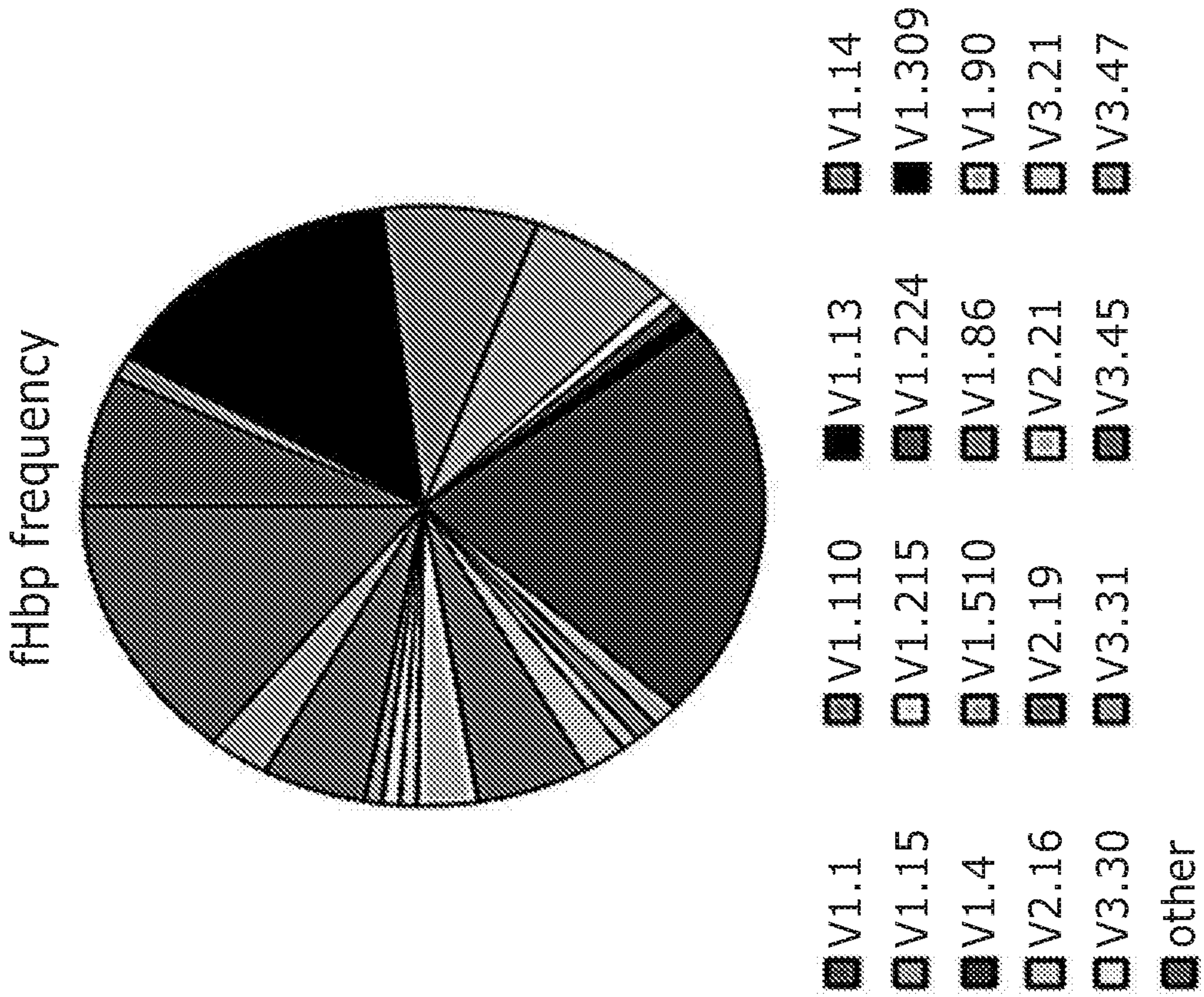


Figure 8B

