Supplementary Information

Controlling septum thickness by a large protein ring

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Supplementary Table 1: Amino acid sequence identity (%) of full length SepF homologues.

	B. subtilis	*B. cereus*	*B. megaterium*	*S. pneumoniae*	*C. perfringens*	*M. tuberculosis*
B. subtilis		56	66	27	34	33
B. cereus			52	29	33	31
B. megaterium				31	42	28
S. pneumoniae					30	30
C. perfringens						29
M. tuberculosis						

Supplementary Table 2: Amino acid sequence identity (%) of the respective core domains of SepF homologues.

	B. subtilis	*B. cereus*	*B. megaterium*	*S. pneumoniae*	*C. perfringens*	*M. tuberculosis*
B. subtilis		76	87	34	40	34
B. cereus			72	35	35	35
B. megaterium				35	43	36
S. pneumoniae					34	30
C. perfringens						34
M. tuberculosis						

Supplementary Table 3: Strains used in this study.

strain name	genotype	references
B. subtilis 168	*trpC2*	(1)
B. subtilis TB92	*trpC2 sepF::spc* (YK204 in 168CA)	(2)
B. subtilis BFA2863	*trpC2 sepF::ery*	(4)
B. subtilis GYQ457	*trpC2 aprE::kan Pspac-sepF(subtilis-cereus)*	this study
B. subtilis GYQ475	*trpC2 aprE::kan Pspac-sepF(subtilis-pneumoniae)*	this study
B. subtilis GYQ544	*trpC2 aprE::kan Pspac-sepF(subtilis-perfringens)*	this study
B. subtilis GYQ474	*trpC2 aprE::kan Pspac-sepF(subtilis-tuberculosis)*	this study
B. subtilis MW18	*trpC2 aprE::kan Pspac-sepF(subtilis) sepF::spc*	this study
B. subtilis GYQ458	*trpC2 aprE::kan Pspac-sepF(subtilis-cereus) sepF::ery*	this study
B. subtilis GYQ759	*trpC2 aprE::kan Pspac-sepF(subtilis-megaterium) sepF::ery*	this study
B. subtilis MW89	*trpC2 aprE::kan Pspac-sepF(subtilis-pneumoniae) sepF::spc*	this study
B. subtilis MW92	*trpC2 aprE::kan Pspac-sepF(subtilis-perfringens) sepF::spc*	this study
B. subtilis MW86	*trpC2 aprE::kan Pspac-sepF(subtilis-tuberculosis) sepF::spc*	this study
E. coli BL21(DE03) pNC2	*pMalC2-sepF(cereus)*	this study
E. coli BL21(DE03) pNC4	*pMalC2-sepF(tuberculosis)*	this study
E. coli BL21(DE03) pNC7	*pMalC2-sepF(perfringens)*	this study
E. coli BL21(DE03) pNC8	*pMalC2-sepF(megaterium)*	this study
E. coli BL21(DE03) pNC9	*pMalC2-sepF(pneumoniae)*	this study
E. coli BL21(DE03) pNC12	*pMalC2-sepF(subtilis)*	(5)
E. coli BL21(DE03) pYQ62	*pMalC2-sepF(subtilis-cereus)*	this study
E. coli BL21(DE03) pYQ94	*pMalC2-sepF(subtilis-tuberculosis)*	this study
E. coli BL21(DE03) pYQ95	*pMalC2-sepF(subtilis-perfringens)*	this study
E. coli BL21(DE03) pYQ96	*pMalC2-sepF(subtilis-pneumoniae)*	this study
E. coli BL21(DE03) pYQ176	*pMalC2-sepF(subtilis-megaterium)*	this study
B. cereus ATCC14579		(6)
B. megaterium DSM509		(7)
S. pneumoniae ATCC6305		(8)
C. perfringens ATCC13124		(9)

Supplementary Table 4: Plasmids used in this study.

