

**Tabel S1: Primers used in this study**

No.	Primers	Sequence 5'-3'
1	<i>SacI</i> -PmpD (aa68-698)-fw	GGAAGTCTTGCGGGGAGCTCCGACAGCCAAGCGGAGGG
2	<i>BamHI</i> -PmpD (aa68-698)-rv	TACCGCTGCCGGATCCACGAAGGGACGAGATAGCTCC
3	<i>SacI</i> -Prn (aa35-574)-fw	GGAAGTCTTGCGGGGAGCTCCATCAAGGTAAGCGGCCGT
4	<i>BamHI</i> -Prn (aa35-574)-rv	TACCGCTGCCGGATCCGGACAACCTCCCTGCCCCG
5	<i>SacI</i> -VacA (aa65-493)-fw	GGAAGTCTTGCGGGGAGCTCCAAACAAGCCGAAGAAGCGAATAAAACC
6	<i>BamHI</i> -VacA (aa65-493)-rv	TACCGCTGCCGGATCCTCCAAACTAATATCGTTATTGAAAGTGGC
7	<i>SacI</i> -PmpD (aa68-629)-fw	CTGCAGGAAGTCTTGCGGGGAGCTCCGACAGCCAAGCGGAG
8	PmpD (aa68-629)-rv	GGGTGGCATCGGAGCTAATCGCGCCGCCGCCTAA
9	“PmpD”-HbpD (840)-fw	GATTAGCTCCGATGCCACCCTGAGTC
10	<i>KpnI</i> -HbpD (921)-rv	CTCCCGCTCCAGATATTGCGGTACCCGTTAAACAGGCTGTA
11	<i>SacI</i> -Prn (aa35-350)-fw	CTGCAGGAAGTCTTGCGGGGAGCTCCATCAAGGTAAGCGGCCGT
12	Prn (aa35-350)-rv	GTGGCATCGGCCAGCGCCACGTCGAG
13	“Prn”-HbpD (840)-fw	GTGGCGCTGGCCGATGCCACCCTGAGTCTGAAC
14	<i>SacI</i> -VacA (aa65-377)-fw	CTGCAGGAAGTCTTGCGGGGAGCTCCAAACAAGCCGAAGAAGCG
15	VacA (aa65-377)-rv	GGGTGGCATCATTTTCCACTAAAAGGGTG
16	“VacA”-HbpD (840)-fw	AGTGGAATGATGCCACCCTGAGTCTGAAC

**Fasta files of amino acid sequences used in this study as expressed in *E. coli*.**

Colorcoding is used to indicate significant features, which is explained under the sequences

**>Amino acid sequence of *C. trachomatis* serovar L2 PmpD (wild-type); (strain 434/Bu/ATCC)**

MSSEKDIKSTCSKFSLSVVAAILASVSGLASCVDLHAGGQSVNELVYVGPQAVLLLDQIRDLFVGSKDS  
QAEGQYRLIVGDPSSFQEKDADTLPGKVEQSTLFSVTNPVVFQGVDDQVSSQGLICSFTSSNLDSR  
DGESFLGVAFVGDSSKAGITLTDVKASLSGAALYSTEDLIFEKIKGGLEFASCSSLEQGGACAAQSILIH  
CQGLQVKHCTTAVNAEGSSANDHLGFGGGAFFVTGSLSGEKSLYMPAGDMVVANCDGAISFEGNSAN  
FANGGAIAASGKVLVANDKKTSTFIENRALSGGAIAASSDIAFQNCALVFKGNCAIGTEDKGSLLGGGA  
ISSLGTVLLQGNHGITCDKNESASQGGAIFGKNCQISDNEGPVVFVFRDSTACLGGAIAAQEIVSIQNNQA  
GISFEGGKASFGGGIACGSFSSAGGASVLGTIDISKNLGAISFSRTLCTTSDLGQMEYQGGGALFGENISL  
SENAGVLTFKDNIVKTFASNGKILGGGAILATGKVEITNNSEGISFTGNARAPQALPTQEEFPLFSKKEG  
RPLSSGYSGGGAILGREVAILHNAAVVFEQNRLQCSEEEATLLGCCGGGAVHGMDSTSIVGNSSVRF  
NNYAMGQGVSGGALLSKTVQLAGNGSVDFSRNIASLGGGALQASEGNCELVDNGYVLFVRDNRGRVY  
GGAISCLRGDVVISGNKGRVEFKDNIATRLYEETVEKVEEVEPAPEQKDNNELSFLGSAEQSFITAAN  
QALFASEDGDLSPSSISSEELAKRRECAGGAIFAQRVIRVDNQEA VVFSNNFSDIYGGAIFTGSLREEDK  
LDGQIPEVLISGNAGDVVFSGNSSKRDEHLPHTGGAICTQNLTISQNTGNVLFYNNVACSGGAVRIED  
HGNVLLAEFGDIVFKGNSSFRAQGSDAIYFAGKESHITALNATEGHAIVFHDALVFENLEERKSAEVL  
LINSRENPGYTGSIRFLEAESKVPQCIHVQQGSLELLNGATLCSYGFKQDAGAKLVLAAGAKLKILDSG  
TPVQQGHAISKPEAEIESSEPEGAHSLWIAKNAQTTPMVVDIHTISVDLASFSSSQEGTVEAPQVIVPG  
GSYVRSSELNLELVNTTGTGYENHALLKNEAKVPLMSFVASGDEASAEISNLSVSDQLQIHVVTPEIEED  
TYGHMGDWSEAKIQDGTLVISWNPTGYRLDPQKAGALVFNALWEEGAVLSALKNARFAHNLTQRM  
EFDYSTNVWGFAGGFRTLSAENLVAIDGYKGAYGGASAGVDIQLMEDFVLGVSGAAFLGKMDSQKF  
DAEVSRRKGVVGSVYTGFLAGSWFFKGQYSLGETQNDMKTRYGVLGESSASWTSRGLADALVEYRS  
LVGPVRPTFYALHFNPHYVEVSYASMKFPGFTEQGREARSFEDASLTNITIPLGMKFELAFIKGQFSEVNS  
LGISYAWAYRKVEGGA VQLLEAGFDWEGAPMDLPRQELRVALENNTEWSSYFSTVLGLTAFCGGFT  
STDSKLGYEANTGLRLIF

