Supporting Information

Neisseria meningitidis type IV pili trigger Ca^{2+}-dependent lysosomal trafficking of the acid sphingomyelinase to enhance surface ceramide levels

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Supporting Information:

- Supplementary Material and Methods (related to mutagenesis and Figure S3)
- Supplementary Table 1
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Material and Methods

Construction of pilus mutant strains in *N. meningitidis*

For generation of the different isogenic pilus mutants in *N. meningitidis* strain 8013/clone 12, a fragment of the gene of interest was amplified from genomic DNA with restriction site containing oligonucleotides (see Table S2 for oligonucleotide details). To generate an isogenic non piliated derivative of 8013/clone12 the *pilE* gene was amplified using olidonucleotides *pilE_Xho*I_F and *pilE_Xba*I_R, cleaved with *Xho*I and *Xba*I, respectively, and cloned into the pTL1 vector, a derivative of the pBluescript vector containing the *Neisseria* uptake sequence (1). The pTL1 plasmid, harboring the *pilE* gene fragment was then transformed into TOP 10 chemically competent *E. coli* cells. After confirmation of positive clones by nucleotide sequencing, an inverse PCR was performed using olidonucleotides *pilE*_inv_F and *pilE*_inv_R harboring an *Avr*II restriction site, the resulting construct was cutted with *Avr*II and a spectinomycin-resistance cassette was inserted via the AvrII sites by replacing about 400 bp of the coding sequence. The resultant construct was amplified in *E. coli* TOP10 before transformation of the plasmid into *N. meningitidis* strain 8013/clone 12. The other isogenic pilus mutants used in this study were generated using the same strategy and appropriate oligonucleotides (Tab. S2). All resulting mutant strains were confirmed by PCR, sequencing, and Western blot analysis.

Western blotting

Bacterial pilus preparations (500ng) were loaded on 12 % sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was carried out for approximately 1 h at 150 V. The gel was then blotted to a nitrocellulose membrane (1 h / 350mA), blocked for 1 h on an orbital shaker with 5 % skim milk in PBST and incubated O/N with the mouse-anti PilE (SM1) (2) antibody (1:4000 in skim milk) at 4°C on an orbital shaker. On the next day, the membrane was washed 3 times with PBST for 10 min. and after incubated with an anti-mouse HRP second antibody for 1 h at room

temperature on an orbital shaker. After washing 3 times with PBST for 10 min. the membrane was incubated with the Clarity Western ECL Substrate (BioRad) for 1 minutes. Protein bands were then visualized with the ChemiDoc.

References

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