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SUPPLEMENTARY INFORMATION

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In vivo TssA proximity labelling during type VI secretion biogenesis reveals TagA as a protein that stops and holds the sheath

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Supplementary Information

In vivo TssA proximity labeling reveals temporal interactions during Type VI secretion biogenesis and TagA, a protein that stops and holds the sheath.

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Supplementary Tables: Strains, Plasmids and Oligonucleotides used in this study.

Supplementary Table 1. Strains used in this study

Name	characteristics	source
DH5α	*E. coli, fhuA2 lacU169 phoA glnV44 Φ80' lacZ(M15) gyrA96 recA relA endA thi1 hsdR17*	New England Biolabs
W3110	*E. coli, F⁻ λ⁻ IN(rrnD-rrnE)1 rph1*	Laboratory collection
BTH101	*E. coli. cya-99 araD139 galE15 galK16 rpsL1 (*Str^R*) hsdR mcrA mcrB*	Karimova *et al.*, 1998
17-2	wild-type strain of Enteroaggregative *E. coli.*	Laboratory collection. From Arlette Darfeuille-Michaud.
17-2 APEX2-TssA	APEX2 inserted downstream of the *tssA* start codon in 17-2.	This study
17-2 APEX2-GspE	APEX2 inserted downstream of the *gspE* start codon in 17-2.	This study
17-2 APEX2:*tssA*	APEX2 inserted downstream of the *tssA* gene in 17-2.	This study
17-2Δ*tssL* APEX2-TssA	APEX2 inserted downstream of the *tssA* start codon in 17-2 Δ*tssL*.	This study
17-2Δ*tssK* APEX2-TssA	APEX2 inserted downstream of the *tssA* start codon in 17-2 Δ*tssK*.	This study
17-2Δ*vgrG* APEX2-TssA	APEX2 inserted downstream of the *tssA* start codon in 17-2 Δ*vgrG*.	This study
17-2Δ*hcp* APEX2-TssA	APEX2 inserted downstream of the *tssA* start codon in 17-2 Δ*hcp*.	This study
17-2Δ*tagA*	deletion of the *tagA* gene in 17-2.	This study
17-2Δ*tagA,* P*sci1-*$_V$*tagA*	insertion of $_V$*tagA* gene under the control of the *sci1* promoter at the *lacZ* locus in 17-2 Δ*tagA*.	This study
17-2Δ*tagA,* P*sci1-tagA*	insertion of the *tagA* gene under the control of the *sci1* promoter at the *lacZ* locus in 17-2 Δ*tagA*.	This study
17-2Δ*sci1*	deletion of the *sci1* gene cluster (*tssB-tssJ*) in 17-2.	Aschtgen *et al.*, 2008
17-2Δ*tssA*	deletion of the *tssA* gene in 17-2.	Brunet *et al*., 2015
17-2 sfGFP-TagA	insertion of sfGFP downstream of the start codon of *tagA* in 17-2.	This study

17-2 sfGFP-TagA, TssK-mCh	insertion of sfGFP downstream of the start codon of *tagA* in 17-2 *tssK-mCherry.*	This study
17-2Δ*sci1* sfGFP-TagA	insertion of sfGFP downstream of the start codon of *tagA* in 17-2 Δ*sci1*.	This study
17-2Δ*tagA* TssB-sfGFP	insertion of sfGFP upstream of the stop codon of *tssB* in 17-2 Δ*tagA*.	This study
17-2Δ*tagA* TssB-sfGFP P*sci1-tagA*	insertion of sfGFP upstream of the stop codon of *tssB* in 17-2 Δ*tagA* P*sci1-tagA*.	This study

Supplementary Table 2. Plasmids used in this study

Name	characteristics	reference
pKD4	Kan^R, Amp^R, FRT recombination sites.	Datsenko and Wanner, 2000
pKD4-Nter-sfGFP	sfGFP inserted on pKD4. For chromosomal insertion of N-terminal sfGFP.	Brunet *et al.*, 2015
pKD4-Cter-mCherry	mCherry inserted on pKD4. For chromosomal insertion of C-terminal mCherry.	Brunet *et al.*, 2015
pcDNA3 APEX2-NES	APEX2-coding vector.	Lam *et al.*, 2014 (Addgene #49386)
pKD4-Nter-APEX2	APEX2 inserted on pKD4. For chromosomal insertion of N-terminal APEX2.	This study
pKD4-P*sci1*	*sci1* promoter inserted on pKD4. For chromosomal insertion of *sci1* promoter.	This study
pKD4-P*sci1*-$_V$TagA	VSV-G-tagged TagA inserted on pKD4-P*sci1*. For chromosomal insertion of VSV-G-tagged TagA under the control of the *sci1* promoter.	This study
pKD4-P*sci1*-TagA	TagA inserted on pKD4-P*sci1*. For chromosomal insertion of TagA under the control of the *sci1* promoter.	This study
pUA66-*rrnB*	Kan^R, *gfpmut2* gene under the control of the constitutive ribosomal *rrnB* promoter	Zaslaver *et al.*, 2006
pKOBEG	Cat^R. Vector encoding the λ-red recombination system under P*araBAD* promoter.	Chaveroche *et al.*, 2000
pCP20	Cat^R. Amp^R. Vector encoding the FRT-specific recombinase.	Datsenko and Wanner, 2000