**>Amino acid sequence of PmpD (full-length)**

MNRIYSLRYSAVARGFIAVSEFARKCVHKSVRRLCFPVLLLIPVLFSAGSLAGTVNNELGSGSGYPYDV  
PDYAGSGTSSSVDLHAGGQSVNELVYVGPQAVLLLDQIRDLFVGSKDSQAEGQYRLIVGDPSSFQEKD  
ADTLPGKVEQSTLFSVTNPVVFQGVDDQDQVSSQGLISSFTSSNLDSPRDGESFLGIAFVGDSKAGITL  
TDVKASLSGAALYSTEDLIFEKIKGGLEFASSSLEQGGASAAQSILIHDSQGLQVKHSTTAVNAEGSSA  
NDHLGFGGGAFFVTGSLSGEKSLYMPAGDMVVANS<sup>SD</sup>GAISFEGNSANFANGGAIAASGKVLVANDK  
KTSFIENRALSGGAIAASSDIAFQNSAELVFKGNSAIGTEDKGS LGGGAISSLGTVLLQGNHGITS<sup>DK</sup>NES  
ASQGAIFGKNS<sup>Q</sup>ISDNEGPVFRDSTA<sup>SL</sup>GGGAIAAAQEIVSIQNNQAGISFEGGKASFGGGIA<sup>SG</sup>SFSSA  
GGASVLGTIDISKNLGAISFSRTLSTT<sup>SD</sup>LGQMEYQGGGALFGENISLSENAGVLT<sup>FK</sup>DNIVKT<sup>FA</sup>SNNGKI  
LGGGAILATGKVEITNNSGGISFTGNARAPQALPTQEEFPLFSKKEGRPLSSGYSGGGAILGREVAILHN  
AAVVFEQNRLQS<sup>SE</sup>EEATLLGSSGGGAVHGMDS<sup>TS</sup>IVGNSSVRFGN<sup>NY</sup>AMGQGVSGGALLSKTVQLA  
GNGSVDFSRNIASLGGGALQASEGNSELVDNGYVLF<sup>RD</sup>NRGRVYGGAISSLRGDVVIS<sup>GN</sup>KGRVEFKD  
NIATRLYVEETVEKVEEVEPAPEQKDNNELSFLGSVEQS<sup>FI</sup>TAA<sup>NQ</sup>ALFAS<sup>ED</sup>GDLSP<sup>ESS</sup>ISSEELAKRR  
E<sup>S</sup>AGGAIFAKRVRIVDNQEAVVFSNNFSDIYGGAIFTG<sup>SL</sup>REEDKLDGQIPEVLISGNAGDVVFSGNSSK  
RDEHLPHTGGGAISTQNL<sup>TS</sup>QNTGNVLFYNNV<sup>AS</sup>SGGAVRIEDHGNVLL<sup>EA</sup>FGGDIVFKGNSS<sup>FRA</sup>QG  
SDAIYFAGKESHITALNATEGHAIVFHDALVFENLKERKSAEVL<sup>LN</sup>SRENPGYTGSIRFLEAESKVPQ<sup>SI</sup>  
HVQQGSLELLNGATL<sup>SS</sup>YGFKQDAGAKLVLAAGSKLKILDSGTPVQGHAIKPEAEIESSEPEGAHSL  
WIAKNAQTTVPMVDIHTISVDLASFSSSQEGTVEAPQVIVPGGSYVRSGELNLELVNTTGTGYENHAL  
LKNEAKVPLMSFVASSDEASAEISNLSVSDLQIHVATPEIEEDTYGHMGDWSEAKIQDGTLVINWNPTG  
YRLDPQKAGALVFNALWEEGAVLSALKNARFAHN<sup>LA</sup>QRMEFDYSTNVWGF<sup>AF</sup>GGFRTLSAENLV<sup>AI</sup>  
DGYKGAYGGASAGVDIQLMEDFVLGVSGAAFLGKMDSQKFDAEVS<sup>RK</sup>GVVGSVYTGFLAGSWFFKG  
QYSLGETQNDMKTRYGV<sup>LG</sup>ESSASWTSR<sup>GV</sup>LADALVEY<sup>RS</sup>LVG<sup>PR</sup>PTFYALHFN<sup>PY</sup>VEVS<sup>YAS</sup>MKF  
PGFTEQGREARSFEDASLTNITPLGMKFELAFIKGQFSEVNSLGISY<sup>AW</sup>EYRKVEGGAVQLLEAGFD  
WEGAPMDLPRQELRVALEN<sup>TE</sup>WSSYFSTVLGLTAF<sup>SG</sup>GFTSTD<sup>SK</sup>LG<sup>YE</sup>ANAGL<sup>RY</sup>SF

***E. coli* Hbp signal peptide**

Hbp N-terminal (passenger domain) ; GTVNN: Edman N-terminal amino acid sequencing result

HA tag;

*C. trachomatis* serovar L2 PmpD (full-length) with 24 cysteine (C) residues replaced by Serine (S): underlined