plasmid	genotype	reference
pMW8	*pAPNC-213-kan-Pspac-sepF(WT)*	this study
pYQ90	*pAPNC-213-kan-Pspac-sepF(subtilis-cereus)*	this study
pYQ97	*pAPNC-213-kan-Pspac-sepF(subtilis-tuberculosis)*	this study
pYQ98	*pAPNC-213-kan-Pspac-sepF(subtilis-perfringens)*	this study
pYQ99	*pAPNC-213-kan-Pspac-sepF(subtilis-pneumoniae)*	this study
pYQ177	*pAPNC-213-kan-Pspac-sepF(subtilis-megaterium)*	this study
pNC1	*pMalC2-sepF(coelicolor3)*	this study
pNC2	*pMalC2-sepF(cereus)*	this study
pNC4	*pMalC2-sepF(tuberculosis)*	this study
pNC5	*pMalC2sepF(coelicolor2)*	this study
pNC6	*pMalC2-sepF(coelicolor1)*	this study
pNC7	*pMalC2-sepF(perfringens)*	this study
pNC8	*pMalC2-sepF(megaterium)*	this study
pNC9	*pMalC2-sepF(pneumoniae)*	this study
pNC12	*pMalC2-sepF(subtilis)*	(5)
pYQ62	*pMalC2-sepF(subtilis-cereus)*	this study
pYQ94	*pMalC2-sepF(subtilis-tuberculosis)*	this study
pYQ95	*pMalC2-sepF(subtilis-perfringens)*	this study
pYQ96	*pMalC2-sepF(subtilis-pneumoniae)*	this study
pYG176	*pMalC2-sepF(subtilis-megaterium)*	this study

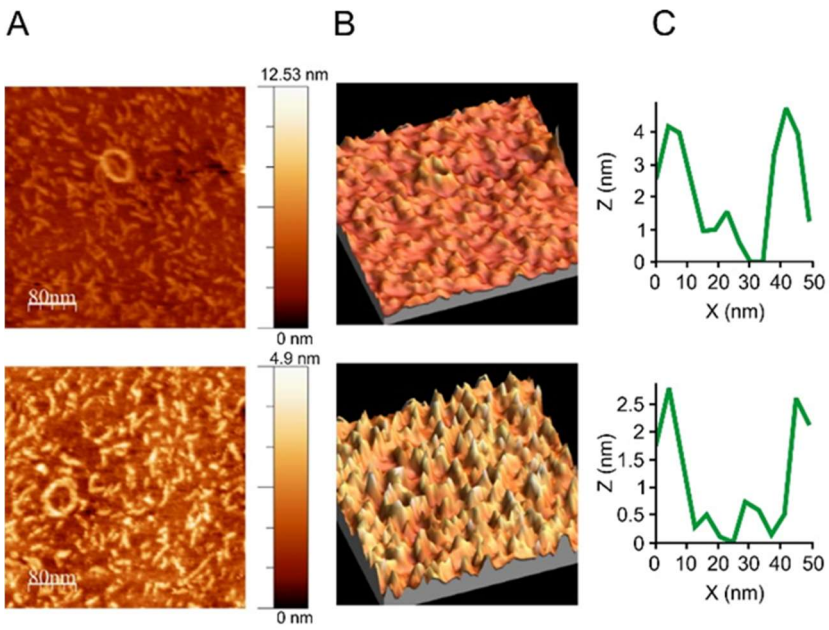
Supplementary Table 5: Primers used in this study.

