

Supplementary table 1. Strains, plasmids and oligonucleotides used in this study.

Strains

Strains	Description and genotype	Source
<i>E. coli</i> K-12		
DH5α	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>	New England Biolabs
T7 Iq pLysS	MiniF <i>lysY lacI^q</i> (Cam ^R) / <i>fhuA2 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10--Tet^S)2 [dcm] R(zgb-210::Tn10--Tet^S) endA1 Δ(mcrC-mrr) 114::IS10</i>	New England Biolabs
BL21(DE3)	<i>fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS λ DE3 = λ sBamHI ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5</i>	New England Biolabs
Enteroaggregative <i>E. coli</i>		
17-2	WT enteroaggregative <i>Escherichia coli</i>	Arlette Darfeuille-Michaud
17-2Δ <i>tssL</i>	17-2 deleted of the <i>tssL</i> gene of the <i>sciI</i> T6SS gene cluster	Aschtgen <i>et al.</i> , 2010
17-2Δ <i>tssM</i>	17-2 deleted of the <i>tssM</i> gene of the <i>sciI</i> T6SS gene cluster	Aschtgen <i>et al.</i> , 2010
17-2 <i>gfp-tssL</i>	<i>gfp-mut2</i> inserted downstream the start codon of <i>tssL</i> in 17-2	This study
17-2 <i>gfp-tssM</i>	<i>gfp-mut2</i> inserted downstream the start codon of <i>tssM</i> in 17-2	This study
17-2 <i>tssJ-gfp</i>	<i>gfp-mut2</i> inserted upstream the stop codon of <i>tssJ</i> in 17-2	This study
17-2 <i>gfp-tssL tssB-mCherry</i>	<i>mCherry</i> inserted upstream the stop codon of <i>tssB</i> in 17-2 <i>gfp-tssL</i>	This study

17-2 <i>gfp-tssM mCherry-tssL</i>	<i>mCherry</i> inserted downstream the start codon of <i>tssL</i> in 17-2 <i>gfp-tssM</i>	This study
17-2 Δ <i>tssM</i> <i>gfp-tssL</i>	<i>gfp-mut2</i> inserted downstream the start codon of <i>tssL</i> in 17-2 Δ <i>tssM</i>	This study
17-2 Δ <i>tssJ</i> <i>gfp-tssL</i>	<i>gfp-mut2</i> inserted downstream the start codon of <i>tssL</i> in 17-2 Δ <i>tssJ</i>	This study
17-2 Δ <i>tssL</i> <i>gfp-tssM</i>	<i>gfp-mut2</i> inserted downstream the start codon of <i>tssM</i> in 17-2 Δ <i>tssL</i>	This study
17-2 Δ <i>tssJ</i> <i>gfp-tssM</i>	<i>gfp-mut2</i> inserted downstream the start codon of <i>tssM</i> in 17-2 Δ <i>tssJ</i>	This study

Plasmids

Vectors	Description	Source
<u>Expression vectors</u>		
pUC-Hcp _{FL}	<i>sci1 hcp</i> gene cloned into pUC12, <i>Plac</i> , C-terminal FLAG epitope	Aschtgen <i>et al.</i> , 2008
pIBA-TssM	<i>sci1 tssM</i> gene cloned into pASK-IBA37(+), <i>Ptet</i> , N-terminal FLAG epitope	This study
<u>Vectors for TssJLM and protein purification</u>		
pRSF-Duet1	Expression vector, <i>lacI</i> , PT7, Kan ^R	Addgene
pRSF-TssJ ^S -FL-H ^M	<i>tssJ</i> -StrepII, FLAG- <i>tssLL</i> and 6×His- <i>tssM</i> cloned into pRSF-Duet1	This study
pETG20A	Gateway destination vector, TRX-6×His followed by a TEV cleavage site	Arie Gerlof
pHEN6-nb25	pHEN6 vector encoding the nb25 nanobody	NGuyen <i>et al.</i> , 2015
pETG20A-TssJ	<i>sci1 tssJ</i> mature form cloned into pETG20A, N-terminal TRX-His-TEV epitope	Felisberto-Rodrigues <i>et al.</i> , 2011
pETG20A-TssMp	<i>sci1 tssM</i> periplasmic domain (aa 386-1129) cloned into pETG20A, N-terminal TRX-His-TEV epitope	Felisberto-Rodrigues <i>et al.</i> , 2011