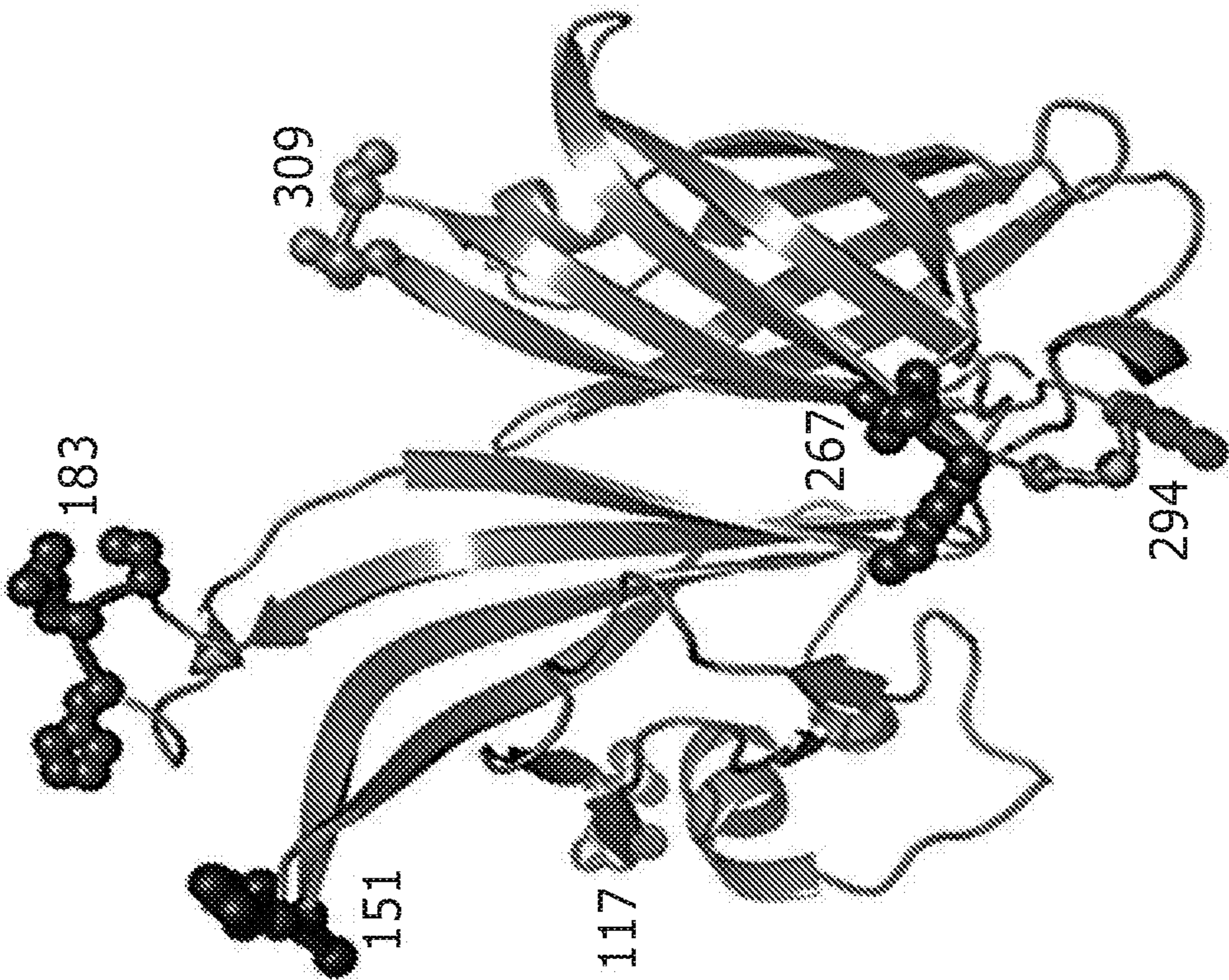


Figure 8A