primer	description	sequence	used for
MW135	pAPNC-213-kan-GA-for	GGCGTTAGCCCAAGCGC	pMW8
MW139	pAPNC-213-kan-GA-rev	ACACCCCCTGTTTCATTTCCCTAGCAGGTCAATTGTGAGCGC	pMW8
MW140	*sepF*-WT-GA-for	GGAAATGAAACAGGGGGTGTACAGCAATGAGTATGAAAAATAAACTGAAAAACTTTTTCTCAATGGAAGATGAAG	pMW8
MW141	*sepF*-WT-GA-rev	GCGCTTGGGCTAACGCCTTACCACCTCTGATGTTCGTCTTCAGATATG	pMW8
eg122	*sepF-cereus*-for	GACGAATTCATGAGTTGGTCAAAAG	pNC2
eg123	*sepF-cereus*-rev	GACTCTAGATTACCACCTCTTTAT	pNC2
inc15	*sepF-pneumoniae*-for	GTAGGAGCCCGGGATGTCTTTAAAAGATAG	pNC9
inc16	*sepF-pneumoniae*-rev	GCTAGACTCTAGATTATCGTACTCTATTTC	pNC9
inc9	*sepF-perfringens*-for	GATTAGCCCGGGATGTGTATGTCAAAAG	pNC7
inc10	*sepF-perfringens*-rev	GACTATCTAGATTATTTTGAAGCCCAGTTG	pNC7
inc7	*sepF-tuberculosis*-for	GGTTACGGAATTCGTGAATAGTCACTGTAG	pNC4
inc8	*sepF-tuberculosis*-rev	GGCGACCGTCTAGACTATTGGTAGGCGTAG	pNC4
inc17	*sepF-coelicolor* 1-for	GTGAGAGGAGGAATTCATGGGATCGGTAC	pNC6
inc18	*sepF-coelicolor* 1-rev	TGTGCGGCTCTAGATCAGCTCTGGTTGAAG	pNC6
inc19	*sepF-coelicolor* 2-for	GAGGACTCCCGGGATGGCCGGCGCGATG	pNC5
inc20	*sepF-coelicolor* 2-rev	ACCGGTAGTCTAGATCAGCTCTGGTTGAAG	pNC5
eg139	*sepF-coelicolor* 3-for	GACGAATTCGTGAAATCGGGGGAGC	pNC1
eg140	*sepF-coelicolor* 3-rev	GACTCTAGATCACACTCCCGGCAC	pNC1
YQ276	*sepF-cereus*-for	AATCCTCTAAAGTGGTGTTGTTAGAACCACGCACATATTCGGA	pYQ62, 90
YQ277	*sepF-cereus*-rev	CGCACCAACAATATCTACATTTTC	pYQ62, 90
YQ278	pMalC2-for	ATGTAGATATTGTTGGTGCGATTTCTGAGCTCATATCTGAAGAC	pYQ62, 90
YQ279	pMalC2-rev	CAACACCACTTTAGAGGATTTCTG	pYQ62, 76, 77, 90
YQ349	pMalC2-rev	TTTCTGAACACTTTGCAAGCTCAC	pYQ94-99
YQ350	*sepF-tuberculosis*-for	GAGCTCATATCTGAAGACGAACATC	pYQ94, 97
YQ351	pMalC2-for	GCTTGCAAAGTGTTCAGAAACTCTCGAAGATCACCACGCT	pYQ94-99
YQ352	*sepF-tuberculosis*-rev	TCGTCTTCAGATATGAGCTCGCGCTCCTCGGGGGACACATC	pYQ94, 97