pETG20A-TssM ₂₆ Ct	<i>sciI tssM</i> C-terminal fragment (aa 869-1107) cloned into pETG20A, N-terminal TRX-His-TEV epitope	This study
Vectors for chromosomal insertions		
pKD4	Kan ^R cassette flanked by FRT recombination sites, used for chromosomal deletion	Datsenko and Wanner, 2000
pKD4 _{Nter-gfp}	<i>gfp-mut2</i> (<i>sf-gfp</i>) gene cloned upstream the Kan ^R cassette in pKD4, used for chromosomal insertion of <i>gfp-mut2</i> (N-terminal GFP)	This study
pKD4 _{Cter-mCherry}	<i>mCherry</i> gene cloned downstream the Kan ^R cassette in pKD4, used for chromosomal insertion of <i>mCherry</i> (C-terminal mCherry)	This study
pCP20	Amp ^R , Cm ^R , FRT recombinase gene	Datsenko and Wanner, 2000

Oligonucleotides

Name	Destination	Sequence (5' → 3')
For strain construction^a		
5- <i>tssB</i> - <i>mCherry</i>	insertion of <i>mCherry</i> at the 3' end of <i>tssB</i>	<u>CCGGCACTGAGTCAGACGCTGCGTGATGAACTGCGTGCACTGGTG</u> <u>CCGAAAAGGCCGGCAGCGGCCGGCGGAGGG</u>
3- <i>tssB</i> - <i>mCherry</i>	insertion of <i>mCherry</i> at the 3' end of <i>tssB</i>	<u>GCAACGTTCTTTTCTTTCTGTACAGACATCAGCATTTTCTCTCGTAA</u> <u>TCCGTTAAACATATGAATATCCTCCTTAGTTCCTATTCGGAAGTTCC</u>
5- <i>gfp</i> - <i>tssL</i>	insertion of <i>gfp-mut2</i> (or <i>mCherry</i>) at the 5' of <i>tssL</i>	<u>CTACACCCCGGCATCGCTGGGAGATGTGAACTGGAACCTTTTGC</u> <u>GGTGCTGCGGACATGACGATTGTGTAGGCTGGAGCTGCTTCGAAGTTCCTATAC</u>

3- <i>gfp-tssL</i>	insertion of <i>gfp-mut2</i> (or <i>mCherry</i>) at the 5' of <i>tssL</i>	<u>TGACCATCAGCCAGCCGGGATAAAAAATCTGTTTCAGCCCGGGAGA TAACAGGTTTATTCCCTCCGCCGGCCGCTGC</u>
5- <i>gfp-tssM</i>	insertion of <i>gfp-mut2</i> at the 5' of <i>tssM</i>	<u>TTCTCATCCGGAGAAGAACATTTTATCAGTACTGTTACATCAGGAA ACCAGAATGAATAACGATTGTGTAGGCTGGAGCTGCTTCGAAGTTCCTATAC</u>
3- <i>gfp-tssM</i>	insertion of <i>gfp-mut2</i> at the 5' of <i>tssM</i>	<u>CACACCAATAAATAACAATCCCCGGTCGCCCAAAGCGACCAGACAG ACAGGCCAGTTTATTCCCTCCGCCGGCCGCTGC</u>
5- <i>tssJ-gfp</i>	insertion of <i>gfp-mut2</i> at the 3' of <i>tssJ</i>	<u>CACGCCCCGTCTTATTGAAGTATCCGGTAACACCCTGACCCTGTTA CCGGTGAAGGATAAAGCAGCGGCCGGCGGAGGG</u>
3- <i>tssJ-gfp</i>	insertion of <i>gfp-mut2</i> at the 3' of <i>tssJ</i>	<u>GTTTAACTCCAGCCCCGCCAGTGA AATTGCCATAGAGAATTTTCATAA AGGGAAGGACGCGGCATCACATATGAATATCCTCCTTAGTTCCTATTCCGAAGTTC</u>