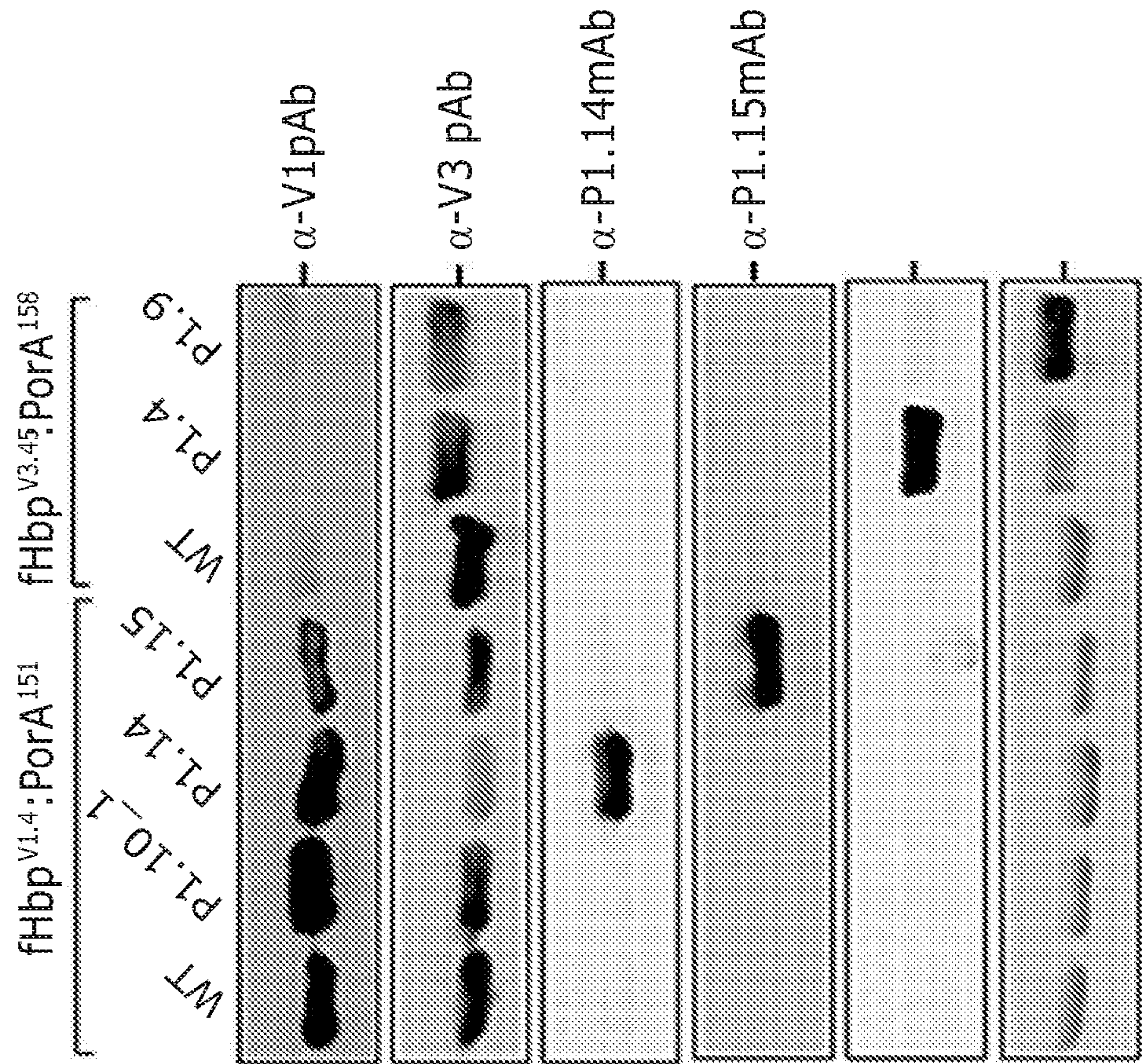


Figure 8D

