

SUPPLEMENTARY INFORMATION

SUPPLEMENTARY METHODS

Preparation of the cells for transmission electron microscopy - Cells grown on coverslips were fixed for 45 minutes with 2.5% glutaraldehyde (50 mM cacodylate pH 7.2, 50 mM KCl, 2.5 mM MgCl₂) at room temperature, then fixed for 2 hours at 4°C with 2% OsO₄ buffered with 50 mM cacodylate (pH 7.2), washed with H₂O and incubated overnight at 4°C with 0.5% uranyl acetate (in H₂O). The cells were dehydrated and embedded in Epon812. Sections stained with uranyl acetate and lead citrate were analyzed with a Zeiss EM10 or EM900 electron microscope (Zeiss, Oberkochen, Germany). Negatives were digitalized by scanning.

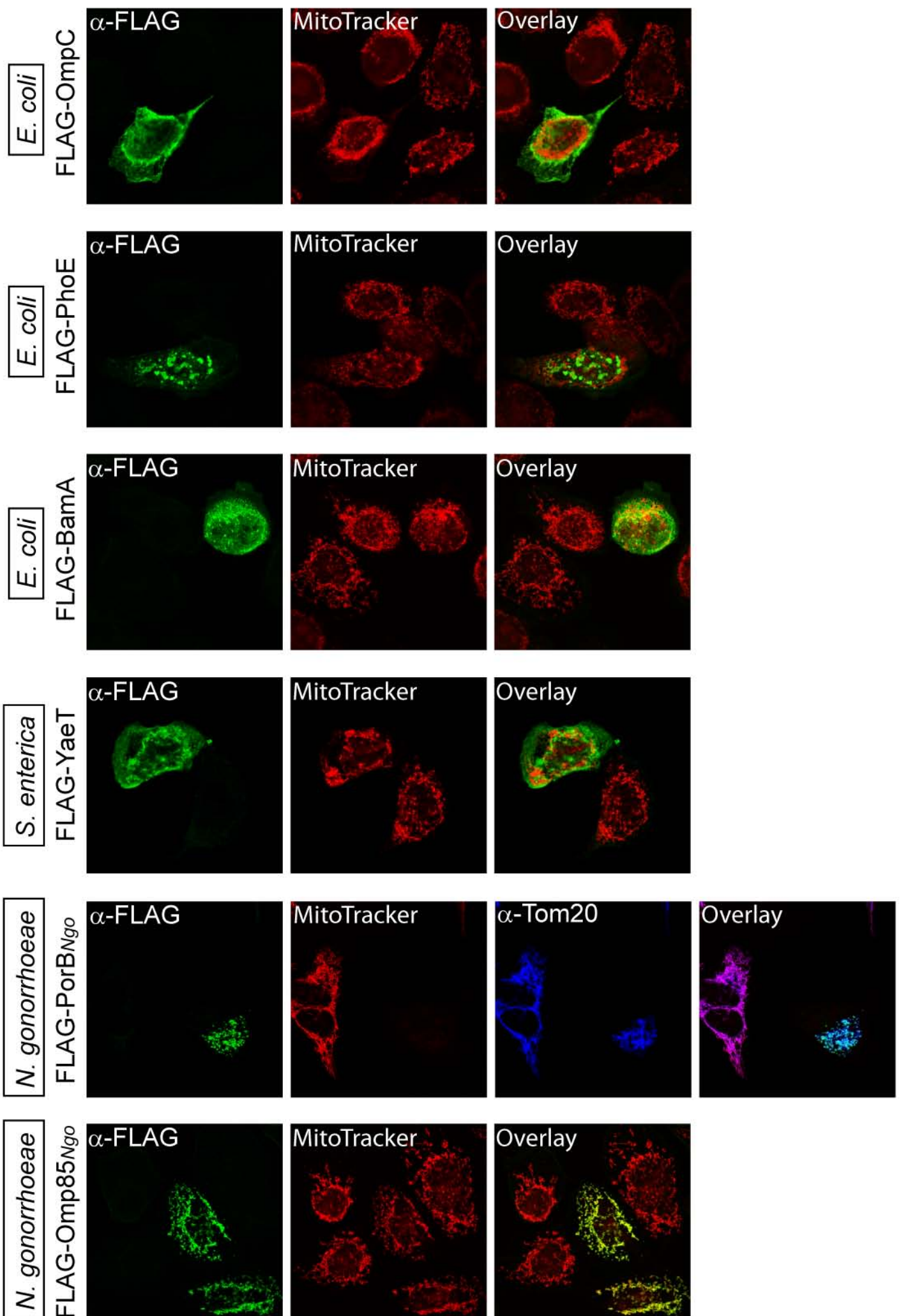
SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Only β -barrel proteins from *Neisseria gonorrhoea* are colocalizing exclusively with mitochondria. Amino-terminally FLAG-tagged β -barrel proteins from enterobacteria *Escherichia coli* (OmpC, PhoE, BamA) and *Salmonella enterica* (YaeT) or from *Neisseria gonorrhoea* (PorB, Omp85) were overexpressed for 24-36 h in HeLa cells grown on cover slips. Cells were then stained with mitochondrial membrane potential-sensitive dye MitoTracker (red), fixed and analyzed by immunofluorescence with antibodies against FLAG-tag (green) and mitochondrial protein Tom20 (blue) and confocal microscopy.

