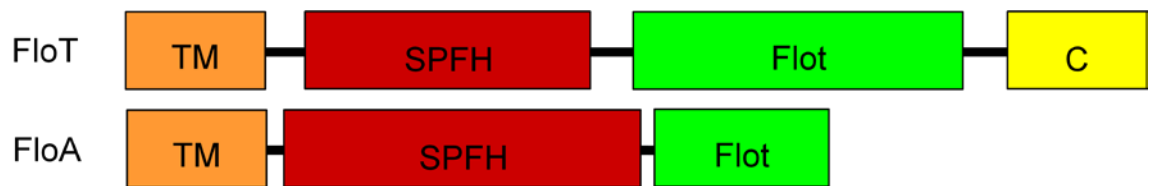
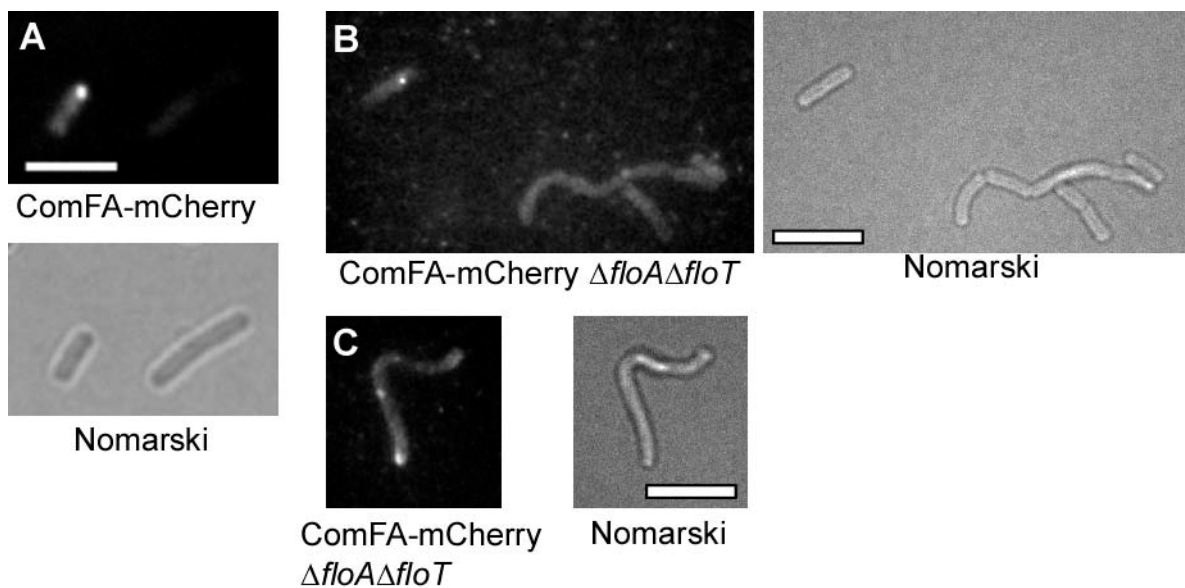


## Supplementary material



Suppl. Fig 1. Domain structure of FloT and of FloA. TM = transmembrane helix, SPFH = Stomatin, Prohibitin, Flotillin and HflK/C domain, Flot = flotillin-domain, C = domain of unknown function.



Suppl. Fig. 2 Fluorescence microscopy of *Bacillus subtilis* cells grown to competence, expressing ComFA-mCherry as a marker for the DNA uptake machinery. A) 20% of wild type cells contain the uptake machinery at the cell pole, B-C) ComFA-mCherry in cells carrying a *floT floA* double deletion. White bars 4  $\mu\text{m}$ .