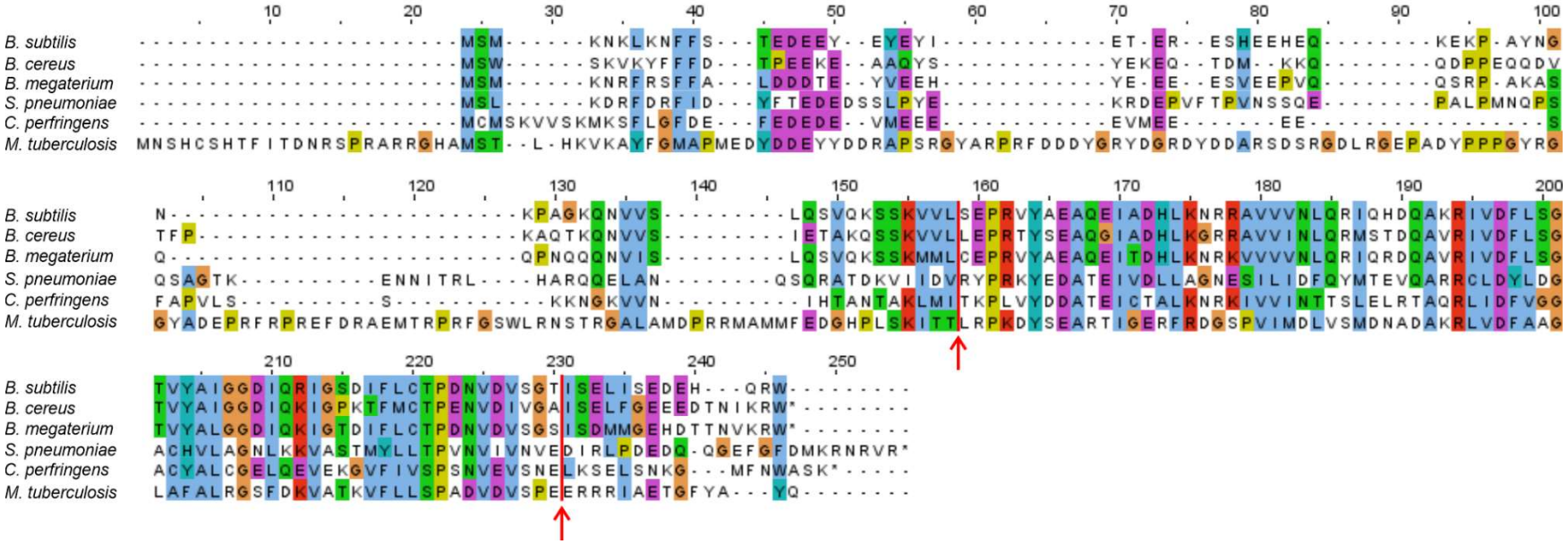
primer	***description***	***sequence***	***used for***
YQ353	*sepF-perfringens*-for	GCTTGCAAAGTGTTCAGAAAACAGCTAAATTAATGATAACTAAACCA	pYQ95, 98
YQ354	*sepF-perfringens*-rev	TCGTCTTCAGATATGAGCTCCTTTAATTCATTGCTTACTTCAAC	pYQ95, 98
YQ355	*sepF-pneumoniae*-for	GCTTGCAAAGTGTTCAGAAAAAGGTCATTATAGATGTTCGTTATC	pYQ96, 99
YQ356	*sepF-pneumoniae*-rev	TCGTCTTCAGATATGAGCTCGATATCTTCAACATTTACAATAACATTC	pYQ96, 99
YQ572	*sepF-megaterium-for*	AATCCTCTAAAGTGGTGTTGTGCGAACCTCGCGTCTATGCCGA	pYQ176, 177
YQ573	*sepF-megaterium-rev*	GCTTCCGCTTACGTCTACATTATC	pYQ176, 177
YQ574	*sepF-megaterium-subtilis-for*	ATGTAGACGTAAGCGGAAGCATTTCTGAGCTCATATCTGAAGAC	pYQ176, 177

Supplementary Table 6: NCBI reference numbers of the *sepF* gene sequences used in this study.

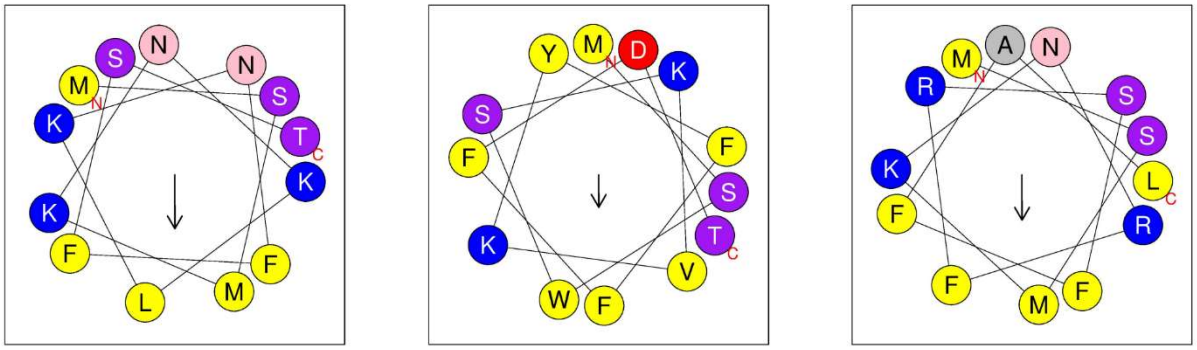
species	NCBI reference number
B. subtilis	SPY12567.1
B. cereus	GCF68736.1
B. megaterium	WP_164796417.1
S. pneumoniae	VDG77796.1
C. perfringens	WP_131442463.1
M. tuberculosis	WP_157756067.1