For plasmid construction^{b,c,d}

IBA37-TssM-FLAG-5	insertion of <i>tssM</i> with N-terminal FLAG epitope into pASK-IBA37	<u>GACAAAAATCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATAT ACAAATGGATTATAAAGACGACGATGACAAAAATAAACTGGCCTGTCTGTCTGGTCGCTTTGGG</u>
IBA37-TssM-3	insertion of <i>tssM</i> with N-terminal FLAG epitope into pASK-IBA37	<u>GATGGTGATGGTGATGCGATCCTCTGCTAGCTCAGTCAGTCTCCTC CACGGTATCCCCGG</u>
KD4-Nt-sfGFP-5	insertion of <i>gfp-mut2</i> into pKD4	<u>CGGAATAGGAACTAAGGAGGATATTCATATGTCTAAAGGTGAAGAAC TGTTACCCG</u>
KD4-Nt-sfGFP-3	insertion of <i>gfp-mut2</i> into pKD4	<u>CTGACATGGGAATTAGCCATGGTCCCCTCCGCCGGCCGCTGCTTTGTAGA GTCATCCATGCCG</u>
KD4-Ct-mCherry-5	insertion of <i>mCherry</i> into pKD4	<u>GCAGCATTACACGTCTTGAGCGATTGCAGCGGCCGGCGGAGG</u>
KD4-Ct- mCherry-3	insertion of <i>mCherry</i> into pKD4	<u>CTTCGAAGCAGCTCCAGCCTACACTTACTTGTACAGCTCGTCCATGCC GCC</u>
5-pETG20-TssM _{26Ct}	insertion of <i>tssM</i> (aa 868-1107) into pETG20A	<u>GACAAGTTTGTACAAAAAAGCAGGCTTAGAAAACCTGTACT TCCAGGGTACGCCCGCGGCAGAAAGTCTG</u>
3-pETG20-TssM _{26Ct}	insertion of <i>tssM</i> (aa 868-1107) into pETG20A	<u>CCACTTTGTACAAGAAAGCTGGGTTTATTCGAAAACCGTTT CCGGCAGAAC</u>
RSF-Js-Fwd	insertion of StrepII-tagged <i>tssJ</i> into pRSF-Duet	<u>ATGGTACATATGATGGCGATTATCGCTGGTAAGGCTGGTGG</u>

RSF-Js-Rev	insertion of StrepII-tagged <i>tssJ</i> into pRSF-Duet	AATCCCATATGGCGATTATCGCTGGTAAGGCTGGTTACGGTC AATCCGCTCGAGTCATTTTTCGAACTGCGGGTGGCTCCATTT ATCCTTACCAGGTAACAGGGTCAGGGTG
RSF-fIL-Fwd	insertion of FLAG-tagged <i>tssL</i> into pRSF-Duet	GCAGTTCGAAAAATGACTCGAGAAGGAGATATAACCATGGA TTATAAAGATGACGATGACAAGAATAAACCTGTTATCTCCCGGGC
RSF-fIL-Rev	insertion of FLAG-tagged <i>tssL</i> into pRSF-Duet	ATGTATATCTCCTTTTATCCCTGCCCGGTAAGCCGTGCCACC TGGTCTG
RSF-hM-Fwd	insertion of 6×His-tagged <i>tssM</i> into pRSF-Duet	GGATAAAAGGAGATATACATATGCATCACCATCATCACCACC ATCACAATAAACTGGCCTGTCTGTCTGGTC
RSF-hM-Rev	insertion of 6×His-tagged <i>tssM</i> into pRSF-Duet	GCGGTTTCTTTACCAGACTCGAGTCAGTCAGTCTCCTCCACG GTATCC