pBAD33	Cat^R. Cloning vector. P*araBAD* promoter.	Guzman *et al.*, 1995
pBAD-$_V$TagA	VSV-G-tagged TagA cloned in pBAD33, under the control of the P*araBAD* promoter.	This study
pBAD-TssB-mCherry	TssB-mCherry cloned in pBAD33, under the control of the P*araBAD* promoter.	Zoued *et al.*, 2013
pT18N	Amp^R, T18 domain of the *Bordetella pertussis* adenylate cyclase.	Karimova *et al.*, 1998
pKT25N	Kan^R, T25 domain of the *Bordetella pertussis* adenylate cyclase.	Karimova *et al.*, 1998
pKT25N-TssA	*tssA* cloned downstream of the T25 domain.	Zoued *et al.*, 2013
pKT18N-TagA	*tagA* cloned downstream of the T18 domain.	This study
pT18-Pal	*pal* devoid of signal sequence cloned downstream of the T18 domain.	Battesti and Bouveret, 2008
pTolB-T25	*tolB* devoid of signal sequence cloned downstream of the T25 domain.	Battesti and Bouveret, 2008

Supplementary Table 3. Oligonucleotides used in this study

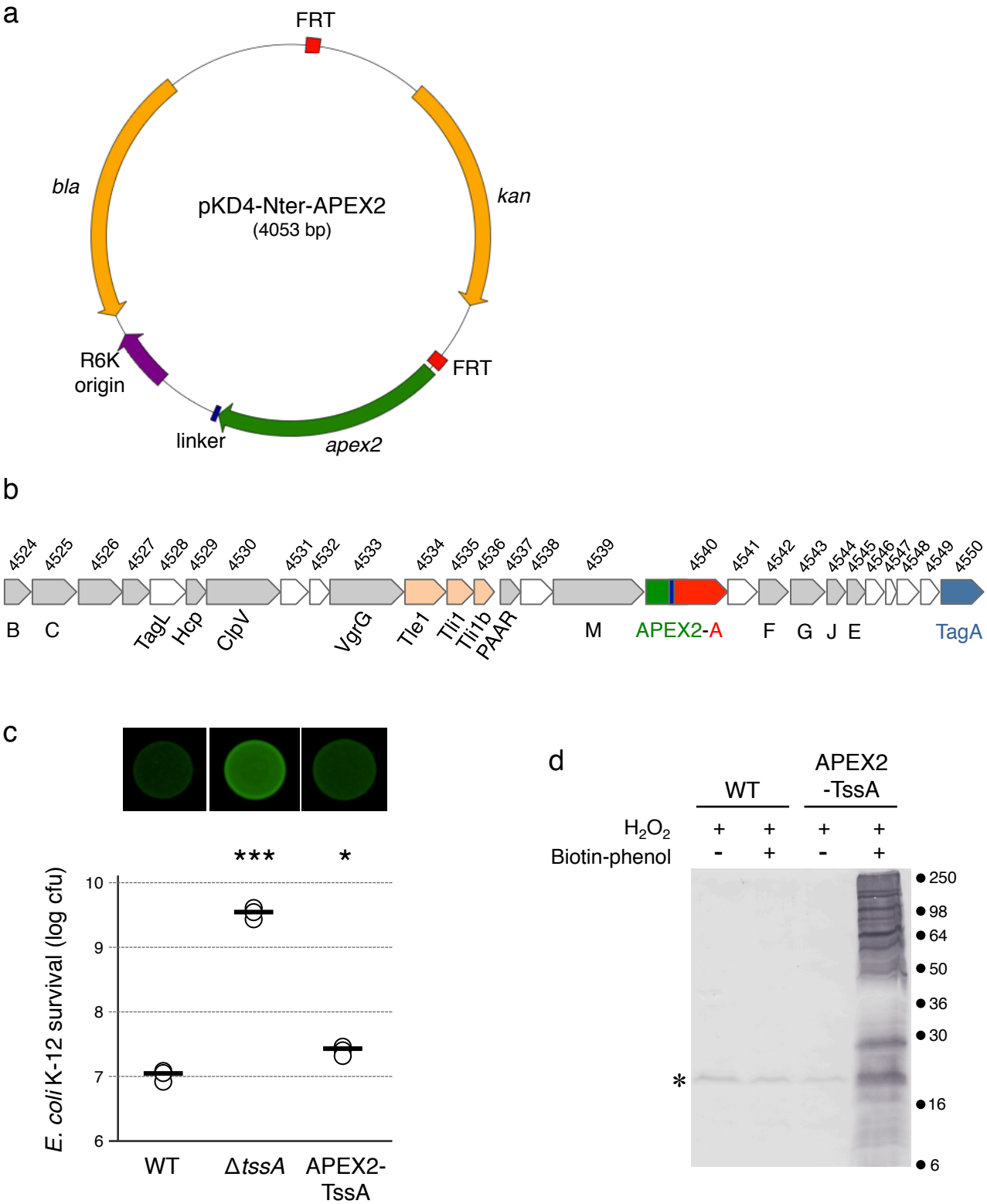
Name	Destination	Sequence (5' to 3')
For strain construction [a]		
5-*APEX2-tssA*	insertion of *apex2* downstream of the *tssA* start codon.	ACGTCAGCGTTTACAGGCAATGACGAAGATGCCGGGGATACCGTGGAGGAGACTGACTGACGATTGTGTAGGCTGGAGCTGCTTCGAAGTTCCTATAC
3-*APEX2-tssA*	insertion of *apex2* downstream of the *tssA* start codon.	CGGTTCAGCCACATCCCGGGGTGTCGTCTGGCATGCACTGAGGAGCGAATGAATGGAAGCCCCTCCGCCGGCCGCTGCCTCC
5-*APEX2-gspE*	insertion of *apex2* downstream of the *gspE* start codon.	GCCTTACCGCCGGAAGTTCGCGCATTCCTCAATGCCGGGAGAACGCGTTATGTGTAGGCTGGAGCTGCTTCG
3-*APEX2-gspE*	insertion of *apex2* downstream of the *gspE* start codon.	CTGTAGGGCAGACGCACGGTGTTAGCGGTGGTTTCCTGTGCTACAGGCACCCCTCCGCCGGCCGCTGCCTCC
5-*APEX2:tssA*	insertion of *apex2* at the *tssA* locus	ACGTCAGCGTTTACAGGCAATGACGAAGATGCCGGGGATACCGTGGAGGAGACTGACTGACGATTGTGTAGGCTGGAGCTGCTTCGAAGTTCCTATAC