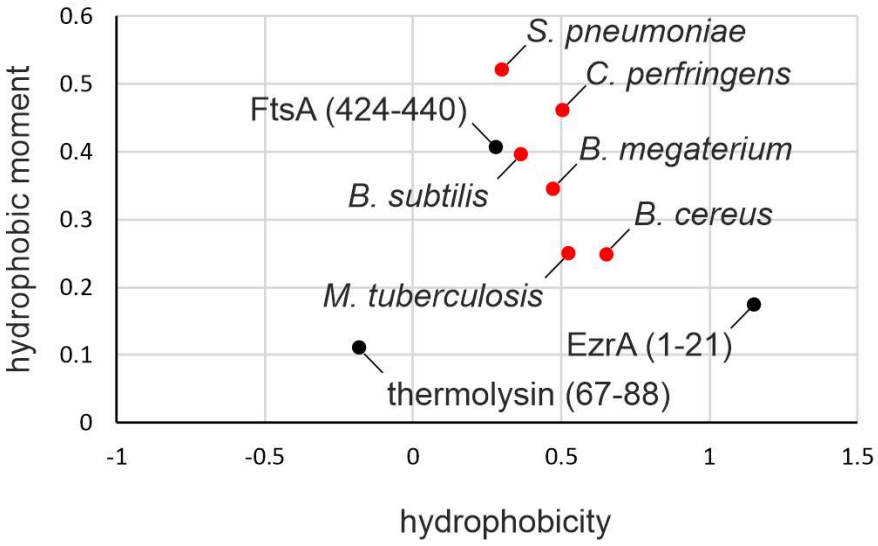
Supplementary Figure 1: Control AFM experiments on graphite surfaces. Control experiments were performed with high speed AFM and conventional AFM (Nanotec Electronica, Tres Cantos) on freshly cleaved highly oriented pyrolytic graphite (HOPG) surfaces (Agar Scientific). For these experiments, purified *B. subtilis* SepF was diluted 1:10 in buffer BF and added onto the HOPG and allowed to settle for ~20-30 min at room temperature prior to imaging in buffer solution (10). This resulted in the same mean ring height (2.9 nm) and comparable standard deviation as for the experiments on mica. Therefore, the data on HOPG and mica were combined in Figure 1D of the main text. **(A)** Plane AFM images of different SepF rings on HOPG imaged in buffer solution with the Nanotec AFM operated in jumping mode at room temperature. Olympus OMCL-RC800PSA rectangular, silicon-nitride cantilevers with a nominal tip radius of 15 nm and a nominal spring constant of 0.05 N/m were used. Images were processed and analyzed with WSxM software (11). **(B)** 3D projections of the same images. **(C)** Height profiles of individual SepF rings shown in A and B.



