

Role and recruitment of the TagL peptidoglycan-binding protein during Type VI secretion system biogenesis

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Supplemental Data

Table S1. Strains, plasmids and oligonucleotides used in this study.

Figure S1. sfGFP-TagL is functional.

Figure S2. Impact of T6SS subunits on TagL localization.

Figure S3. Impact of TagL on T6SS subunits localization.

Figure S4. Impact of TagL on TssA and TssM recruitment and dynamics.

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STRAINS

Strains	Description and genotype	Source
<i>Escherichia coli</i> K12		
DH5 α	F-, Δ (<i>argF-lac</i>)U169, <i>phoA</i> , <i>supE44</i> , Δ (<i>lacZ</i>)M15, <i>relA</i> , <i>endA</i> , <i>thi</i> , <i>hsdR</i>	Laboratory collection
W3110	F-, lambda-IN(<i>rrnD-rrnE</i>)1 <i>rph-1</i>	Laboratory collection
Enteroaggregative <i>Escherichia coli</i>		
17-2	Wild-type	Laboratory collection
17-2 Δ <i>tagL</i>	Deletion of the <i>tagL</i> gene (EC042_4528) in 17-2	Aschtgen et al., 2010a
17-2 Δ <i>tssL</i>	Deletion of the <i>tssL</i> gene (EC042_4527) in 17-2	Aschtgen et al., 2010a
17-2 Δ <i>tssL-tagL</i>	Deletion of the <i>tssL</i> (EC042_4527) and <i>tagL</i> (EC042_4528) genes in 17-2	This study
17-2 Δ <i>tssM</i>	Deletion of the <i>tssM</i> gene (EC042_4539) in 17-2	Aschtgen et al., 2010a
17-2 Δ <i>tssJ</i>	Deletion of the <i>tssJ</i> gene (EC042_4544) in 17-2	Aschtgen et al., 2008
17-2 Δ <i>mltE</i>	Deletion of the <i>mltE</i> gene (EC042_1244) in 17-2	Santin et al., 2017
17-2 Δ <i>tssK</i>	Deletion of the <i>tssK</i> gene (EC042_4526) in 17-2	Zoued et al., 2013
17-2 Δ <i>tssA</i>	Deletion of the <i>tssA</i> gene (EC042_4540) in 17-2	Zoued et al., 2016
17-2 Δ <i>tssBC</i>	Deletion of the <i>tssB</i> (EC042_4524) and <i>tssC</i> (EC042_4525) genes in 17-2	Brunet et al., 2015
17-2 sfGFP-TagL	Insertion of the <i>sfGFP</i> coding sequence at the 5' of <i>tagL</i> in 17-2	This study
Δ <i>tssL</i> sfGFP-TagL	Insertion of the <i>sfGFP</i> coding sequence at the 5' of <i>tagL</i> in 17-2 Δ <i>tssL</i>	This study
Δ <i>tssM</i> sfGFP-TagL	Insertion of the <i>sfGFP</i> coding sequence at the 5' of <i>tagL</i> in 17-2 Δ <i>tssM</i>	This study
Δ <i>tssJ</i> sfGFP-TagL	Insertion of the <i>sfGFP</i> coding sequence at the 5' of <i>tagL</i> in 17-2 Δ <i>tssJ</i>	This study
Δ <i>mltE</i> sfGFP-TagL	Insertion of the <i>sfGFP</i> coding sequence at the 5' of <i>tagL</i> in 17-2 Δ <i>mltE</i>	This study
Δ <i>tssK</i> sfGFP-TagL	Insertion of the <i>sfGFP</i> coding sequence at the 5' of <i>tagL</i> in 17-2 Δ <i>tssK</i>	This study
Δ <i>tssA</i> sfGFP-TagL	Insertion of the <i>sfGFP</i> coding sequence at the 5' of <i>tagL</i> in 17-2 Δ <i>tssA</i>	This study
Δ <i>tssBC</i> sfGFP-TagL	Insertion of the <i>sfGFP</i> coding sequence at the 5' of <i>tagL</i> in 17-2 Δ <i>tssBC</i>	This study
17-2 TssB-mCh	Insertion of the <i>mCherry</i> coding sequence at the 3' of <i>tssB</i> in 17-2	Durand et al., 2015
17-2 sfGFP-TssL	Insertion of the <i>sfGFP</i> coding sequence at the 5' of <i>tssL</i> in 17-2	Durand et al., 2015