Fig. S2. Presence of Omp85_{NgO} in mitochondria does not affect their function or morphology. Cells from a cell line that, upon induction by doxycyclin (Dox) overexpresses Omp85 from *Neisseria gonorrhoea* with an amino-terminal Myc-tag (*myc-Omp85*_{NgO}) were analyzed by immunofluorescence (upper part) or transmission electron microscopy (lower part, EM). For immunofluorescence, cells were decorated with antibodies directed against c-myc (green) or mitochondrial protein Tom20 (red).

Fig. S3. Deletion of the β -sorting signal from Omp85_{NgO} affects its mitochondrial localization. FLAG-Omp85_{NgO}-12, an amino-terminally FLAG-tagged construct of Omp85_{NgO} lacking the last 12 amino acids was overexpressed in HeLa cells. Cells were stained with MitoTracker (red) and antibodies against the FLAG-tag (green) and mitochondrial protein Tom20 (blue).

Fig. S4. Sequence alignment of PorB proteins from different *Neisseria* sp. used in this study. Sequences were aligned using ClustalW2 program.



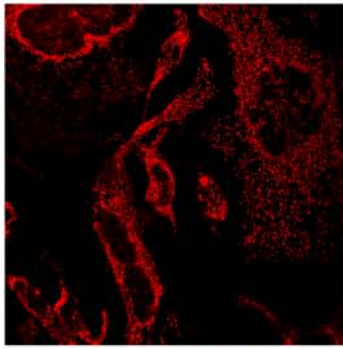
Supplementary Figure 1

myc-Omp85_{Ngo} -Dox

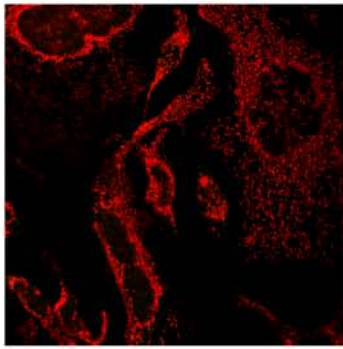
α -c-myc



α -Tom20

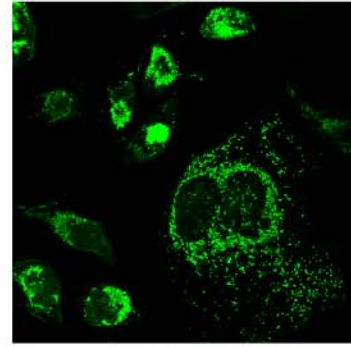


Overlay

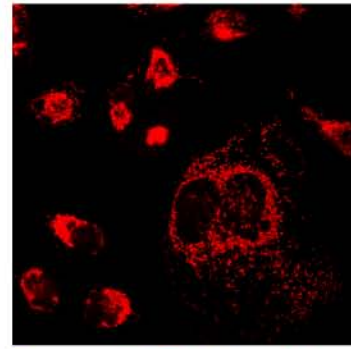


myc-Omp85_{Ngo} +Dox

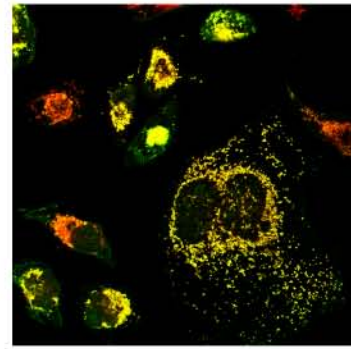
α -c-myc



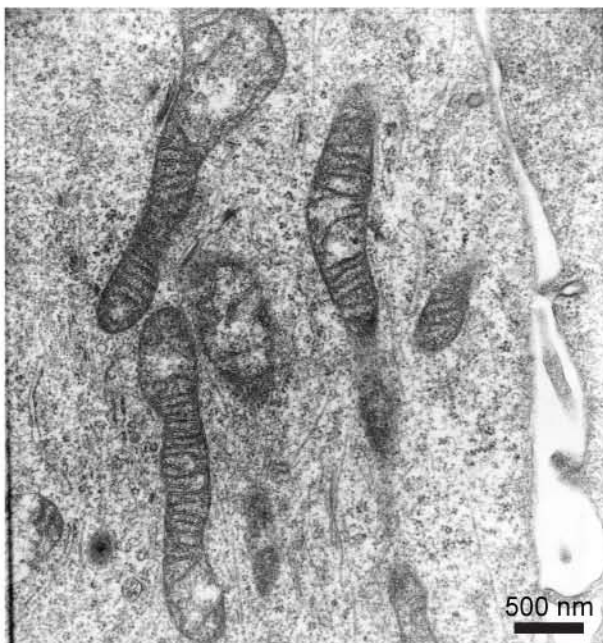
α -Tom20



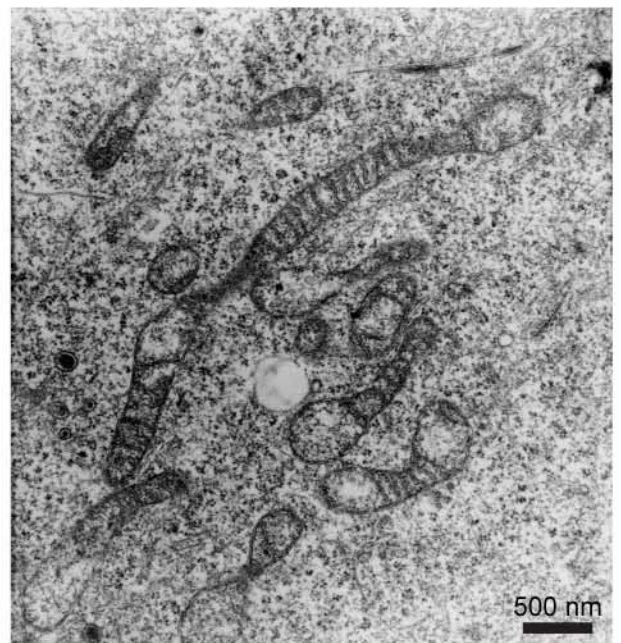
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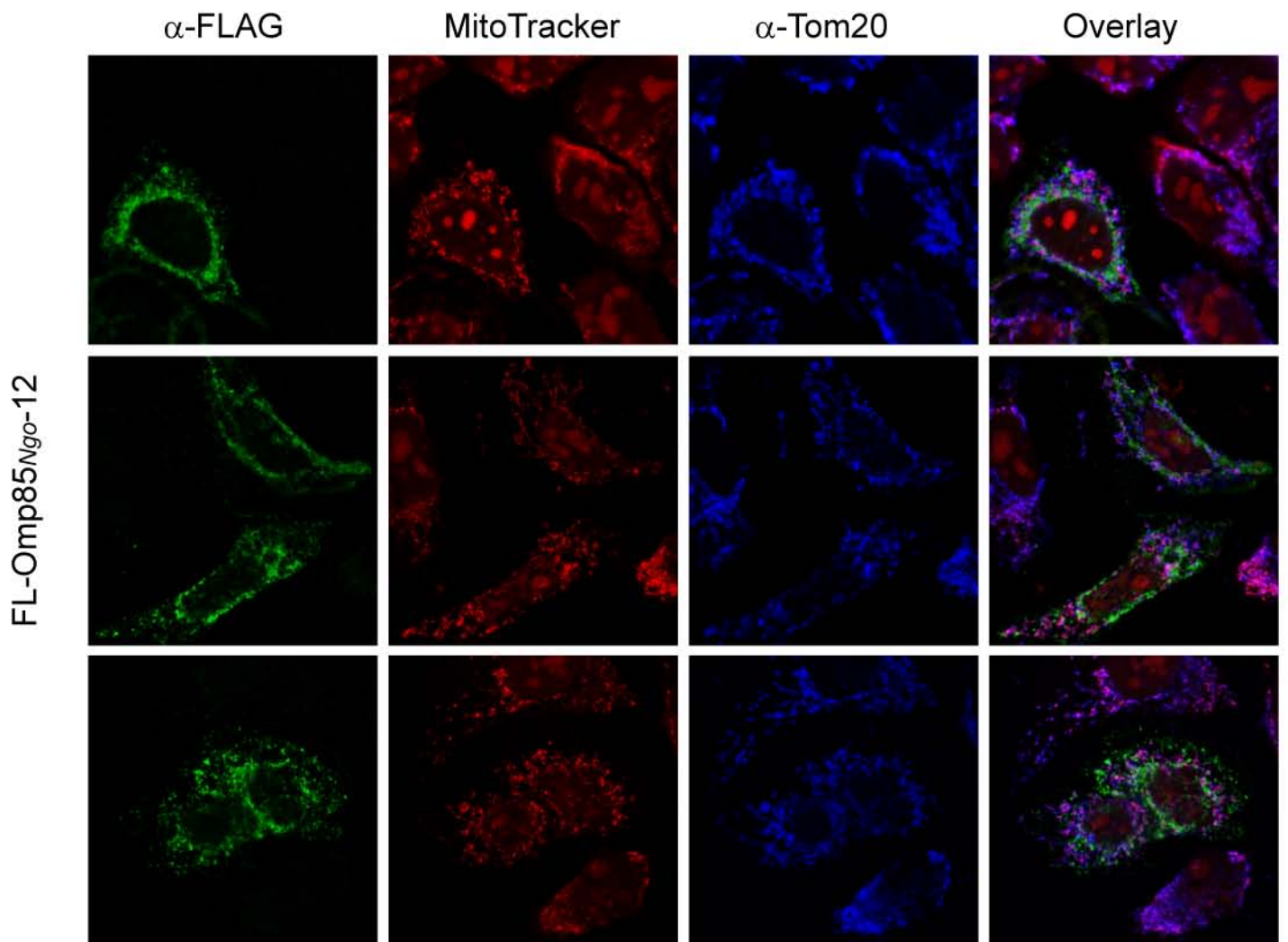
EM