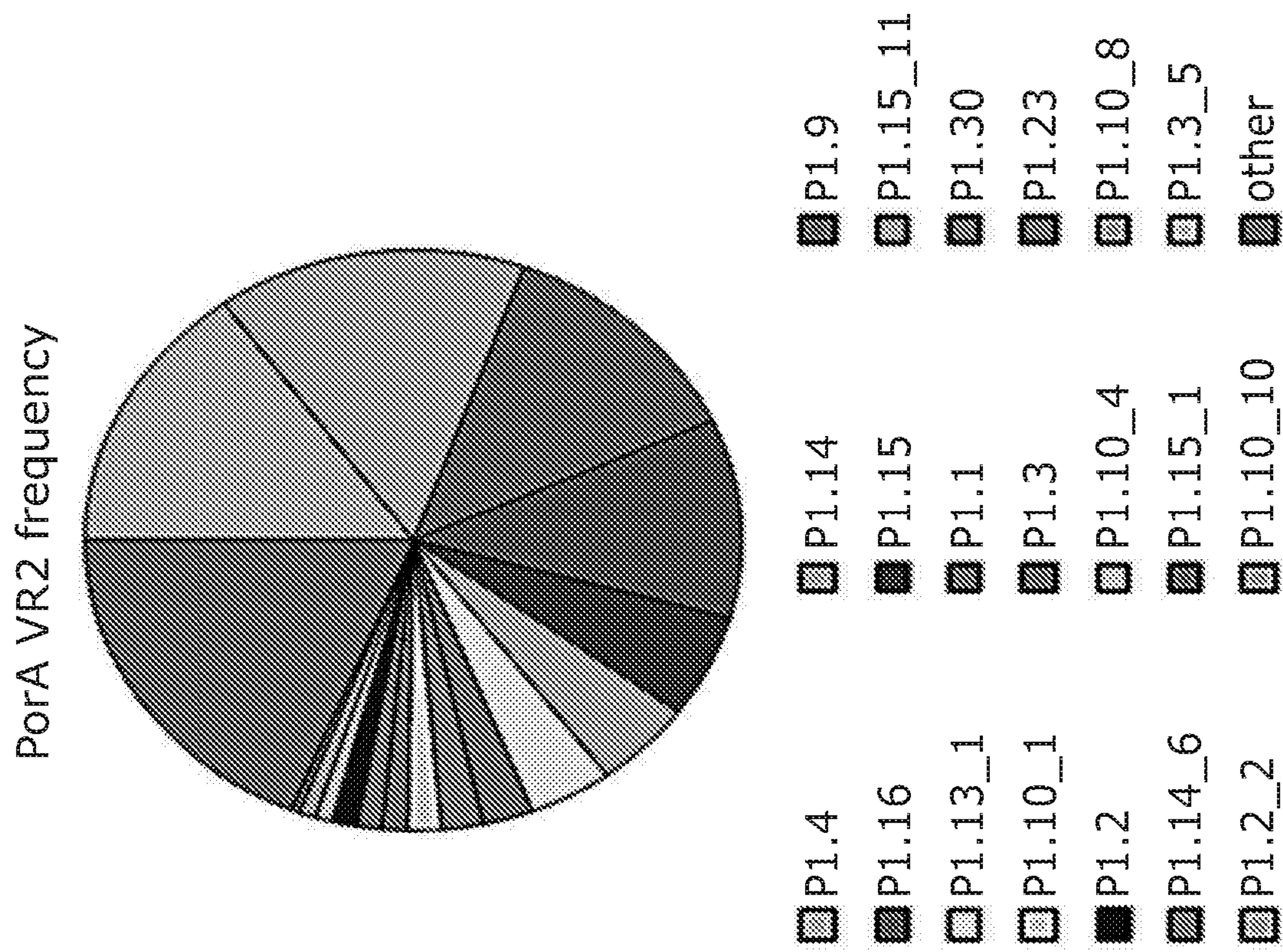


Figure 8C