17-2 mCh-TssL	Insertion of the <i>mCherry</i> coding sequence at the 5' of <i>tssL</i> in 17-2	Durand et al., 2015
17-2 sfGFP-TssM	Insertion of the <i>sfGFP</i> coding sequence at the 5' of <i>tssM</i> in 17-2	Durand et al., 2015
17-2 sfGFP-TssA	Insertion of the <i>sfGFP</i> coding sequence at the 5' of <i>tssA</i> in 17-2	Zoued et al., 2016
17-2 TssK-sfGFP	Insertion of the <i>sfGFP</i> coding sequence at the 3' of <i>tssK</i> in 17-2	Brunet et al., 2015
17-2 TssB-mCh sfGFP-TagL	Insertion of the <i>sfGFP</i> coding sequence at the 5' of <i>tagL</i> in 17-2 TssB-mCh	This study
17-2 mCh-TssL sfGFP-TagL	Insertion of the <i>sfGFP</i> coding sequence at the 5' of <i>tagL</i> in 17-2 mCh-TssL	This study
$\Delta tagL$ TssB-mCh	Deletion of the <i>tagL</i> gene (EC042_4528) in 17-2 TssB-mCh	This study
$\Delta tagL$ sfGFP-TssL	Deletion of the <i>tagL</i> gene (EC042_4528) in 17-2 sfGFP-TssL	This study
$\Delta tagL$ sfGFP-TssM	Deletion of the <i>tagL</i> gene (EC042_4528) in 17-2 sfGFP-TssM	This study
$\Delta tagL$ sfGFP-TssA	Deletion of the <i>tagL</i> gene (EC042_4528) in 17-2 sfGFP-TssA	This study
$\Delta tagL$ TssK-sfGFP	Deletion of the <i>tagL</i> gene (EC042_4528) in 17-2 TssK-sfGFP	This study

PLASMIDS

Plasmid	Description and main characteristics	Source
pKD4	Kan ^R cassette flanked by FRT sites, R6K origin, Kan ^R , Amp ^R	Datsenko and Wanner, 2000
pKD4-Nter-sfGFP	<i>sfGFP</i> -coding sequence cloned downstream the Kan ^R cassette in pKD4	Durand et al., 2015
pFPV-mCherry	<i>mCherry</i> under <i>rpsM</i> promoter, Amp ^R	Drecktrah et al., 2008
pASK-IBA37(+)	Expression vector, AHT-inducible, ColE1 origin, Amp ^R	IBA technology
pIBA-TagL	<i>tagL</i> gene cloned into pASK-IBA37(+), N-terminal 6×His tag coding sequence	Aschtgen et al., 2010a
pIBA-TagL Δ_p	TAA stop codon inserted at codon Met-366 in pIBA-TagL	This study
pIBA-TssL	<i>tssL</i> gene cloned into pASK-IBA37(+), C-terminal FLAG epitope coding sequence	Aschtgen et al., 2012
pIBA-TagL-TssL	6×His <i>tagL</i> and <i>tssL</i> -FLAG cloned into pASK-IBA37(+)	This study
pIBA-TssL-PG	wild-type PGB domain of <i>tagL</i> (residues 365-576) fused to the C-terminus of <i>tssL</i>	Aschtgen et al., 2012
pIBA-TssL-PG*	substituted PGB domain of <i>tagL</i> fused to the C-terminus of <i>tssL</i>	This study
pMS600	pOK12 vector, <i>EcoRI-XbaI-XhoI</i> linker, P15A origin, Kan ^R	Aschtgen et al., 2008
pOK-Hcp-HA	<i>hcp</i> gene cloned into pMS600, N-terminal HA epitope coding sequence	Aschtgen et al., 2010a
pOK-TssL-HA	<i>tssL</i> gene cloned into pMS600, N-terminal HA epitope coding sequence	Aschtgen et al., 2010a
pOK-TssL _C	<i>tssL_C</i> (residues 1-184) cloned into pMS600, N-terminal HA epitope coding sequence	This study

