

Table S1. Strains used in this study. Natural *N. meningitidis* serogroup B strains used in Figure 1 and their main characteristics are indicated. For each of them a *nhba* deletion mutant was generated. * Unassigned. ° Kan, kanamycin, Ery, erythromycin, Clm, chloramphenicol.

Natural Strains	Description	Clonal Complex	CREN	NHBA Peptide Variant	NHBA Isoform
M11205	Clinical isolate, USA 2003	41/44	-	p0001	Long
M11822	Clinical isolate, USA 2004	41/44	-	p0001	Long
NGH38	Clinical isolate, Norway 1988	ua *	-	p0002	Long
MC58	Laboratory-adapted reference strain, UK 1985	32	+	p0003	Long
M10935	Clinical isolate, USA 2003	35	-	p0058	Long
M14933	Clinical isolate, USA 2006	32	+	p0003	Long
M10713	Clinical isolate, USA 2003	41/44	-	p0010	Short
M03279	Clinical isolate, USA 1997	41/44	-	p0011	Short
N16/97	Clinical isolate, Norway 2007	41/44	-	p0029	Long
M11204	Clinical isolate, USA 2003	41/44	-	p0029	Long
M10282	Clinical isolate, USA 2003	41/44	-	p0029	Long
M07-0240679	Clinical isolate, UK 2007	269	-	p0017	Short
M11719	Clinical isolate, USA 2003	162	-	p0020	Short
M16452	Clinical isolate, USA 2007	41/44	-	p0144	Short
M18070	Clinical isolate, USA 2008	162	-	p0020	Short
8047	Clinical isolate, USA 1978	11	-	p0020	Short
Engineered Strains		Description			
<i>C_nhba</i>		Complemented <i>nhba</i> mutant of MC58; EryR and KanR ° [39]			
<i>Ptac_nhba</i>		Complemented mutant of MC58 expressing <i>nhba</i> (MC58 sequence) under the control of an IPTG-inducible <i>Ptac</i> promoter; EryR and ClmR ° [39]			

Pwt_ <i>mCherry</i>	Complemented mutant of MC58 expressing <i>mCherry</i> reporter gene under the control of <i>nhba</i> intergenic region (MC58 sequence); EryR and ClmR
Ptac_ <i>mCherry</i>	Complemented mutant of MC58 expressing <i>mCherry</i> reporter gene under the control of an IPTG-inducible Ptac promoter; EryR and ClmR

Table S2. Oligonucleotides used in this study.

Plasmid	Description	Application
NMB2132RT-F	GGCTTGTATTTTGGCCCTTC	qRT-PCR
NMB2132RT-R	GCATCTTCCTTGCCTCTGT	
mCherry_RT1 fw	GTCAGTTTCAGGTAGTCCG	qRT-PCR
mCherry_RT1 REV	AGCCCTCAATTCATGTACGG	
pRTNM16sII.F1	GTGGGGAATTTTGGACAATG	qRT-PCR
pRTNM16sII.R1	CAACAGCCTTTTCTTCCCTG	
mCherry Fw	TCCAAAGGCGAGGAAGATAAC	Generation of Pwt_mCherry fusion: insert PCR to amplify the <i>mCherry</i> gene codon-optimized for <i>N. meningitidis</i>
mCherry Rev	CACGGATCCCTGCAGTTATTTGTAC AGTTCGTCCATG	
CmR Fw	CTGCAGGGATCCGTGATATAG	Generation of Pwt_mCherry fusion: vector PCR on pBS_ <i>nhba</i> background
Pwt_14aa Rev	CTTCCTCGCCTTTGGAGGCAAAAAT ACAAGCCATTGC	
Insert Fw	CGAAAGTGGGAATCTAGACATTATC GGCGTGATTGAG	Subcloning of Pwt_mCherry fusion into pComCImRP _{int} plasmid
Insert Rev	GAGCATAAAATTTTAGTAACCTATG TTTTTATTCAGCAAGTCTTGTAATTC	
Vector Fw	GAATTACAAGACTTGCTGAATAAAA ACATAGGTTACTAAAATTTTATGCTC	Subcloning of Pwt_mCherry fusion into pComCImRP _{int} plasmid
Vector Rev	CTGAATCACGCCGATAATGTCTAGA TTCCCACTTTCG	
NmmCh_NdeI Fw	GAAACACATATGATGGTGCCAAAG GCGAG	Cloning <i>mCherry</i> gene into pCOM_Ptac plasmid
NmmCh_NsiI Rev	GTGTCAATGCATTTATTTGTACAGTT CGTCCATGCC	

Table S3. Plasmid used in this study.

Plasmid	Description	Antibiotic Resistance	Reference or Source
pBluescript (pBS)	Cloning vector	Amp	Stratagene
pBS-UDgna2132	Plasmid for the deletion of the <i>nhba</i> gene by homologous recombination	Amp, Ery	[39]
pBS_ <i>nhba</i>	pBS derivative for the <i>in-locus</i> complementation of the <i>nhba</i> gene (MC58 sequence)	Amp, Clm	[37]
pComClmR-Pind	Plasmid for the allelic replacement at chromosomal location between ORFs <i>NMB1428</i> and <i>NMB1429</i> and inducible expression under control of Ptac promoter and lacI repressor	Amp, Clm	[54]
pCOM-C <i>nhba</i>	Plasmid for the <i>ex-locus</i> complementation of the <i>nhba</i> null mutant. Derivative of pComClmR-Pind containing a copy of <i>nhba</i> (MC58 sequence) under control of the <i>nhba</i> intergenic region.	Amp, Kan	[39]
pCOM-Ptac_ <i>nhba</i>	Plasmid for the <i>ex-locus</i> complementation of the <i>nhba</i> null mutant. Derivative of pComClmR-Pind containing a copy of <i>nhba</i> (MC58 sequence) under control of Ptac promoter and lacI repressor.	Amp, Clm	[39]
pBS_14aa_ <i>mCherry</i>	Derivative of pBS_ <i>nhba</i> . Construct for the <i>in-locus</i> complementation of the <i>mCherry</i> reporter gene under the control of the <i>nhba</i> intergenic region	Amp, Clm	This study
pCOM_14aa_ <i>mCherry</i>	Construct for the <i>ex-locus</i> complementation of the <i>mCherry</i> reporter gene under the control of the <i>nhba</i> intergenic region. Derivative of pComClmR-Pind	Amp, Clm	This study
pCOM_Ptac_ <i>mCherry</i>	Construct for complementation of the <i>mCherry</i> reporter gene under the control of an IPTG-inducible Ptac promoter. Derivative of pComClmR-Pind	Amp, Clm	This study

References

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