For site-directed mutagenesis^e

A-TssJ C1S	Cys1-to-Ser substitution in <i>tssJ</i>	TTATCAGGATCCGGTCTGACGCAAAGAGTGGCAGACGGTACGGTATCTGC
B-TssJ C1S	Cys1-to-Ser substitution in <i>tssJ</i>	GTCAGACCCGGATCCTGATAACGACAGGGAAAACAACGCAATAAT
A-TssM-C727S	Cys727-to-Ser substitution in <i>tssM</i>	GAATACGCTGGCGGTTTCAGGGATCCACTGGCCAGCCCCGGGAAG
B-TssM-C727S	Cys727-to-Ser substitution in <i>tssM</i>	CTTCCCGGGGCTGGCCAGTGGATCCCTGAACCGCCAGCGTATTC
A-TssM-T972C	Thr972-to-Cys substitution in <i>tssM</i>	ATAGCGGATGTGGCGTTCACCTGTGGTAACGCGGGGCTGCATTTTG
B-TssM-T972C	Thr972-to-Cys substitution in <i>tssM</i>	CAAAATGCAGCCCCGCGTTACCACAGGTGAACGCCACATCCGCTAT
A-TssM-V989C	Val989-to-Cys substitution in <i>tssM</i>	CGCCCGGGAAGTGTGCGCGGTTGTATGCAGACGACGCTGATAAC
B-TssM-V989C	Val989-to-Cys substitution in <i>tssM</i>	GTTATCAGCGTCGTCTGCATACAACCGGCAGCAGTTCGCCGGGCG
A-TssM-N1005C	Asn1005-to-Cys substitution in <i>tssM</i>	GATAATCAGAACTGATTTATGTTTGTTCAGATGCCGGTATGGAAGCG
B-TssM-N1005C	Asn1005-to-Cys substitution in <i>tssM</i>	CGCTTCCATAACGGCATCTGACAAACATAAATCAGTTTCTGATTATC
A-TssM-T1019C	Thr1019-to-Cys substitution in <i>tssM</i>	GATTTACCTGGCCGGCTGATTGTGAAGCACCTGGCGCCAGTTTAAAG
B-TssM-T1019C	Thr1019-to-Cys substitution in <i>tssM</i>	CTTAAACTGGCGCCAGGTGCTTACAATCAGCCGGCCAGGTAAATC
A-TssM-T1035C	Thr1035-to-Cys substitution in <i>tssM</i>	GGGTAAGCACTCAGGCCGTGTCCTCCGTCAGTATGCAGACCTGCCGGG
B-TssM-T1035C	Thr1035-to-Cys substitution in <i>tssM</i>	CCCGGCAGGTCTGCATACTGACGGGACACGGCCTGAGTGCTTTACCC
A-TssM-V1062C	Val1062-to-Cys substitution in <i>tssM</i>	GACGGAAAGCCGCACCGGTGTTTGGCAGTGGCTGGAGCCTGAG
B-TssM-V1062C	Val1062-to-Cys substitution in <i>tssM</i>	CTCAGGCTCCAGCCACTGGCAAACACCGGTGCGGCTTTCCGTC
A-TssM-G1075C	Gly1075-to-Cys substitution in <i>tssM</i>	CTGAGCTGGCAGGCGCAGGACTGTCGTATGCTGAATTACACACTGC
B-TssM-G1075C	Gly1075-to-Cys substitution in <i>tssM</i>	GCAGTGTGTAATTCAGCATAACGACAGTCCTGCGCCTGCCAGCTCAG

A-TssM-V1092C	Val1092-to-Cys substitution in <i>tssM</i>	GGGGAAGGGCCGCTTTGTTGCTGAAACTCCGCAATTTTG
B-TssM-V1092C	Val1092-to-Cys substitution in <i>tssM</i>	CAAATTTGCGGAGTTTCAGCAAACAAGCGGCCCTTCCCC
A-TssM-S1109C	Ser1109-to-Cys substitution in <i>tssM</i>	GAAACGGTTTTTCGAACTCTGCGGCACGTCAGCGTTTAC
B-TssM-S1109C	Ser1009-to-Cys substitution in <i>tssM</i>	GTAAACGCTGACGTGCCG <u>CAG</u> AGTTTCGAAAACCGTTTC

^a Sequences corresponding to the downstream and upstream regions of the gene to be deleted underlined.

^b Sequence annealing on the target plasmid underlined.

^c Restriction sites in **Bold**.

^d FLAG, StrepII or 6× His tag coding sequence *italicized*.

^e mutagenized codon underlined.