OLIGONUCLEOTIDES

Oligonucleotide	Sequence (5' to 3')
Strain construction^a	
Del-4527-5/DW	<u>CATCGCTGGGAGATGTGAAACTGGAAC</u> TTTTGCGGTGCTGCGGACATGAATGTGTAGGCTGGAGCTGCTTCG
Del-4528-5/DW	TCTTCTGTGCTTGCAGACCAGGTGGCACGGCTTACCGGGCAGGGATAACCTGTGTAGGCTGGAGCTGCTTCG
Del-4528-3/DW	<u>ATATCTGCACCGCCATCATCTTTCAGCCACAGATAAACTGGAATTGCCATCATATGAATATCCTCCTTAGTTCC</u>
5-sfGFP-TagL-DW	<u>GTGTGTGCTTTCTTCTGTGCTTGCAGACCAGGTGGCACGGCTTACCGGGCAGGGATAACCCGATTGTGTAGGCT</u> GGAGCTGCTTCGAAGTTCCTATAC
3-sfGFP-TagL-DW	<u>AATCAGCCACAGTGTCAAGAACAGACTCAGTATTGTCAGCCAGAGACGGGAACGTACGTGCCCTCCGCCGGCC</u> GCTGC
Plasmid construction^{b,c,d,e}	
5'-TssL-pIBATagL	<u>GACAAAAATCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACAAATGAATAAACCTGTTATCTCCCGG</u> GC
3'-TssL-pIBATagL	<u>GATGGTGATGGTGATGCGATCCTCTGCTAGCTTATTTATCATCGTCGTCTTTATAATCTCCCTGCCCGGTAAGCCG</u>
5'EcoRI-4527	GTCGgaattcTACTTTCTACACCCCGGCATCGC
3'XhoI-4527-deltaTM	CTGCctcgagCAACCAGTACATCGTTCTGCCAGCAC
4527-Fus-PG(28)-5	<u>GCTGGCTGGTCTCTGGTGTGTGCTTTCTTCTCTGGTTCGCAGCGTGGGGGAAC</u>
4527-Fus-PG(28)-3	<u>GCGTGCCACCTGGTCTGCAAGCAC</u> <u>TTAGCAACACCCGGGGCAGCACTCCGTTATGTTTTCTGATGCGCCCTCTTC</u>
Site-directed mutagenesis^{f,g}	
A-TagL-M366Stop	CTGACGGCCTGGCACCGG ^{taa} CGCGGATATTCATCTG
B-TagL-M366Stop	CAGATGAATATCCGCG ^{tta} CCGGTGCCAGGCCGTCAG

^a 50-bp upstream/downstream regions of the site of deletion/insertion underlined.

^b restriction site in lower case (*Eco*RI at the 5' end, GAATTC; *Xho*I at the 3' end, CTCGAG).

^c sequence annealing on target vector underlined.

^d FLASH-tag coding sequence italicized.

^e FLAG-tag coding sequence italicized.

^f mutagenized codons in lower case.

^g base substitutions underlined.