Hbp (RYSF) conserved C-terminal motif

**>Amino acid sequence of HbpD(Ad1)-PmpD (aa68-698)**

MNRIYSLRYSAVARGFIAVSEFARKCVHKSVRRLCFPVLLLIPVLFSAGSLAGSSDSQAEGQYRLIVGDP  
SSFQEKDADTLPGKVEQSTLFSVTNPVVFQGVDDQDQVSSQGLISSFTSSNLDSPRDGESFLGIAFVGDS  
SKAGITLTDVKASLSGAALYSTEDLIFEKIKGGLEFASSSSLEQGGASAAQSILIHDSQGLQVKHSTTAVN  
AEGSSANDHLGFGGGAFFVTGSLSGEKSLYMPAGDMVVANS<sup>SD</sup>GAISFEGNSANFANGGAIAASGKVLV  
VANDKKTSFIENRALSGGAIAASSDIAFQNSAELVFKGNSAIGTEDKGS LGGGAISSLGTVLLQGNHGIT  
SDKNESASQGAIFGKNS<sup>Q</sup>ISDNEGPVFRDSTA<sup>SL</sup>GGGAIAAAQEIVSIQNNQAGISFEGGKASFGGGIA  
SGSFSSAGGASVLGTIDISKNLGAISFSRTLSTT<sup>SD</sup>LGQMEYQGGGALFGENISLSENAGVLT<sup>FK</sup>DNIVKT  
FASNGKILGGGAILATGKVEITNNSGGISFTGNARAPQALPTQEEFPLFSKKEGRPLSSGYSGGGAILGRE  
VAILHNA<sup>AA</sup>VVFEQNRLQS<sup>SE</sup>EEATLLGSSGGGAVHGMDS<sup>TS</sup>IVGNSSVRFGN<sup>NY</sup>AMGQGVSGGALLS  
KTVQLAGNGSVDFSRNIASLGGGALQASEGNSELVDNGYVLF<sup>RD</sup>NRGRVYGGAISSLR<sup>SGSG</sup>NDAPV  
TFRTSEGGALEWSFNSS<sup>TG</sup>AGALTQGT<sup>TTY</sup>AMHGQQGNDLNAGKNLIFQGGQNGQINLKDSVSQGAGS  
LTF<sup>RD</sup>NYTVTTSNGSTWTGAGIVVDNGVSVNWQVNGVKGDNLHKIGEGTLTVQGTGINEGGLKVG  
GKVVLNQQADNKGQVQAFSSVNIASGRPTVVLTDERQVNPDTVSWGYRGGTLDVNGNSLTFHQLKA

ADYGAVLANNVDKRATITLDYALRADKVALNGWSESGKGTAGNLYKYNNPYTNTTDDYFILKQSTYG  
YFPTDQSSNATWEFVGHSQGDAQKLVAADRFTAGYLFHGQLKGNLNVDNRLPEGVTGALVMDGAAD  
ISGTFQTENGRLTLQGHPVIHAYNTQSVADKLAASGDHSLVTQPTSFSQEDWENRSFTFDRLSLKNTDF  
GLGRNATLNTTIQADNSSVTLGDSRVFIDKNDGQGTAFMLEEGTSVATKDADKSVFNGTVNLDNQSVL  
NINDIFNGGIQANNSTVNISSDSAVLGNSTLTSTALNLNKGANALASQSFVSDGPVNISDATLSLNSRPD  
EVSHTLLPVYDYAGSWNLKGDDARLNVGPYSMLSGNINVQDKGTVTLGGEGELSPDLTLQNQMLYSL  
FNGYRNIWSGSLNAPDATVSM TDTQWSMNGNSTAGNMKLNRTIVGFNGGTSPFTTLTTDNLDAVQSA  
FVMRTDLNKADKL VINKSATGHDNSIWVNFLLKKPSNKDTLDIPLVSAPEATADNLFRASTRVVGFS  
DVTPILSVRKEDGKKEWVLDGYQVARNDGQGKAAATFMHISYNNFITEVGS LNKRMGDLRDINGEAGTW  
VRLLNGSGSADGGFTDHYTLLQMGADRKHGELGSMDLFTGVMATYTDTDASADLYSGKTKSWGGGFY  
ASGLFRSGAYFDVIAKYIHNNENKYDLNFAGAGKQNFRRSHSLYAGAEVGYRYHLTDTTFVEPQAE  
LVWGRLQGQTFNWNDSGMDVSMRRNSVNPLVGRTGVVSGKTFSGKDWSLTARAGLHYEFDLTDSAD  
VHLKDAAGEHQINGRKDSRMLYGVGLNARFGDNTRLGLEVERSAFGKYNTDDAINANIRYSF

*E. coli* Hbp signal peptide

*C. trachomatis* PmpD (aa68-698)

Flexible linker

*E. coli* HbpD

**>Amino acid sequence of HbpD(Ad1)-Prn (aa35-574)**

MNRIYSLRYSAVARGFIAVSEFARKCVHKSVRRLCFPVLLIPVLFSAAGSLAGSSIKVSGRQAQGILLENP  
AAELQFRNGSVTSSGQLSDDGIRRF LGTVTVKAGKLVA DHATLANVGDTWDDDGIALYVAGEQAQAS  
IADSTLQGAGGVQIERGANVTVQRSAIVDGG L HIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAV  
SVLGASELTLDGGHITGGRAAGVAAMQGA VVHLQRATIRRGDAPAGGAVPGGAVPGGAVPGGFGPG  
GFGPVLDGWYGVVDVSGSSVELAQ SIVEAPELGA AIRVGRGARVTVSGGSL SAPHGNVIETGGARRFAP  
QAAPLSITLQAGAHAQ GKALLYRVLPEPVKLTLTG GADAQGDIVATELPSIPGTSIGPLDVALASQARW  
TGATRAVDLSIDNATWVMTDNSNVGALRLASDGSVDFQQP AEAGRFKVLTVNTLAGSGLFRMNVFA  
DLGLSDKLVVMQDASGQHRLWVRNSGSE PASANTLLL VQTPLGSAATFTLANKDGKVDIGTYRYRLA  
ANGNGQWSLVGAKAPPAPKPAPQPGPQP PPQPQPEAPAPQPPAGRELSGSGSNDAPVTFRTSEGG  
ALEWSFNSSGTGAGALTQGTTTYAMHGQQGNDLNAGKNLIFQQQNGQINLKDSVSQGAGSLTFRDNYT  
VTTNNGSTWTGAGIVVDNGVSVNWQVNGVKGDNLHKIGEGTLTVQGTGINEGGLKVG DGKVVNLNQ  
ADNKGQVQAFSSVNIASGRPTVVLTDERQVNPDTVSWGYRGGTLDVNGNSLTFHQLKAADYGAVLA  
NNVDKRATITLDYALRADKVALNGWSESGKGTAGNLYKYNNPYTNTTDDYFILKQSTYGYFPTDQSSN  
ATWEFVGHSQGDAQKLVAADRFTAGYLFHGQLKGNLNVDNRLPEGVTGALVMDGAADISGTFQTEN  
GRLTLQGHPVIHAYNTQSVADKLAASGDHSLVTQPTSFSQEDWENRSFTFDRLSLKNTDFGLGRNATL  
NTTIQADNSSVTLGDSRVFIDKNDGQGTAFMLEEGTSVATKDADKSVFNGTVNLDNQSVLNINDIFNGG  
IQANNSTVNISSDSAVLGNSTLTSTALNLNKGANALASQSFVSDGPVNISDATLSLNSRPDEVSH TLLPV  
YDYAGSWNLKGDDARLNVGPYSMLSGNINVQDKGTVTLGGEGELSPDLTLQNQMLYSLFNGYRNIW  
SGSLNAPDATVSM TDTQWSMNGNSTAGNMKLNRTIVGFNGGTSPFTTLTTDNLDAVQSAFVMRTDLN  
KADKL VINKSATGHDNSIWVNFLLKKPSNKDTLDIPLVSAPEATADNLFRASTRVVGFS DVTPILSVRKE  
DGKKEWVLDGYQVARNDGQGKAAATFMHISYNNFITEVGS LNKRMGDLRDINGEAGTWVRLLNGSG  
SADGGFTDHYTLLQMGADRKHGELGSMDLFTGVMATYTDTDASADLYSGKTKSWGGGFYASGLFRSG  
AYFDVIAKYIHNNENKYDLNFAGAGKQNFRRSHSLYAGAEVGYRYHLTDTTFVEPQAE LVWGRLQGQTF  
NWNDSGMDVSMRRNSVNPLVGRTGVVSGKTFSGKDWSLTARAGLHYEFDLTDSADVHLKDAAGEH  
QINGRKDSRMLYGVGLNARFGDNTRLGLEVERSAFGKYNTDDAINANIRYSF