3-*APEX2:tssA*	insertion of *apex2* at the *tssA* locus	CCCGGGGTGTCGTCTGGCATGCACTGAGGAGCGAATGAATGGAAGCCATTAGCACTTCCTCCTTAAGCTTACTCCAGGGGAGGCAGCTGCAGGGC
5-Δ*tagA*	*tagA* deletion	CACTGATGTTCTTTTCGTCACTGTTAATCATGATTTAATACAGCAACACCGAATCTGCCGTGTGTAGGCTGGAGCTGCTTCG
3-Δ*tagA*	*tagA* deletion	AGAAGAGAAGCACTATACTCTGCAGTGCCTTTAGAAAATTATTCTCCAAAAGATGGAATACATATGAATATCCTCCTTAGTTCC
5-*gfp-tagA*	insertion of *gfp-mut2* downstream of the *tagA* start codon.	CTTTTCGTCACTGTTAATCATGATTTAATACAGCAACACCGAATCTGCCGCGATTGTGTAGGCTGGAGCTGCTTCGAAGTTCCTATAC
3-*gfp-tagA*	insertion of *gfp-mut2* downstream of the *tagA* start codon.	TCCGGTAATGACCGGGGGTCACCACCGGTTTTCAGTTTCACTTCAGAAGTCCCTCCGCCGGCCGCTGC
5-*tssK-mCherry*	insertion of *mCherry* upstream of the *tssK* stop codon.	TTCTACACCCCGGCATCGCTGGGAGATGTGAAACTGGAACTTTTTGCGGTGCTGCGGACAGCAGCGGCCGGCGGAGGG
3-*tssK-mCherry*	insertion of *mCherry* upstream of the *tssK* stop codon.	GGCTGACCATCAGCCAGCCGGGATAAAAAATCTGTTCAGCCCGGGAGATAACAGGTTTATTCATATGAATATCCTCCTTAGTTCCTATTCCGAAGTTCC
5-P*sci1-tagA*	insertion of P*sci1-V-tagA or* P*sci1-tagA* at the *lacZ* locus	TGGACCATTTCGGCACAGCCGGGAAGGGCTGGTCTTCATCCACGCGCGCGCGATTGTGTAGGCTGGAGCTGCTTCGAAGTTCCTATAC
3-P*sci1-tagA*	insertion of P*sci1-V-tagA or* P*sci1-tagA* at the *lacZ* locus	GAAGGCGGCGGAGCCGACACCACGGCCACCGATATTATTTGCCCGATGTATCACTTCATGTCCCCTTGCGTCAAAACCCAG

For plasmids construction [b, c]		
KD4-Nt-APEX2-5	insertion of *APEX2* into pKD4	*CGGAATAGGAACTAAGGAGGATATTCAT*ATGGGAAAGTCTTACCCAACTGTGAGTGC
KD4-Nt-APEX2-3	insertion of *APEX2* into pKD4	*CGGCTGACATGGGAATTAGCCATGGTC*CCCTCCGCCGGCCGCTGCGTCCAGGGTCAGGCGCTCC
KD4-P*sci1*-5	insertion of the *sci1* promoter into pKD4	*CGGAATAGGAACTAAGGAGGATATTCAT*CGCACCATGATCGTCTCTGTATCG
KD4-P*sci1*-3	insertion of the *sci1* promoter into pKD4	*CGGCTGACATGGGAATTAGCCATGGTC*CATGGCTCTCTCCTGTGAAACCTG