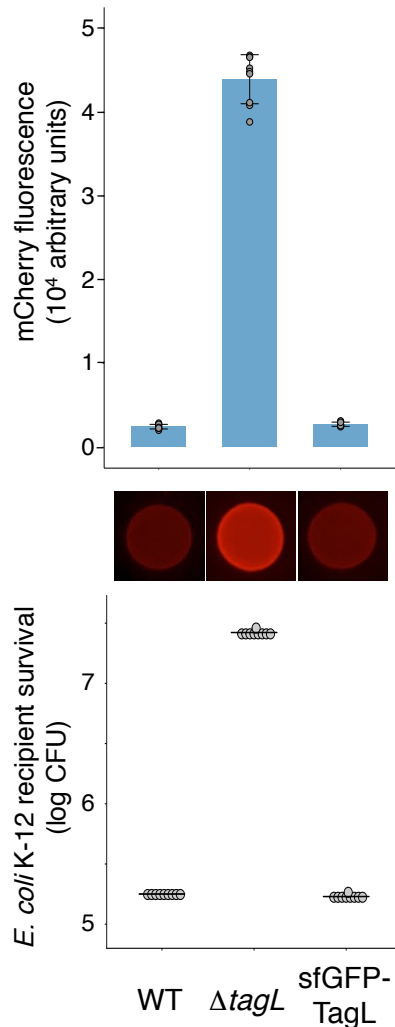


Figure S1. sfGFP-TagL is functional. *E. coli* K-12 recipient cells (W3110 mCherry, AmpR) were mixed with the indicated attacker cells, spotted onto SIM agar plates and incubated for 4 h at 37 °C. The image of a representative bacterial spot is shown below the graph reporting the fluorescence level of the spots (in arbitrary units). The average fluorescence and standard deviation are indicated, as well as the values from nine independent measurements (grey dots). The lower graph reports the number of surviving *E. coli* recipient cells [counted on selective ampicillin medium; in \log_{10} of colony-forming units (cfu)]. The grey dots correspond to the nine values from three independent biological replicates. The average is indicated by the horizontal bar.