*E. coli* Hbp signal peptide

*B. pertussis* Prn (aa35-574)

GSGSG: flexible linker

*E. coli* HbpD

**>Amino acid sequence of HbpD(Δd1)-VacA (aa65-493)**

MNRIYSLRYSAVARGFIAVSEFARKCVHKSVRRLCFPVLLLIPVLFSAGSLAGSSKQAEAEANKTPDKPD  
KVVRIQAGKGFNEFPNKEYDLYKSLSSKIDGGWDWGNAARHYWVKGGQWNKLEVDMKDAVGTY  
KLSGLRNFTGGDLVDNMQKATLRLGQFNNGSFTSYKDSADRTTRVNFNAKNISIDNFVEINNRRVGSGA  
GRKASSTVLTQLASEGITSSKNAEISLYDGATLNLASNSVKLNGNVWMGRLQYVGYLAPSYSTINTS  
KVQGEVDFNHLTVGDQNAQAAGIIASNKTHIGTLDLWQSAGLNIIAPPEGGYKDKPNSTTSQSGTKND  
KKEISQNNNSNTEVINPPNNTQKTETETPTQVIDGPFAGGKDTVVNIFHLNTKADGTIKVGGFKASLTN  
AAHLNIGKGGVNLNQNQASGRITLLVENLTGNITVDGPLRVNNQVGGYALAGSSANFEFKAGVDTKNGT  
ATFNNDISLGGSGSGNDAPVTFRTSEGGALEWSFNSSSTGAGALTQGTTTYAMHGQQGNDLNAGKNLIF  
QGQNGQINLKDSVSQAGSLTFRDNYTVTTSNGSTWTGAGIVVDNGVSVNWQVNGVKGDNLHKIGE  
GTLTVQGTGINEGGLKVGDKVVLNQQADNKGQVQAFSSVNIASGRPTVVLTDERQVNPDTVSWGY  
RGGTLDVNGNSLTFHQLKAADYGAVLANNVDKRATITLDYALRADKVALNGWSESGKGTAGNLYKY  
NNPYTNTTDYFILKQSTYGYFPTDQSSNATWEFVGHSSQGDAQKL VADRFNTAGYLFHGQLKGNLNVD  
NRLPEGVTGALVMDGAADISGFTTQENGRLTLQGHPIHAYNTQSVADKLAASGDHSLVTQPTSFSQE  
DWENRSFTFDRLSLKNTDFGLGRNATLNTTIQADNSSVTLGDSRVFIDKNDGQGTAFMLEEGTSVATKD  
ADKSVFNGTVNLNQSVLNINDIFNGGIQANNSTVNISSDSAVLGNSTLTSTALNLNKGANALASQSFV  
SDGPVNISDATLSLNSRPDEVSHITLLPVYDYAGSWNLKGDDARLNVGPYSMLSGNINVQDKGTVTLG  
GEGELSPDLTLQNQMLYSLFNNGYRNISWGSLSNAPDATVSMTDTQWSMNGNSTAGNMKLNRTIVGFNG  
GTSPFTTLTDDNLDAVQSAFVMRTDLNKAADKL VINKSATGHDNSIWVNFLLKKPSNKDTLDIPLVSAPEA  
TADNLFRASTRVVGFSVDTPILSVRKEDGKKEWVLDGYQVARNDGQGKAAATFMHISYNNFITEVGS  
NKRMGDLRDINGEAGTWVRLNNGSGSADGGFTDHYTLLQMGADRKHELGSMDLFTGVMATYTDTD  
ASADLYSGKTKSWGGGFYASGLFRSGAYFDVIAKYIHNNKYDLNFAGAGKQNFRRSHSLYAGAEEVGY  
RYHLTDTTTFVEPQAEVLVWGRLLQGGTFNWNDSGMDVSMRRNSVNPLVGRGTGVVSGKTFSGKDWSLTA  
RAGLHYEFDLTDSDVHLKDAAGEHQINGRKDSRMLYGVGLNARFGDNTRLGLEVERSAFGKYNTD  
DAINANIRYSF

*E. coli* Hbp signal peptide

*H. pylori* VacA (aa65-493)