KD4-P*sci1*-TagA-5	insertion of *tagA* into pKD4-P*sci1*	*CAGGTTTCACAGGAGAGAGCCATG*ACTTCTGAAGTGAAACTGAAAACCGGTGG
KD4-P*sci1*-$_V$TagA-5	insertion of VSV-G-tagged *tagA* into pKD4-P*sci1*	*CAGGTTTCACAGGAGAGAGCCATG***TATACAGATATTGAAATGAATAGATTAGGAAAA**ACTTCTGAAGTG
KD4-P*sci1*-TagA-3	insertion of *tagA* or VSV-G-tagged *tagA* into pKD4-P*sci1*	*CGGCTGACATGGGAATTAGCCATGGTC*TCACTTCATGTCCCCTTGCGTCAAAAC
T18N-5-TagA-Ct	insertion of *tagA* downstream of the T18 domain	*CGCCACTGCAGGGATTATAAAGATGACGATGACAAG*ACTTCTGAAGTGAAACTGAAAACCGGT
T25T18N-3-TagA	insertion of *tagA* downstream of the T18 or T25 domain	*CGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTAG*TTACTTCATGTCCCCTTGCGTCAAAAC
BAD33-VSVG-TagA-5	insertion of *tagA* into pBAD33	*CTCTCTACTGTTTCTCCATACCCGTTTTTTTGGGCTAGC*AGGAGGTATTACACCATG**TATACAGATATTGAAATGAATAGATTAGGAAAA**ACTTCTGAAGTGAAACTGAAAACCGGTGG
BAD33-VSVG-TagA-3	insertion of *tagA* into pBAD33	*GGTCGACTCTAGAGGATCCCCGGGTACC*TCACTTCATGTCCCCTTGCGTCAAAAC

[a] sequence corresponding to the downstream or upstream region of the gene to be deleted, disrupted or of the site of insertion underlined.
[b] sequence annealing on the target plasmid *italicized*.
[c] VSV-G epitope coding sequence in **bold**


a
FRT
bla
kan
pKD4-Nter-APEX2
(4053 bp)
R6K
origin
FRT
linker
apex2
b
4524
4525
4526
4527
4528
4529
4530
4531
4532
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4534
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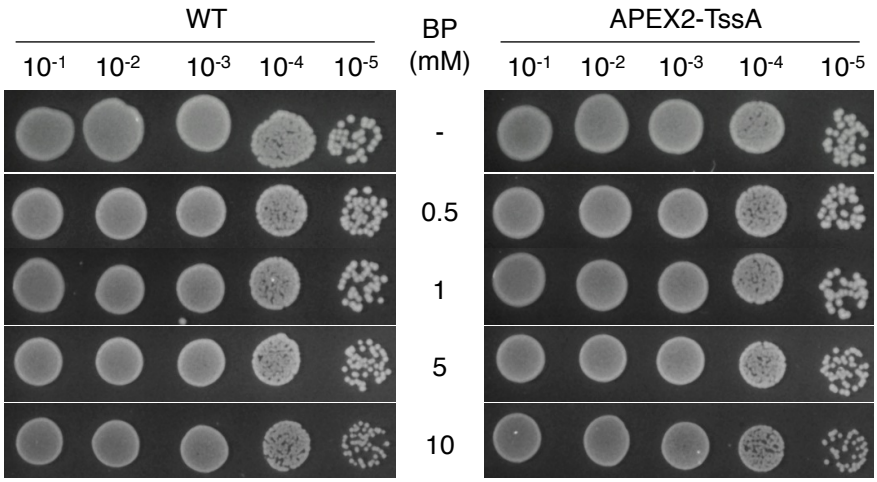
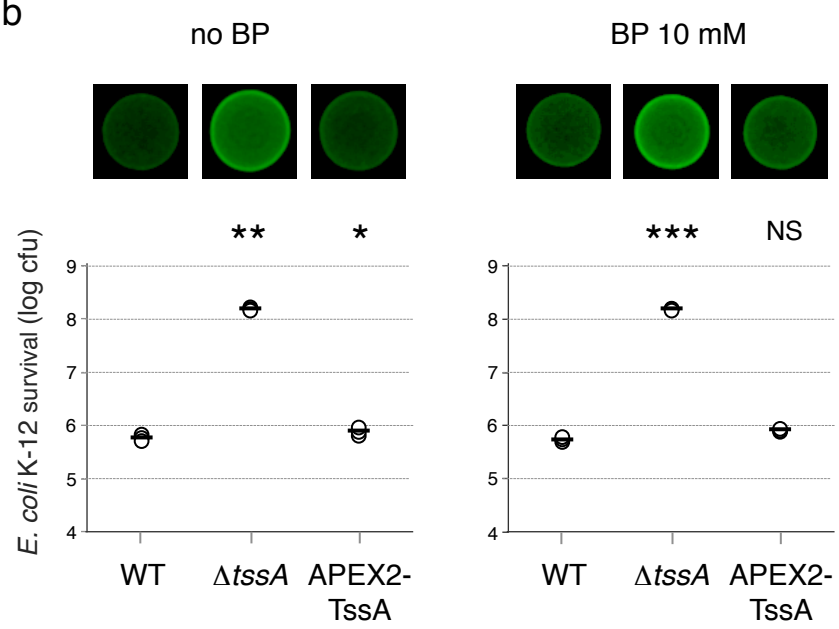
4546
4547
4548
4549
4550
B
C
TagL
Hcp
ClpV
VgrG
Tle1
Tli1
Tli1b
PAAR
M
APEX2-A
F
G
J
E
TagA
c
***
*
E. coli K-12 survival (log cfu)
10
9
8
7
6
WT
ΔtssA
APEX2-
TssA
d
WT
APEX2
-TssA
H2O2
+
+
+
+
Biotin-phenol
-
+
-
+
*
250
98
64
50
36
30
16
6