Suppl. Table S1: oligonucleotides used in this study

description	Sequence (5' to 3')	Restriction site	direction
5' megaprimer for <i>yqfA</i> deletion	GAACAACCTGCACCATTGCAAGACCACTCCGGCTGCCAAAGC		down
5' megaprimer for <i>yqfA</i> deletion	GAGATGGAAGATGCCGCAG		up
3' megaprimer for <i>yqfA</i> deletion	TTGATCCTTTTTTTATAACAGGAATTTCGACCCTTCGGATGAAGACC		up
3' megaprimer for <i>yqfA</i> deletion	TAAATTTACTTCTTACCCGCCG		down
5' megaprimer for <i>yqfB</i> deletion	GAACAACCTGCACCATTGCAAGACCGCTGCAATAATAAGCGGATTG		down
5' megaprimer for <i>yqfB</i> deletion	CAGCTTTGGCAGCCGGAGTG		up
3' megaprimer for <i>yqfB</i> deletion	TTGATCCTTTTTTTATAACAGGAATTCCTCCACGGGCGAAAAAACC		up
3' megaprimer for <i>yqfB</i> deletion	GCGCGGGCTAACATATTTTTC		down
For c terminal FloT fusion	ACTGGGCCCCGAGCAGCTGAGAAAGC	<i>ApaI</i>	up
For c terminal FloT fusion	CCTGAATTCCTCCACCCTCTGATTTTTGGATCGTTTTGG	<i>EcoRI</i>	down
For c terminal YuaF fusion	TCAGGGCCCCGCGGGCAGCCTTACGCTG	<i>ApaI</i>	up

For c terminal YuaF fusion	TCAGAATTCCCCACCAATGGGTTCATGCGGAGTAAC	<i>EcoRI</i>	down
For FloT $\Delta$ flot –YFP fusion	TCAGGGCCCCAAAAGAAATTCTTCGCCGTG	<i>ApaI</i>	up
For FloT $\Delta$ flot –YFP fusion	TCAGAATTCCCCCCTCCTGAAAAGTTCTCAAGCATT	<i>EcoRI</i>	down
For FloT $\Delta$ flot $\Delta$ C – YFP fusion	TCAGGGCCCGAAGCGTTAATTGTAACAGGG	<i>ApaI</i>	up
For FloT $\Delta$ flot $\Delta$ C – YFP fusion	TCAGAATTCCCCCCCCGGTTTCCCTAATGATTCAAG	<i>EcoRI</i>	down
For c terminal YqeZ fusion	TCAGGGCCCATCCGGTCATTGTCCCTATTC	<i>ApaI</i>	up
terminal YqeZ fusion	TCAGAATTCCCCGCCAATTTCTCTCACGACAATGCG	<i>EcoRI</i>	down
Disruption of the <i>yqeZ</i> gene	TCA GGG CCC GCG CTC GCA ATG GCT GAC C	<i>ApaI</i>	up
Disruption of the <i>yqeZ</i> gene	TCA GAA TTC GCT GAG GCG ATC AAG AGA G	<i>EcoRI</i>	down
For C terminal YqfA fusion	TACGGGCCCCGGGGGAGCAGGGGAAG	<i>ApaI</i>	up
For C terminal YqfA fusion	TACGAATTCCCCGCTCCTGATTTGCGGTCTTCATCCG	<i>EcoRI</i>	down
For yqfA $\Delta$ flot –YFP fusion	TCAGGGCCCCGCGGGACTTAATGTTGGAAC	<i>ApaI</i>	up
For yqfA $\Delta$ flot –YFP fusion	TCAGAATTCCCCGCCCCCTAAAATTGCCCCGATGTTTTTG	<i>EcoRI</i>	down

## Supplementary movies

Movie 1: TIRF-M time lapse microscopy of cells expressing FloT-YFP from the original gene locus. Cells grown in S750 minimal medium. Images taken every 3 s, 6 frame/s

Movie 2: TIRF-M time lapse microscopy of cells expressing FloT-YFP from the original gene locus. Cells grown in S750 minimal medium. Images taken every 3 s, 6 frame/s

Movie 3: TIRF-M time lapse microscopy of cells expressing FloA-YFP from the original gene locus. Cells grown in LB rich medium. Images taken every 3 s, 6 frame/s

Movie 4: Epi-fluorescence time lapse microscopy of cells expressing FloA-YFP from the original gene locus. Cells grown in S750 minimal medium. Images taken every 3 s, 6 frame/s

Movie 5: TIRF-M time lapse microscopy of cells expressing FloT-YFP from the original gene locus, 60 min after addition of FCCP (to dissipate the membrane potential). Images taken every 3 s, 6 frame/s

Movie 6: TIRF-M time lapse microscopy of cells expressing NfeD2-YFP from the original gene locus. Cells grown in S750 minimal medium. Images taken every 3 s, 6 frame/s

Movie 7: 3 D reconstruction of Z-stacks taken through an S2 cell expressing FloT-YFP.