GSGSG: flexible linker

*E. coli* HbpD

**>Amino acid sequence of HbpD(840)-PmpD (aa68-629)**

MNRIYSLRYSAVARGFIAVSEFARKCVHKSVRRLCFPVLLLIPVLFSAGSLAGSSDSQAEGQYRLIVGDP  
SSFQEKDADTLPGKVEQSTLFSVTNPVVFQGVDDQDQVSSQGLISSFTSSNLDSPRDGESFLGIAFVGDS  
SKAGITLTDVKASLSGAALYSTEDLIFEKIKGGLEFASSSSLEQGGASAAQSILIHDSQGLQVKHSTTAVN  
AEGSSANDHLGFGGGAFFVTGSLSGEKSLYMPAGDMVVANS DGAISFEGNSANFANGGAIAASGKVL  
VANDKKTSFIENRALS GGAIAASSDIAFQNSAELVFKGNSAIGTEDKGS LGGGAISSD ATLSLNSRPDEV  
SHITLLPVYDYAGSWNLKGDDARLNVGPYSMLSGNINVQDKGTVTLGGEGLSPDLTLQNQMLYSLFN  
GYRNISWGSLSNAPDATVSMTDTQWSMNGNSTAGNMKLNRTIVGFNGGTSPFTTLTDDNLDAVQSAFV  
MRTDLNKAADKL VINKSATGHDNSIWVNFLLKKPSNKDTLDIPLVSAPEATADNLFRASTRVVGFSVDTP  
ILSVRKEDGKKEWVLDGYQVARNDGQGKAAATFMHISYNNFITEVGS LNKRMGDLRDINGEAGTWVR

LLNGSGSADGGFTDHYTLLQMGADRKHELGSMDLFTGVMATYTDTDASADLYSGKTKSWGGGFYAS  
GLFRSGAYFDVIAKYIHNENKYDLNFAGAGKQNFRRSHSLYAGAEVGYRYHLTDTTTFVEPQAELVWGR  
LQGQTFNWNDSGMDVSMRRNSVNPLVGRGTGVVSGKTFSGKDWSLTARAGLHYEFDLTDSADVHLKD  
AAGEHQINGRKDSRMLYGVGLNARFGDNTRLGLEVERSAFGKYNTDDAINANIRYSF

*E. coli* Hbp signal peptide

*C. trachomatis* PmpD (aa68-698)

*E. coli* HbpD(840)

**>Amino acid sequence of HbpD(840)-Prn (aa35-350)**

MNRIYSLRYSAVARGFIAVSEFARKCVHKSVRRLCFPVLLLIPVLFSAGSLAGSSIKVSGRQAQGILLENP  
AAELQFRNGSVTSSGQLSDDGIRRFLLGTVTVKAGKL VADHATLANVGD TWDDDGIALYVAGEQAQAS  
IADSTLQGAGGVQIERGANVTVQRSAIVDGGGLHIGALQSLQPEDLPPSRVVLRDTNVTAVPASGAPAAV  
SVLGASELTLDGGHITGGRAAGVAAMQGA VVHLQRATIRRGDAPAGGAVPGGAVPGGAVPGGFGPG  
GFGPVLDGWYGVVDVSGSSVELAQSI VEAPELGA AIRVGRGARVTVSGGSL SAPHGNVIETGGARRFAP  
QAAPLSITLQAGAHAQ GKALLYRVLPEPVKLTLTG GADAQGDIVATELPSIPGTSIGPLDVALADATLSL  
NSRPDEVSH TLLPVYDYAGSWNLKGDDARLNVGPYSMLSGNINVQDKGT VTLGGEGELSPDLTLQNQ  
MLYSLFNGYRNIWSGSLNAPDATVSM TDTQWSMNGNSTAGNMKLNRTIVGFNGGTSPFTTLTTDNLD  
AVQSAFVMRTDLN KADKLVINKSATGHDNSI WVNFLKKPSNKDTLDIPLVSAPEATADNLFRASTRVV  
GFSDVTPILSVRKEDGKKEWVLDGYQVARNDGQ GKAAATFMHISYNNFITEVGS LNKRMGDLRDING  
EAGTWVRLNNGSGSADGGFTDHYTLLQMGADRKHELGSMDLFTGVMATYTDTDASADLYSGKTKS  
WGGGFYASGLFRSGAYFDVIAKYIHNENKYDLNFAGAGKQNFRRSHSLYAGAEVGYRYHLTDTTTFVEP  
QAELVWGR LQGQTFNWNDSGMDVSMRRNSVNPLVGRGTGVVSGKTFSGKDWSLTARAGLHYEFDLT  
DSADVHLKDAAGEHQINGRKDSRMLYGVGLNARFGDNTRLGLEVERSAFGKYNTDDAINANIRYSF

*E. coli* Hbp signal peptide

*B. pertussis* Prn (aa35-350)

*E. coli* HbpD(840)