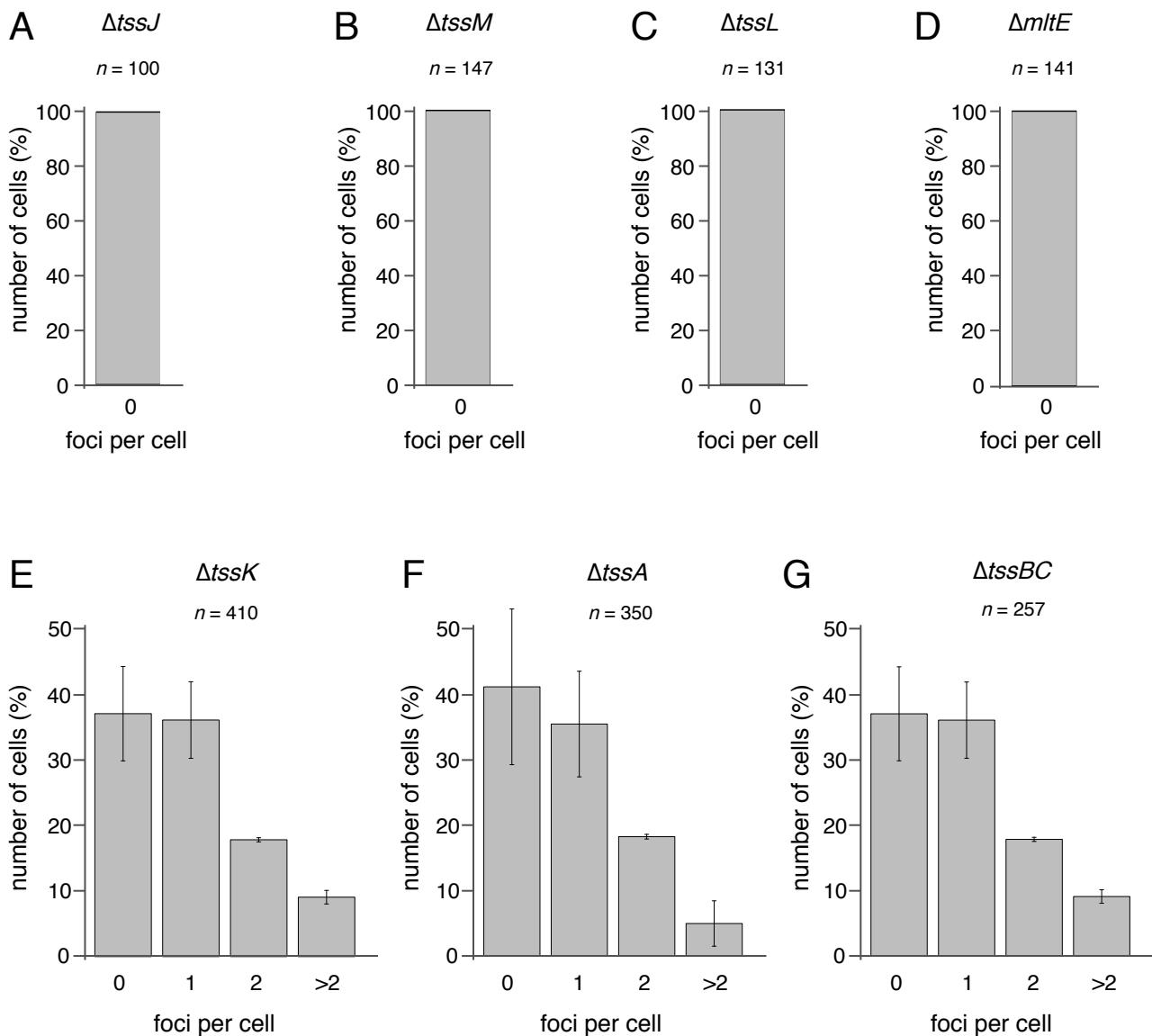


Figure S2. Impact of T6SS subunits on TagL localization. Number of sfGFP-TagL foci per cell in $\Delta tssJ$ (A), $\Delta tssM$ (B), $\Delta tssL$ (C), $\Delta mltE$ (D), $\Delta tssK$ (E), $\Delta tssA$ (F), and $\Delta tssBC$ (G) cells. The number of analyzed cells (n) from representative fields of three biological replicates is indicated on top of each graph.