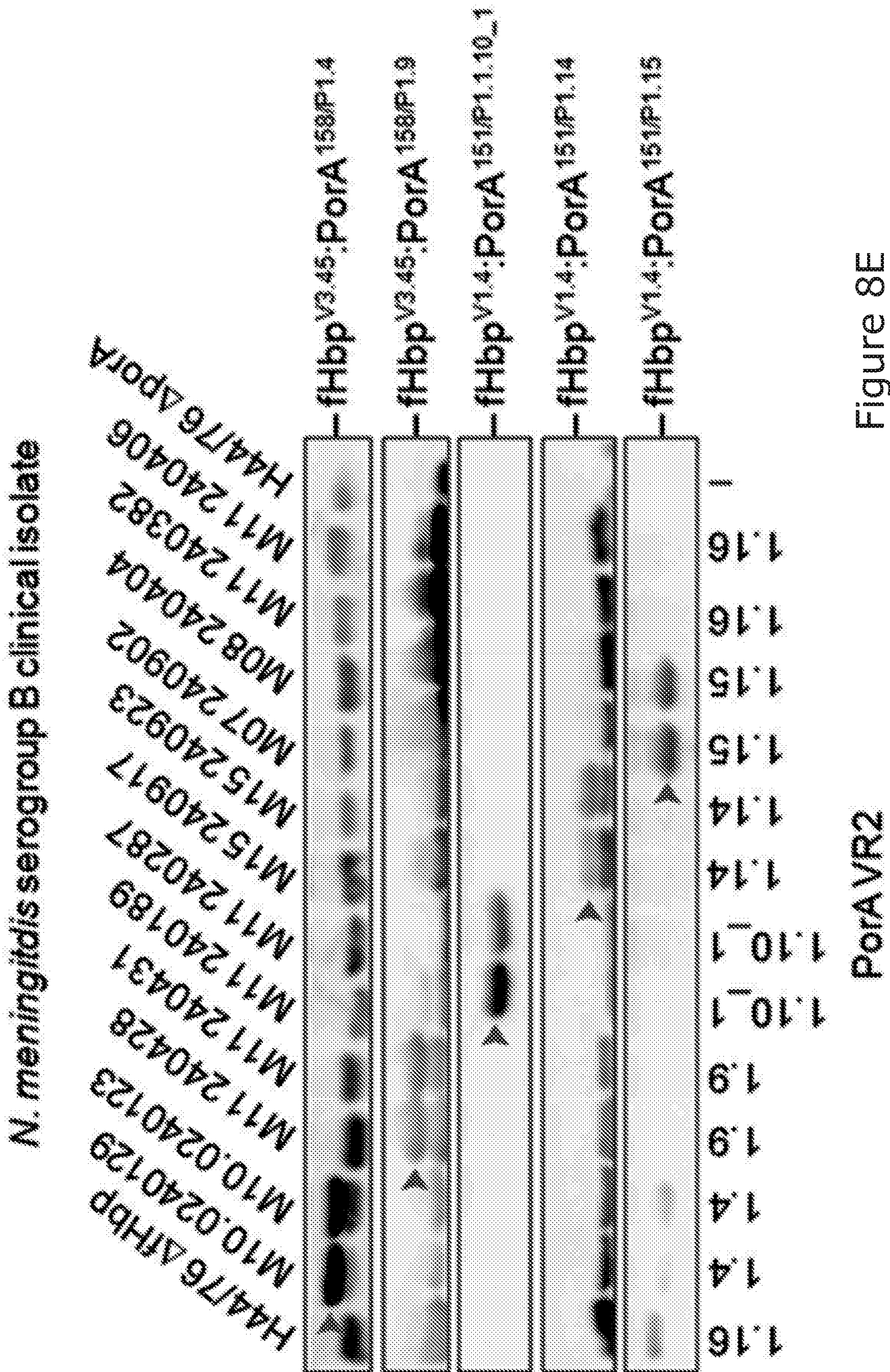


Figure 8E