Supplementary Figure 1 | Construction and function of the APEX2-TssA fusion.
a, Map of the pKD4-Nter-APEX2 vector (Addgene #112868). The different features are indicated: resistance genes (*bla*, *kan*; orange), origin of replication (R6K, purple), FRT sites for Flp-dependent recombination (FRT, red), APEX2-coding sequence (*apex2*, green) and the sequence coding a 3×Ala-3×Gly linker in frame with *apex2* (blue). **b,** Genetic organization of the T6SS gene cluster of the wild-type strain engineered to produce the APEX2-TssA fusion. Names are indicated below each gene (B corresponds to TssB). T6SS core components are shaded grey, toxin and immunity are shown in light orange, and accessory components and genes of unknown function are shaded white. The *apex2-tssA* fusion is colored green (*apex2*), blue (linker) and red (*tssA*). The *tagA* gene, identified in this study is shaded in light blue. **c,** The APEX2-TssA fusion is capable of conferring T6SS-dependent antibacterial activity. *E. coli* K-12 competitor cells (W3110 gfp^+, kan^R) were mixed with the indicated attacker cells, spotted onto SIM agar plates, and incubated for 4 h at 37 °C. A representative image of the fluorescent spot is shown on top. The number of surviving *E. coli* competitor cells (counted on selective kanamycin medium) is indicated in the lower graph (in $\log_{10}$ of colony-forming unit). The circles indicate the values from three independent assays, and the average is indicated by the bar. Statistical significance relative to the wild-type strain is indicated (*p*-values; NS, non significant; *, $p < 0.05$; ***, $p < 0.001$, two-sided Student's *t*-test; *p*-values Δ*tssA*, 3.8×10^{-5}; APEX2-TssA, 0.018). Anti-bacterial activity assays have been performed in triplicate with identical results. **d,** The APEX2-TssA fusion is capable of biotinylating proteins in presence of biotin phenol and H_2O_2. Cell lysates of the wild-type strain and its APEX2-TssA derivatives were treated with H_2O_2 in presence (+) or absence (-) of biotin phenol. The total proteins were separated by SDS-PAGE and biotinylated proteins were detected using streptavidin coupled to phosphatase alkaline. The star (*) indicates the biotin carboxyl carrier protein (BCCP), the major naturally biotinylated protein in *E. coli*. Molecular weight markers (in kDa) are indicated on right. The uncropped blot is shown in Supplementary Fig. 7. The experiment has been performed in duplicate with identical results.

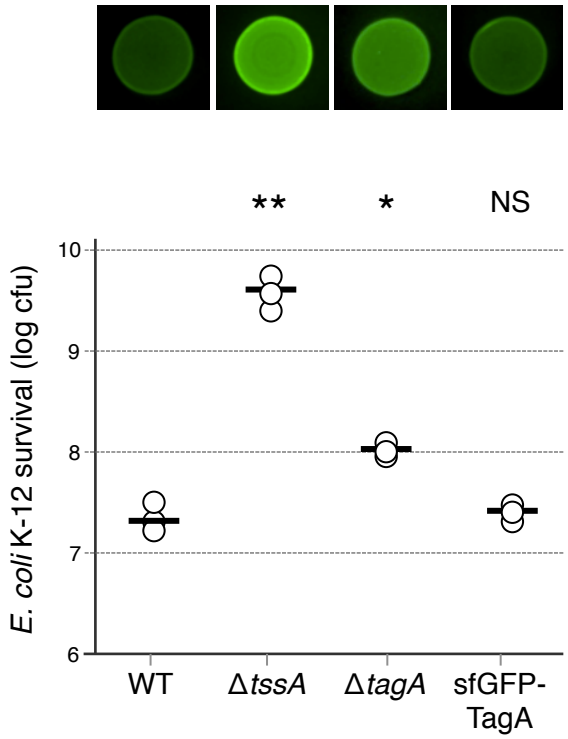
a


WT
APEX2-TssA
BP (mM)
10-1 10-2 10-3 10-4 10-5
10-1 10-2 10-3 10-4 10-5
-
0.5
1
5
10