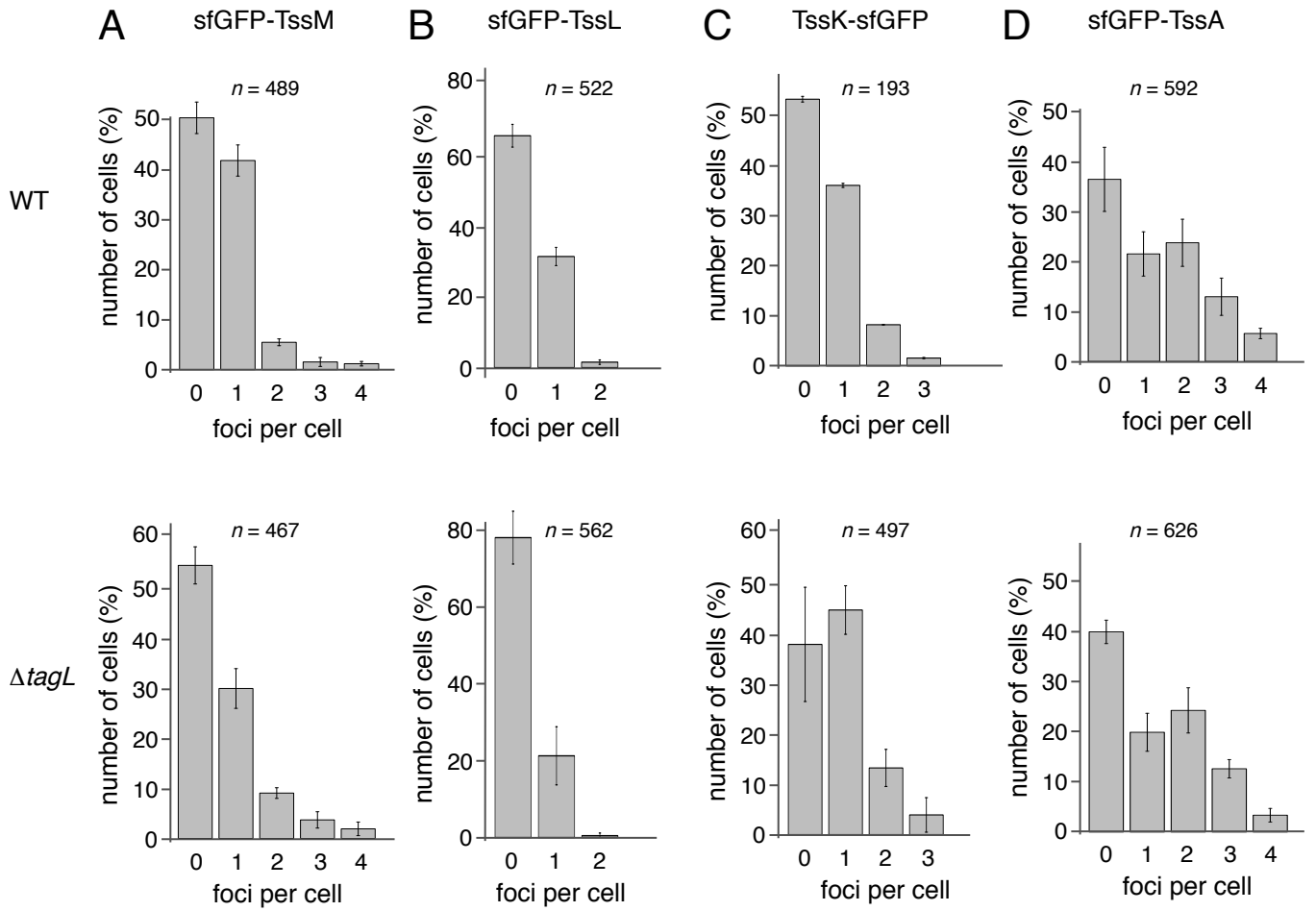


Figure S3. Impact of TagL on T6SS subunits localization. Number of sfGFP-TssM (A), sfGFP-TssL (B), TssK-sfGFP (C), and sfGFP-TssA (D) foci per cell in wild-type (upper graphs) and $\Delta tagL$ (lower graphs) cells. The number of analyzed cells (n) from representative fields of three biological replicates is indicated on top of each graph.

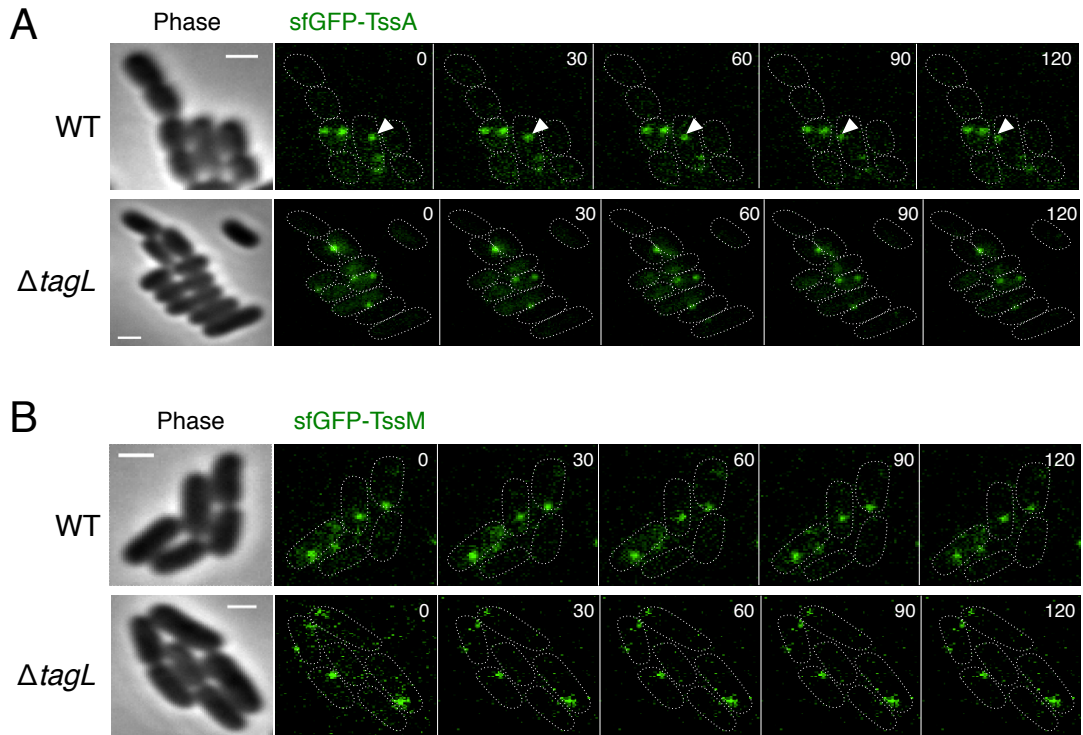


Figure S4. Impact of TagL on TssA and TssM recruitment and dynamics. Representative fluorescence microscopy time-lapse recordings of wild-type (WT, upper panels) or $\Delta tagL$ (lower panels) cells producing sfGFP-TssA (A) or sfGFP-TssM (B). Individual images were taken every 30 s. White arrowheads indicate the localization and dynamics of a TssA focus. Scale bar is 1 μm .