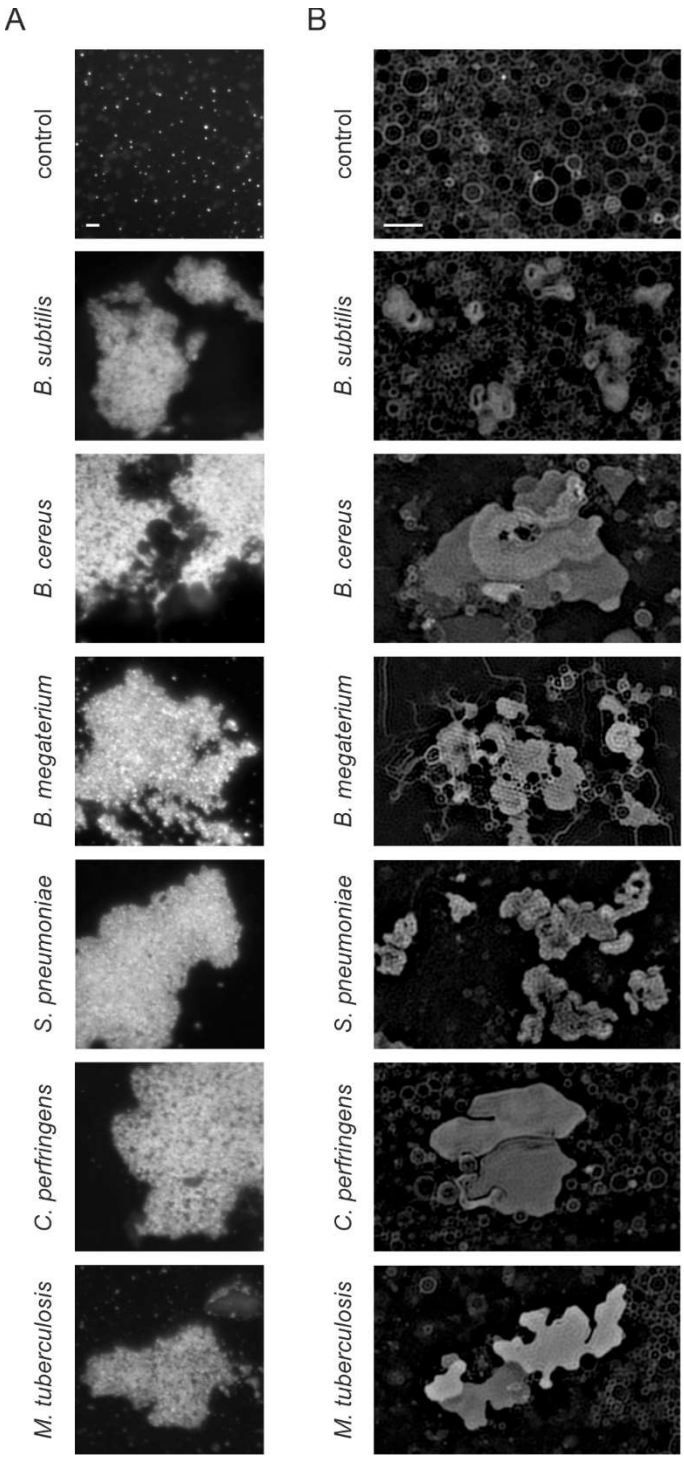
Supplementary Figure 2: Sequence alignment of SepF homologues. Sequences were aligned with Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). Arrows mark the beginning and end of the highly conserved core domain.



Supplementary Figure 3: Comparison of the amphipathic helices of SepF variants. Helical wheel projections show the first 13 amino acids of the different proteins were created with HeliQuest (https://heliquest.ipmc.cnrs.fr/). One-letter code for amino acids is used. Hydrophobic residues are depicted in yellow, positively charged amino acids in dark blue, negatively charged amino acids in red, glycine and alanine in gray, serine and threonine in purple, asparagine in light pink, and histidine in light blue. N- and C-termini are indicated with red letters. Arrows indicate the hydrophobic moments of the respective helices.