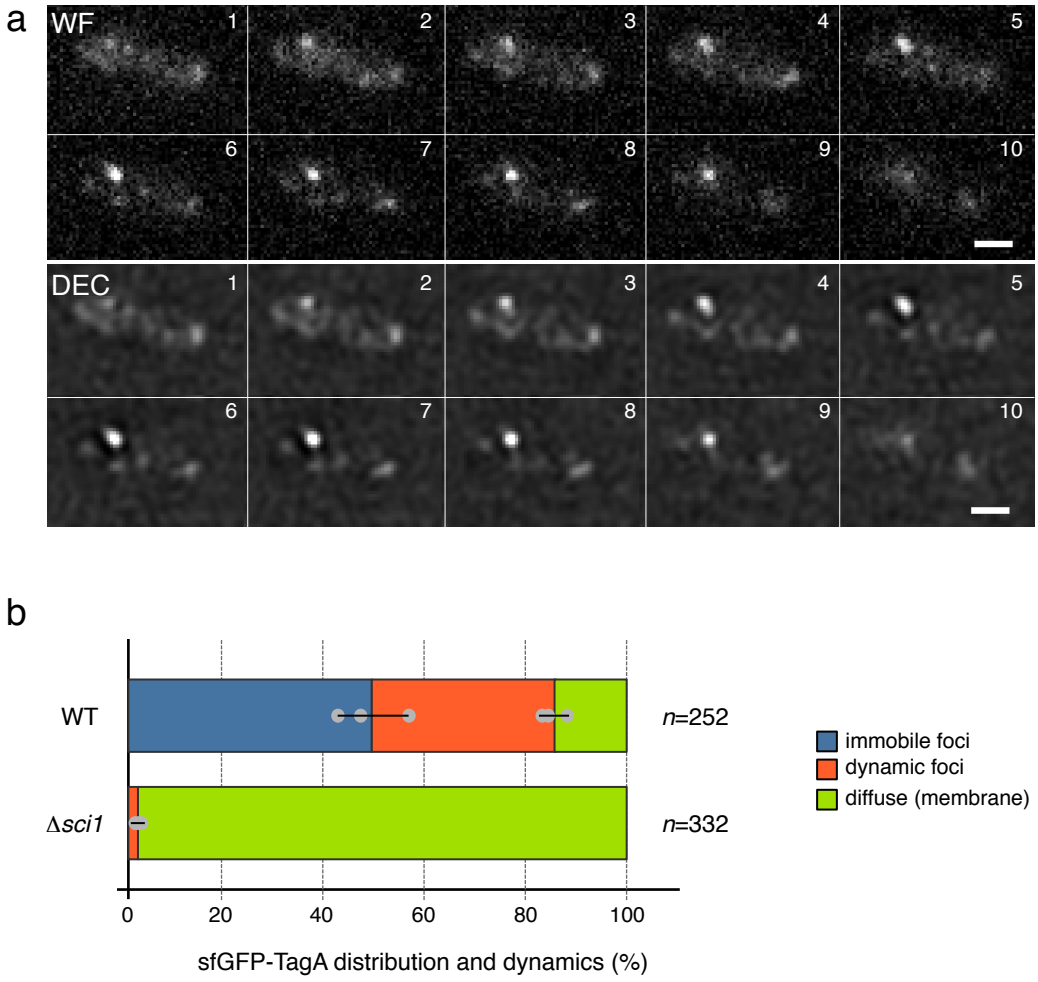
b


no BP
BP 10 mM
**
*
***
NS
E. coli K-12 survival (log cfu)
9
8
7
6
5
4
WT
ΔtssA
APEX2-TssA
WT
ΔtssA
APEX2-TssA

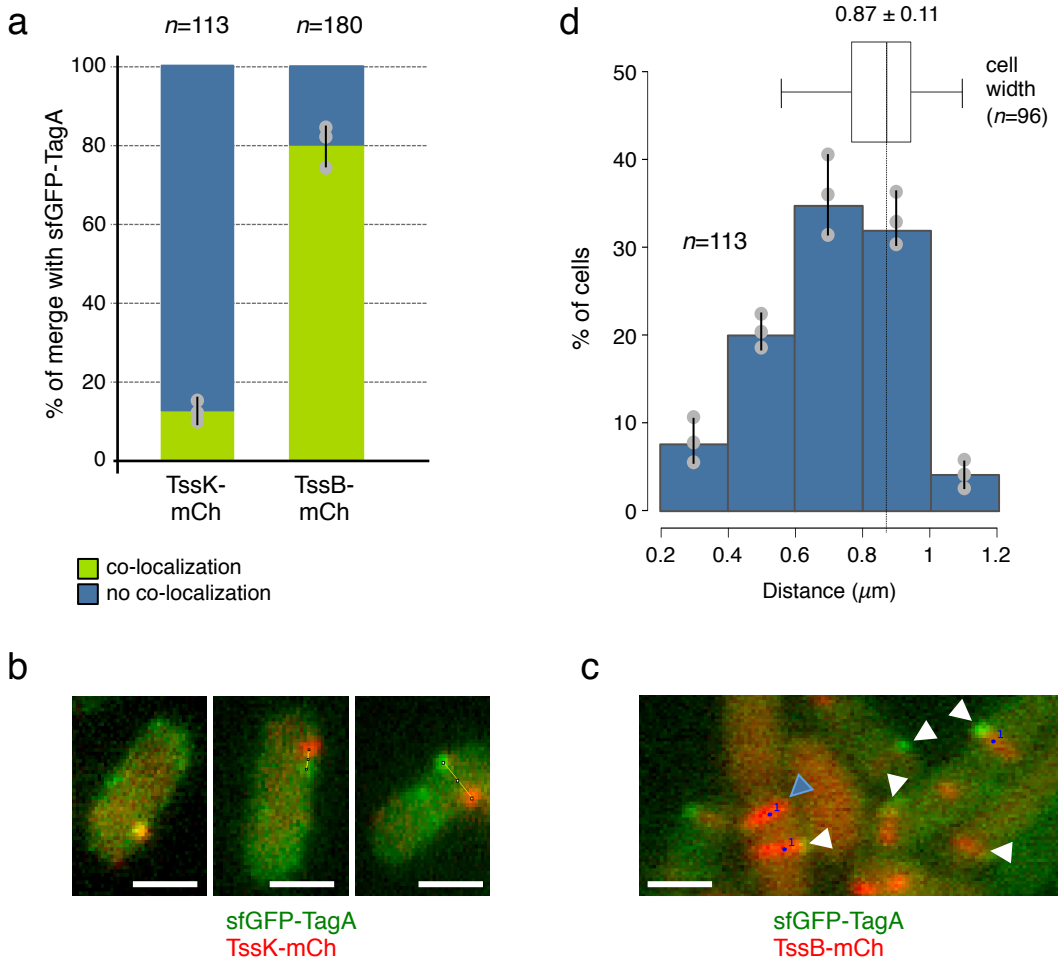
Supplementary Figure 2 | Biotin-phenol does not impact bacterial growth and T6SS activity.
a, Biotin-phenol does not impact bacterial cell growth. Serial 10-fold dilutions of wild-type and APEX2-TssA cell cultures were spotted on SIM plates supplemented with the indicated concentration of biotin-phenol (BP, in mM). The experiment has been performed with a technical triplicate with identical results. **b,** Biotin-phenol does not impact T6SS activity. *E. coli* K-12 competitor cells (W3110 *gfp*$^{+}$, kan^{R}) were mixed with the indicated attacker cells, spotted onto SIM agar plates supplemented with 10 mM biotin-phenol (BP 10 mM) or not (no BP), and incubated for 4 h at 37 °C. A representative image of the fluorescent spot is shown on top. The number of surviving *E. coli* competitor cells (counted on selective kanamycin medium) is indicated in the lower graph (in $\log_{10}$ of colony-forming unit). The circles indicate the values from three independent assays, and the average is indicated by the bar. Statistical significance relative to the wild-type strain is indicated (*p*-values; NS, non significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, two-sided Student's *t*-test; *p*-values in absence of BP: Δ*tssA*, 0.00187; APEX2-TssA, 0.045; *p*-values in presence of BP: Δ*tssA*, 0.00085; APEX2-TssA, 0.079). Anti-bacterial activity assays have been performed in triplicate with identical results.