**>Amino acid sequence of HbpD(840)-VacA (aa65-377)**

MNRIYSLRYSAVARGFIAVSEFARKCVHKSVRRLCFPVLLLIPVLFSAGSLAGSSKQAE EANKTPDKPD  
KVVRIQAGKGFNEFPNKEYDLYKSLLSSKIDGGWDWGNAARHYWVKGGQWNKLEVDMKDAVGTY  
KLSGLRNFTGGDL DVNMQKATLRLGQFN GNSFTSYKDSADRTTRVNFNAKNISIDNFVEINN RVGSGA  
GRKASSTVLT LQASEGITSSKNAEISLYDGATLNLASNSVKLNGNVWMGRLQYVGAYLAPSYSTINTS  
KVQGEVDNFNHLTVGDQNA AQAGIIASNKTHIGTLDLWQSAGLNIIAPPEGGYKDKPNSTTSQSGTKND  
KKEISQNNNSNTEVINPPNNTQKTET EPTQVIDGPFAGGKDTVVNIFHLNTKADGTIKVGGFKASLTN  
AAHLNIGKGGVNL SNQASGR TLLVEN DATLSLNSRPDEVSH TLLPVYDYAGSWNLKGDDARLNVGPY  
SMLSGNINVQDKGT VTLGGEGELSPDLTLQNQMLYSLFNGYRNIWSGSLNAPDATVSM TDTQWSMNG  
NSTAGNMKLNRTIVGFNGGTSPFTTLTTDNLD AVQSAFVMRTDLN KADKLVINKSATGHDNSI WVNFL  
KKPSNKDTLDIPLVSAPEATADNLFRASTRVVGFS DVTPILSVRKEDGKKEWVLDGYQVARNDGQ GK  
AAATFMHISYNNFITEVGS LNKRMGDLRDINGEAGTWVRLNNGSGSADGGFTDHYTLLQMGADRKHE  
LGSM DLFTGVMATYTDTDASADLYSGKTKSWGGGFYASGLFRSGAYFDVIAKYIHNENKYDLNFAGA  
GKQNFRRSHSLYAGAEVGYRYHLTDTTTFVEPQAELVWGR LQGQTFNWNDSGMDVSMRRNSVNPLVGR  
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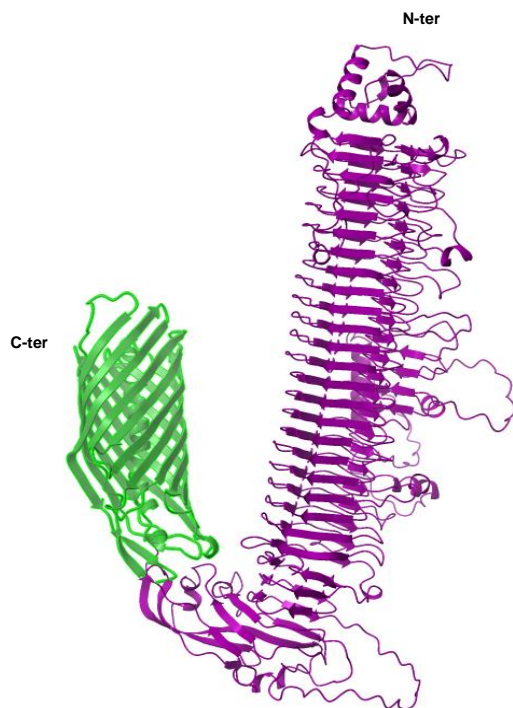
*E. coli* Hbp signal peptide

*H. pylori* VacA (aa65-377)

*E. coli* HbpD(840)

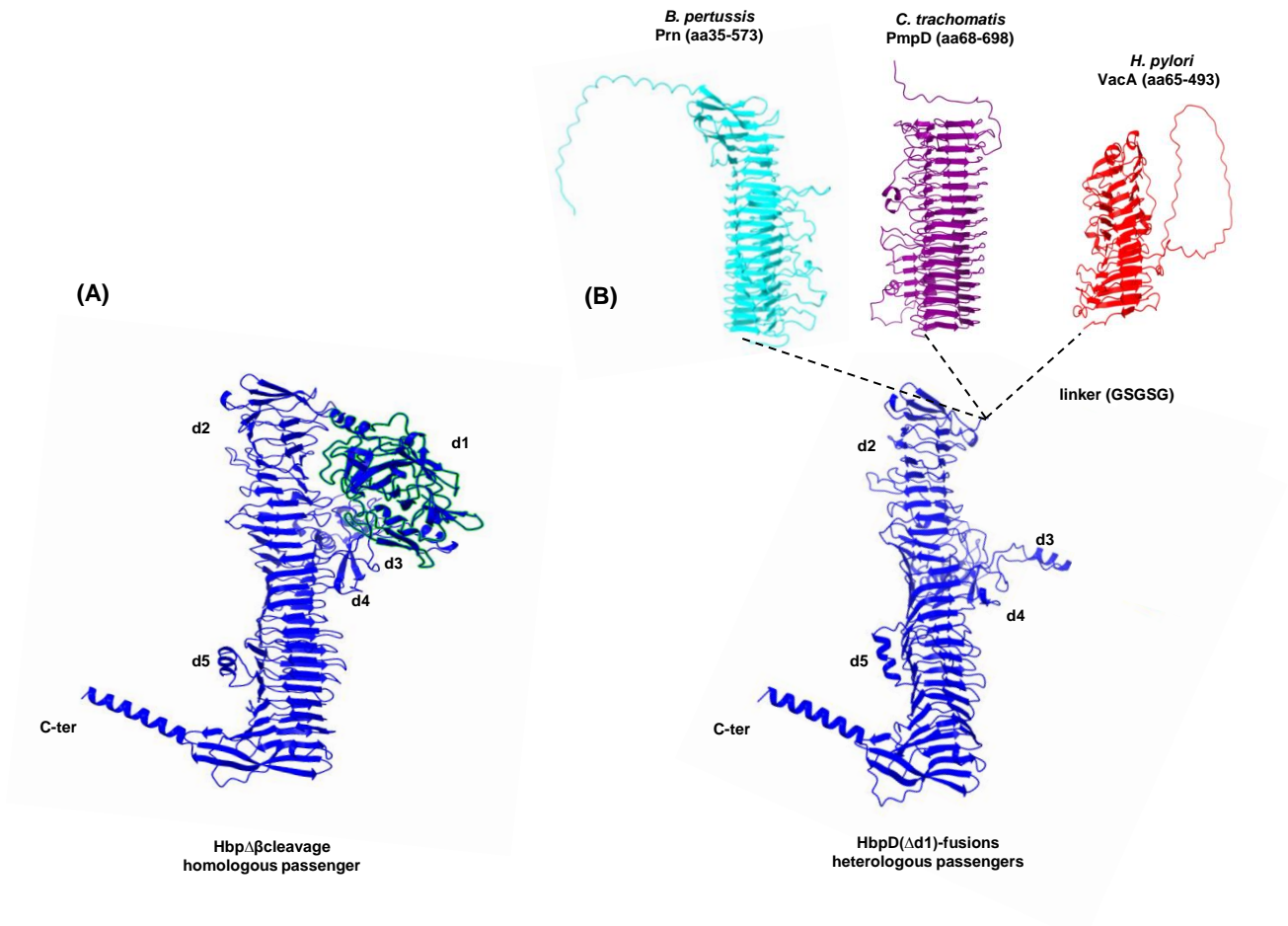
## Supplimentary figures

**Fig. S1**



**Fig. S1:** Structural model of *C. trachomatis* PmpD (FL), serovar L2 (strain 434/Bu/ATCC VR-902B). Cartoon representation for the N-terminal  $\beta$ -helical passenger (purple) and C-terminal  $\beta$ -barrel of PmpD (FL), predicted by AlphaFold2.