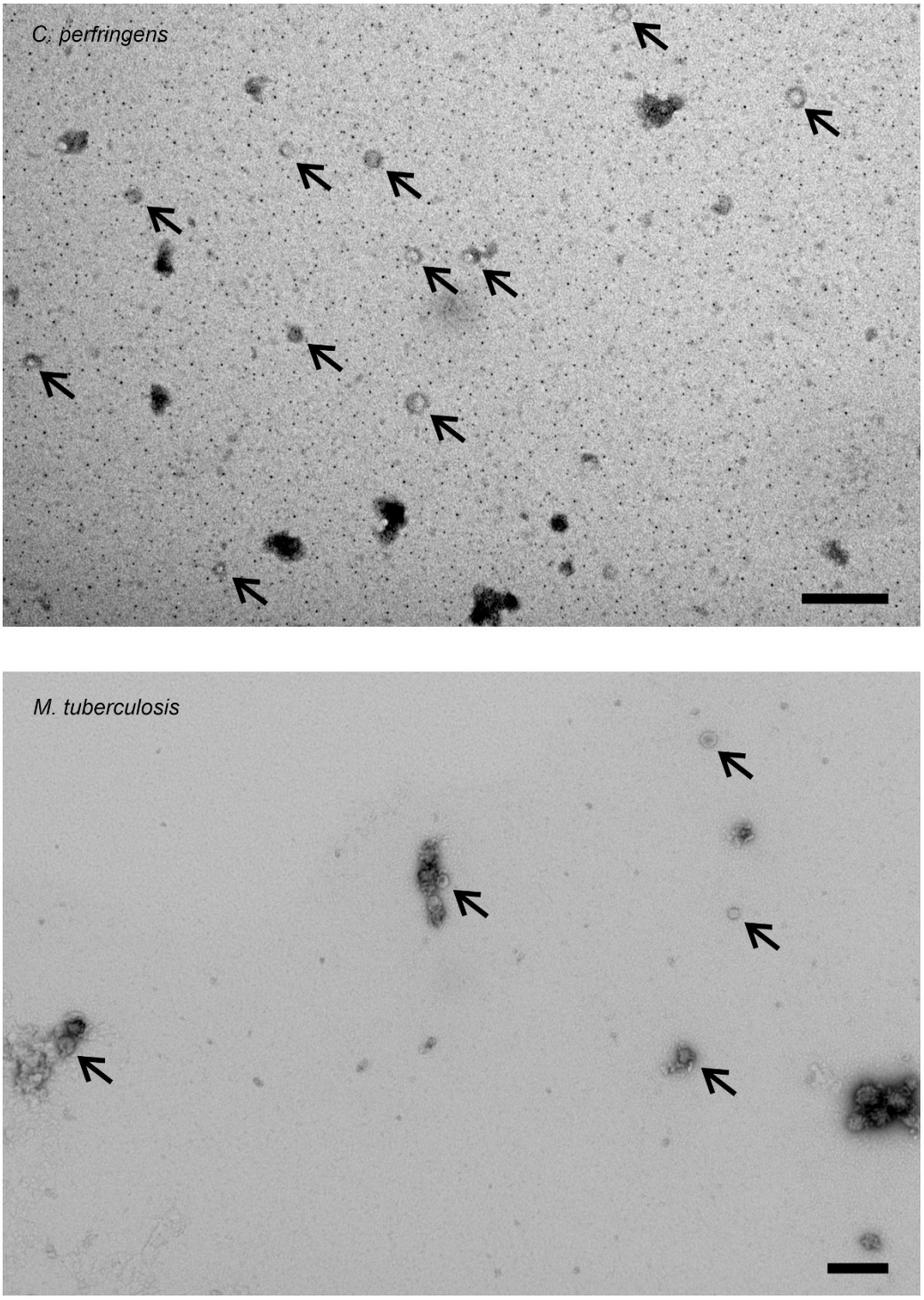


Supplementary Figure 4: Hydrophobic moment plot of the putative N-terminal α-helices of SepF homologues. High H values indicate strong hydrophobicity. The hydrophobic moment (μH) is a measure for the amphipathicity of an α-helix (high values indicating strong amphipathicity). The plot of H and μH indicates the probability of an α-helix to reside in the globular or surface-exposed domains of a protein and can be used to predict transmembrane or amphipathic membrane-binding helices. Black dots indicate known globular (*Bacillus thermoproteolyticus* thermolysin, aa 67-88), transmembrane (*B. subtilis* EzrA, aa 1-21) and amphipathic (*B. subtilis* FtsA, aa 424-440) helices for comparison. N-terminal α-helices of SepF homologues are depicted in red.

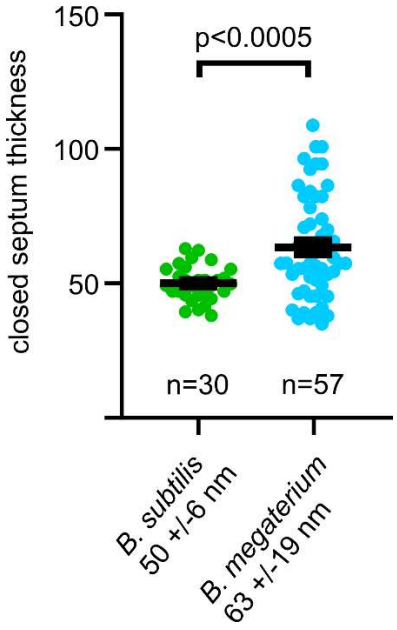


Supplementary Figure 5: Liposome aggregation and deformation by the different SepF variants. **(A)** Fluorescence light microscopy images of small liposomes (200 nm) stained with Nile red. **(B)** SIM images of large liposomes (800 nm) stained with