Supplementary Figure 3 | sfGFP-TagA is functional.
Antibacterial competition assay. *E. coli* K-12 competitor cells (W3110 *gfp*$^{+}$, kan^{R}) were mixed with the indicated attacker cells, spotted onto SIM agar plates, and incubated for 4 h at 37 °C. A representative image of the fluorescent spot is shown on top. The number of surviving *E. coli* competitor cells (counted on selective kanamycin medium) is indicated in the lower graph (in $\log_{10}$ of colony forming units). The circles indicate the values from three independent assays, and the average is indicated by the bar. Statistical significance relative to the wild-type strain is indicated (*p*-values; NS, non significant; *, $p < 0.05$; **, $p < 0.01$, two-sided Student's *t*-test; *p*-values: Δ*tssA*, 0.00246; Δ*tagA*, 0.036; GFP-TagA, 0.117). The experiment has been performed in triplicate with identical results.

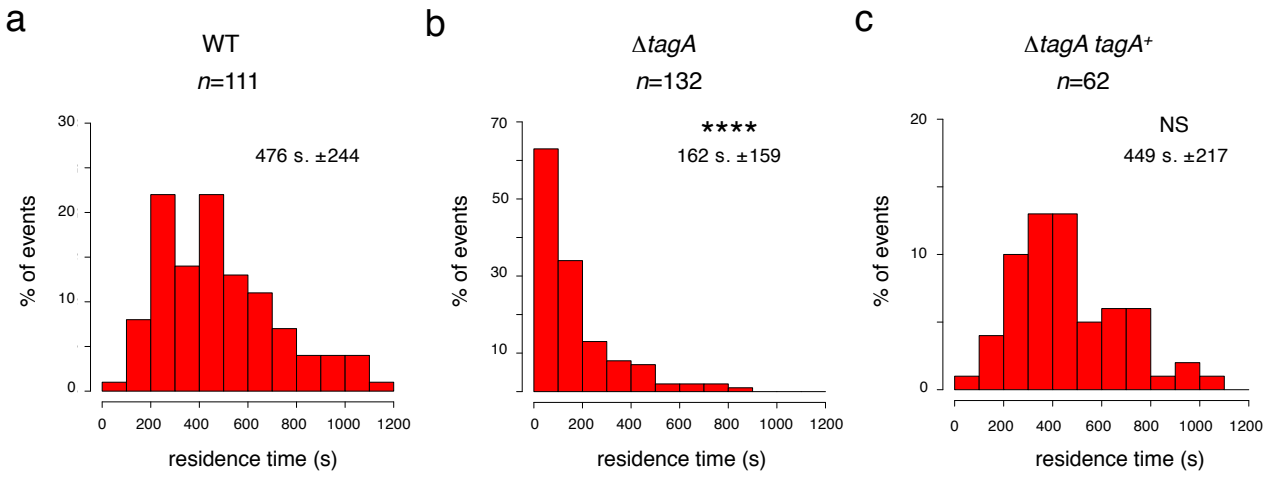


Supplementary Figure 4 | sfGFP-TagA localization and dynamics.
a, Deconvolution analyses of cells producing sfGFP-TagA. Widefield (WF) image and deconvolution analysis (DEC) of a representative wild-type EAEC cell producing sfGFP-TagA (1 to 10, from top to bottom). Scale bar, 1 µm. The deconvolution analysis has been performed in duplicate with identical results. **b,** sfGFP-TagA dynamics. Statistical analyses of the distribution of sfGFP-TagA dynamics in wild-type (WT) and Δ*sci1* (*i.e.*, lacking all T6SS genes) cells. The percentage of immobile foci (blue), dynamic foci (red) or of membrane-associated diffuse patches (green) is indicated. Standard deviation is indicated by the horizontal bar, and dot plots (grey circles) are overlaid. The number of cells analyzed (*n*) is indicated for each strain (from three biological replicates) on right.