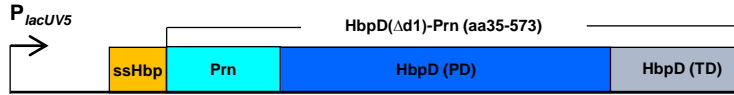
**Fig. S2**



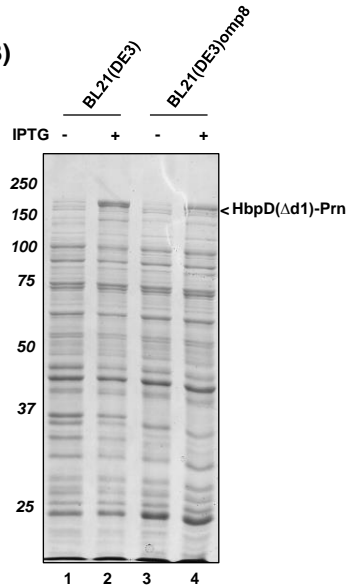
**Fig. S2:** Structural models of HbpΔβcleavage and HbpD(Δd1)-fusion passengers. Cartoon representation of homologous passenger HbpΔβcleavage, containing five side-domains d1-d5 (A) and of heterologous passengers HbpD(Δd1)-PmpD (aa68-698), HbpD(Δd1)-Prn (aa35-573) and HbpD(Δd1)-VacA (aa65-493) (B) predicted by AlphaFold2. In the panel B, the flexible fusion linker (GSGSG) between HbpD(Δd1) and the fused truncated passengers were indicated.

## Fig. S3

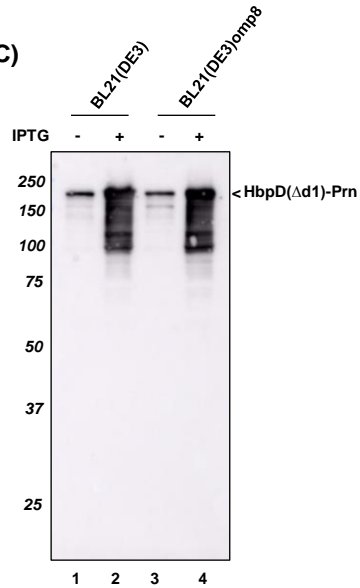
(A)



(B)

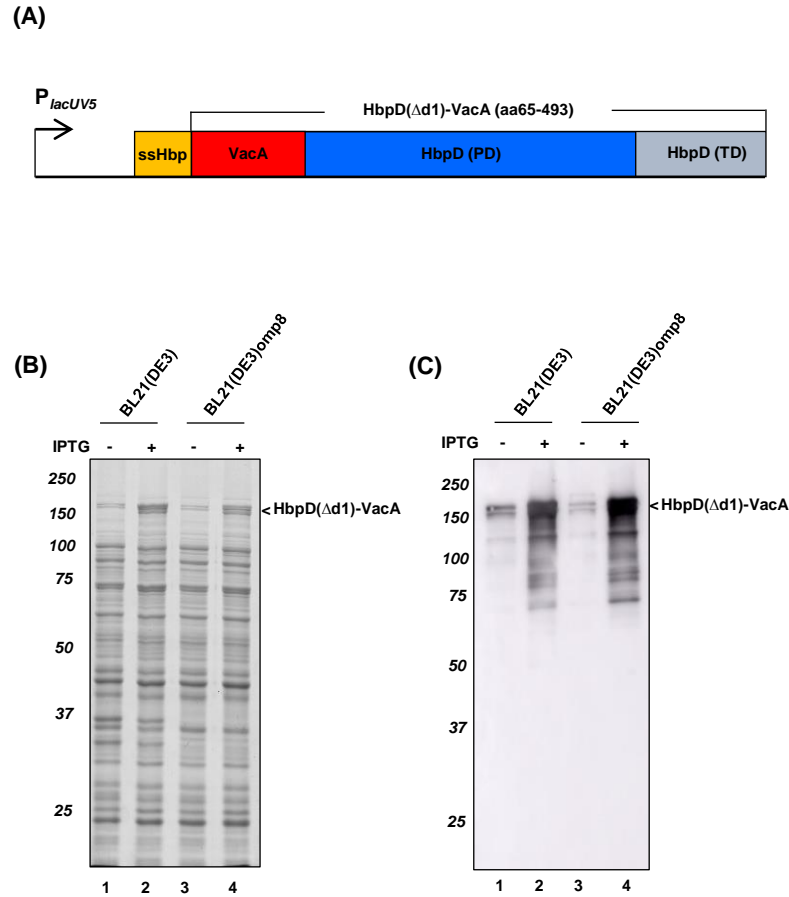


(C)