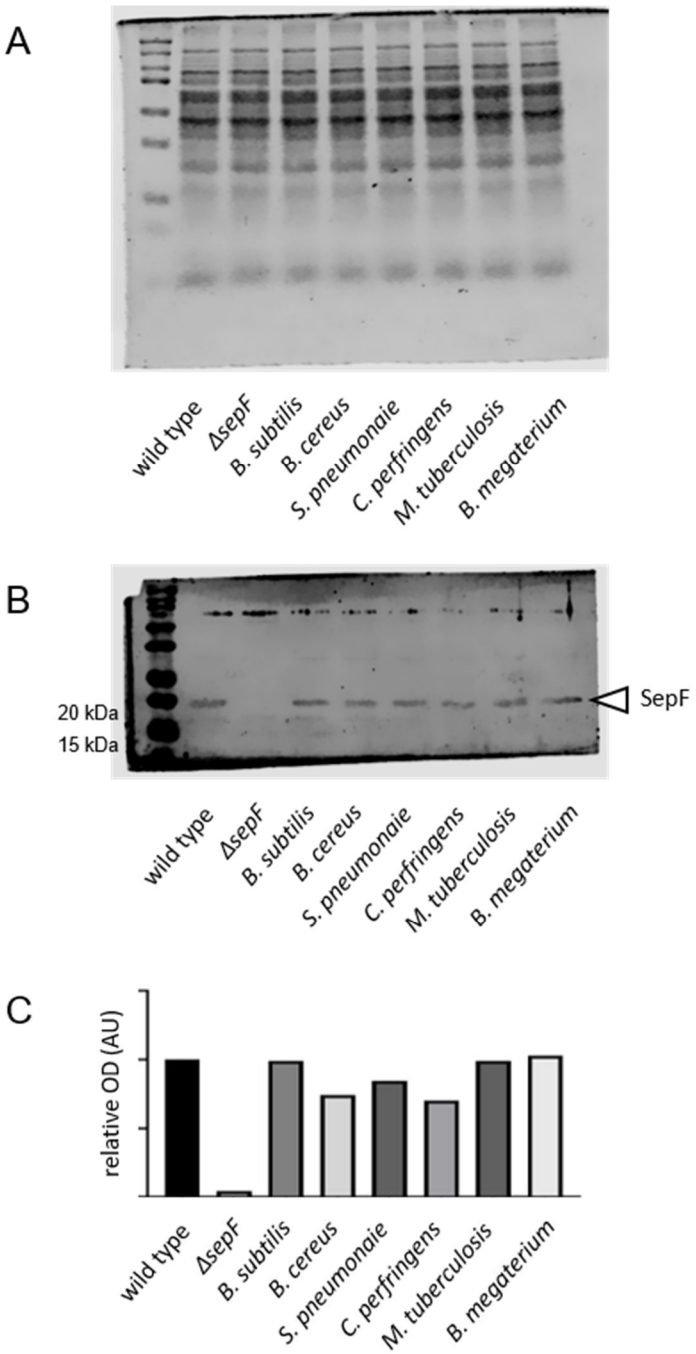
mitotracker green. 0.25 µg/ml of the different SepF proteins (species names indicated) were mixed with 2 mg/ml liposomes prepared from *E. coli* polar lipid extract. Scale bars 1 µm.



Supplementary Figure 6: Overview pictures of SepF rings from *C. perfringens* and *M. tuberculosis*. Arrows indicate SepF rings. Scale bars 200 nm.



Supplementary Figure 7: Closed septum thickness of *B. subtilis* and *B. megaterium.*



Supplementary Figure 8: Western Blot showing expression levels of chimeric SepF variants. SepF chimera proteins were expressed from the IPTG-inducible *Pspac* promoter using 100 µM IPTG in a *ΔsepF B. subtilis* background. Cultures were grown until an OD_{600} of 0.4 prior to protein analysis. Wild type (168), *ΔsepF* (BFA2863), *ΔsepF* complemented with IPTG-inducible *B. subtilis* wild type *sepF* (MW18), and

ΔsepF expressing chimera SepF proteins containing the core domain of the respective SepF proteins from *B. cereus* (GYQ458), *S. pneumoniae* (MW89), *C. perfringens* (MW92), *M. tuberculosis* (MW86), and *B. megaterium* (GYQ759) were used (see Table S3 for strain details). **(A)** Loading control. The SDS PAGE gel was stained with coomassie brilliant blue. **(B)** Western blot of a parallel gel. SepF was detected with a specific polyclonal antibody (5). A representative blot out of three independent replicates is shown. **(C)** Densitometric quantification of SepF bands.

Supplementary References

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