EM



Supplementary Figure 2



Supplementary Figure 3

PorB *N. gonorrhoeae* DVTLYGTIKAGVETSRVSAHHGA-----QADRVKTATEIADLGSKIGFKGQEDLGNGLKA 55
 PorB *N. lactamica* DVTLYGTIKAGVETVRYTVKHTDG-----KVTEVKTGSEIADFGSKIGFKGQEDLGNGLKA 55
 PorB *N. sicca* DVTLYGQIKAGVEVSKVNLGKKTATALGKEKSSKTATEIADLGSRVGFKGHEHLGSNLNA 60
 PorB *N. cinerea* DVTLYGQVKAGVEISKVKVGKAT-----SKTATEIADYGSRIGFKGHEHLGSNLNA 51
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PorB *N. gonorrhoeae* IWQLEQKAYVSGTDTGWGNRQSFIFGLKGGFGKVRVGRLLNLIKDTGG-FNPWE-GKS--Y 111
 PorB *N. lactamica* IWQLEQNASIAGTDSGWGNKQSFIFGLKGGFGTVRAGNLNSILKSTGDNVNAWESGKATED 115
 PorB *N. sicca* IWQVEQKTSIAGGDKEWASRESFIGLEGGFGKVRAGKLNSTVKDSSDNVDQWES---NNG 117
 PorB *N. cinerea* IWQVEQNTSIAGGDSGFANRESFIGLEGGFGKVRAGNLNTALKDSSDSFDPWESGAANAD 111
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PorB *N. gonorrhoeae* YSGLSNIAQPEERHVSRYDSPEFAGFSGSVQYVPNDNSGKNR-----SESYHAGFN 163
 PorB *N. lactamica* VLQVSKIGAPEHRYASVRYDSPEFAGFSGSVQYAPKDNNGAN-----GESYHVGLN 166
 PorB *N. sicca* ALGLSVFTRVDKRAVSRYDSPVFAGFSASVQYTPRDNATPGDKYTHEVPSQDTYYAGLN 177
 PorB *N. cinerea* ALQLGKIKRVDTRKVSRYDTPVFGGFSASVQYQPRDNANPGDKYTHTVKSRESYDLGLN 171
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PorB *N. gonorrhoeae* YKNSGFFVQYAGSYKR-----HNYTT-----EKHQVHRLVGGYDHDALYASV 205
 PorB *N. lactamica* YQNSGFFAQYAGLQFQ-----HGEGTKATVGEPEVKLQVHRLVGGYDNDALYASV 216
 PorB *N. sicca* YENSGFFGQYAGGFRK--NLKTG--KDasGKDTYEN---KDGQVHRLVAGYDANNLFVSV 230
 PorB *N. cinerea* YENSGFFGRYAGSYAKRADLDAGYLDAFNGNTTLAAGAYKDHQAHRLTAGYDANNLMVAV 231
 *:***** :*** : : . :. * .***.*** : * .*:

PorB *N. gonorrhoeae* AVQQQD-----AKLTWRDDNSHN-----SQTEVA 229
 PorB *N. lactamica* AVQQQD-----AKLAAAP-NSHN-----SQTEVA 239
 PorB *N. sicca* AGQYAKNWETLGDYAQAQSNVVDAAHMAYPAGKTKQLNTVFGTDADLNNKGVETVEVA 290
 PorB *N. cinerea* VGQYEG---FKADVAGAKKN-----ERTEVG 254
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PorB *N. gonorrhoeae* TTVAAYRFGNVTPRVSYAHGFKGSVYDAD-HDNTYDQVVVGAEYDFSKRTSALVSAGWLQK 288
 PorB *N. lactamica* ATVAAYRFGNVTPRVSYAHGFKGTVAKAD-GDNRYDQVVVGAEYDFSKRTSALVSAGWLQE 298
 PorB *N. sicca* ATAAAYRAGNVTPRVSYAHGFKAKVDGEKLGKGTQYDQVIVGADYDFSKRTTALVSTGWLRG 350
 PorB *N. cinerea* ATAAAYRFGNVTPRVSYAHGFKAKEDGVKQNSAYNQVIVGADYDFSKRTSALLSAGWLKE 314
 :*.*** *****. . . . **.:*****:*****:***:***:

PorB *N. gonorrhoeae* GK-GAEKFVATVGGVGLRHKF 308
 PorB *N. lactamica* GK-GAGKTVSTASTVGLRHKF 318
 PorB *N. sicca* AESGSHKVETISGLVGLRHKF 371
 PorB *N. cinerea* GK-GDYKYEKTAGTVGLRHKF 334
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Supplementary Figure 4