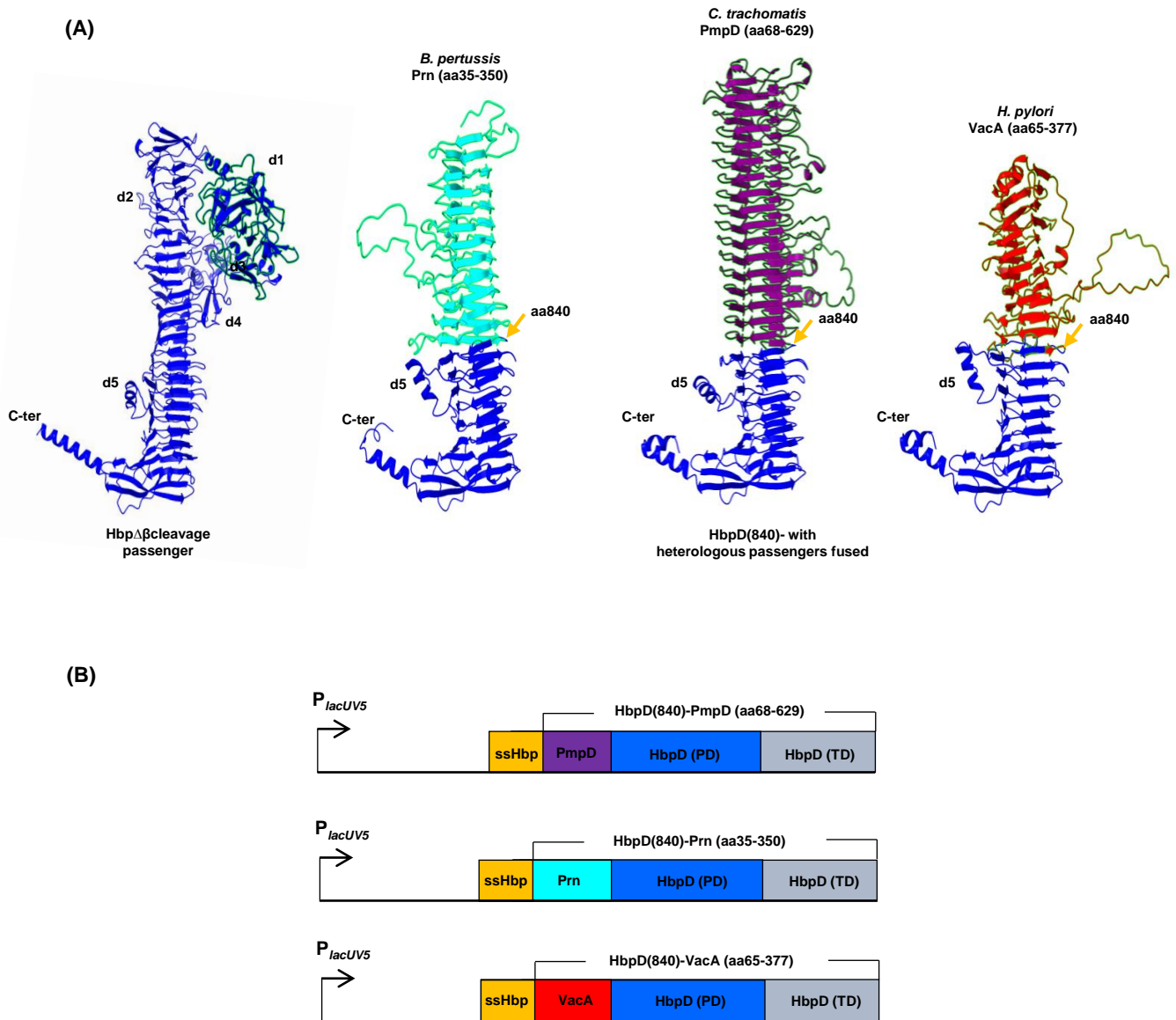
**Fig. S3:** Expression of HbpD(Δd1)-Prn in different *E. coli* strains. (A) Schematic representation of pEH3-HbpD(Δd1)-Prn (aa35-573) construct in which truncated Prn (aa35-573) was replaced for domain 1 (Δd1) in the HbpD(Δd1)-based platform under control of the *lacUV5* promoter and the signal sequence of *E. coli* Hbp (ssHbp) was fused to the fusion of HbpD(Δd1)-Prn at its N-terminus. (B and C) BL21(DE3) and BL21(DE3)omp8 cells were induced for HbpD(Δd1)-Prn expression with (+) or without (-) 0.1 mM IPTG for 2 h and whole cells were analyzed by SDS-PAGE followed by Coomassie staining and immunoblotting using antiserum against Hbp. MW (kDa) markers were indicated at the left side of panels, the identified protein bands were indicated at the right side of panels.

# Fig. S4



**Fig. S4:** Expression of HbpD( $\Delta$ d1)-VacA in different *E. coli* strains. (A) Schematic representation of pEH3-HbpD( $\Delta$ d1)-VacA (aa65-493) construct in which truncated VacA (aa65-493) was replaced for domain 1 ( $\Delta$ d1) in the HbpD( $\Delta$ d1)-based platform under control of the *lacUV5* promoter and the signal sequence of *E. coli* Hbp (ssHbp) was fused to the fusion of HbpD( $\Delta$ d1)-VacA at its N-terminus. (B and C) BL21(DE3) and BL21(DE3)omp8 cells were induced for HbpD( $\Delta$ d1)-VacA expression with (+) or without (-) 0.1 mM IPTG (2 h) and whole cells were analyzed by SDS-PAGE followed by Coomassie staining and immunoblotting using antiserum against Hbp. MW (kDa) markers were indicated at the left side of panels, the identified protein bands were indicated at the right side of panels.

**Fig. S5**



**Fig. S5:** Structural models and constructions of heterologous HbpD(840)-fusions. **(A)** Cartoon representation of conformations predicted by Alfafold 2 of HbpΔβcleavage, containing a passenger with five side-domains d1-d5 and of three expressed versions of HbpD(840) constructs with heterologous passenger fragments fused: HbpD(840)-PmpD (aa68-629), HbpD(840)-Prn (aa35-350) and HbpD(840)-VacA (aa65-377). The fused partner was linked at the position of residue 840 (arrow) in the Hbp passenger. **(B)** Schematic representation of the constructs in pEH3-HbpD(840)-PmpD (aa68-629), pEH3-HbpD(840)-Prn (aa35-350) and pEH3-HbpD(840)-VacA (aa65-377). Sequences encoding truncated passenger fragments of PmpD (aa68-629), Prn (aa35-350) and VacA (aa65-377) were included in between those encoding the Hbp signal sequence (ssHbp) and HbpD(840) and resulted in passengers as modelled in (A).