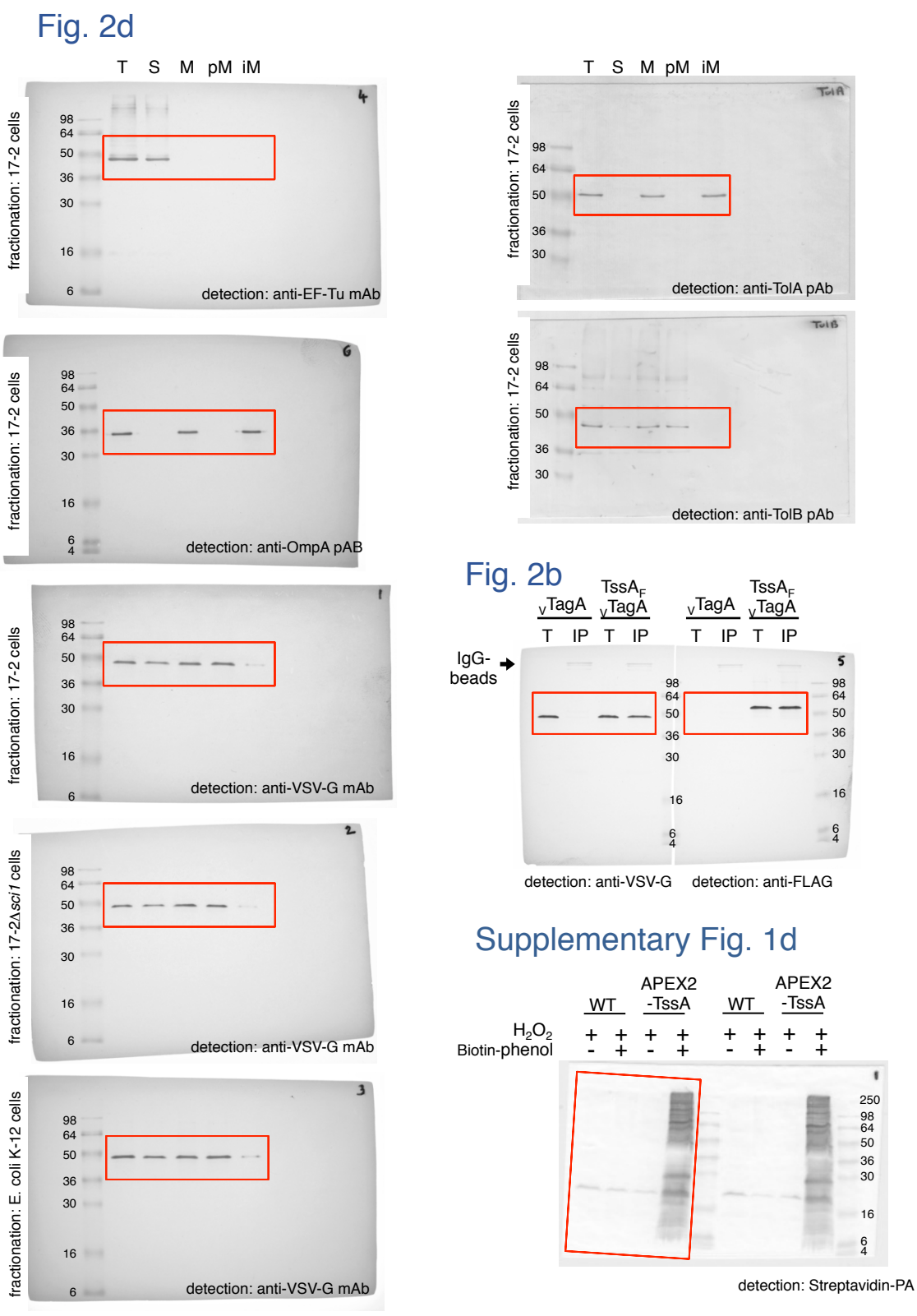


Supplementary Figure 5 | Statistical analyses of co-localization of TagA with TssK and the distal end of the elongated sheath.

a, Statistical analyses of the distribution of co-localization of sfGFP-TagA with TssK-mCherry or with the distal end of TssB-mCherry sheaths is represented as the percentage of analyzed cells (green, overlap; blue, no overlap). The number of cells analyzed is indicated on top (*n*, from three independent biological replicates). Standard deviation are indicated by the vertical bar, and dot plots (grey circles) are overlaid. Exemples of the different events are shown in panels **b** (TssK-mCherry; left, overlap; middle and right, no overlap) and **c** (TssB-mCherry; white arrowhead, overlap; blue arrowhead, no overlap). Scale bars, 1 µm. Co-localization recordings have been performed three and five times for TssK-mCherry and TssB-mCherry, respectively, with identical results. **d,** Statistical analyses of the distribution of the distance between TssK-mCherry and sfGFP-TagA foci. The number of foci is plotted against the distance (in mm). The number of cells analyzed (n = 113) is indicated (from three biological replicates, bars represent the average, standard deviation are indicated, dot plots (grey circles) are overlaid). A box-and-whisker representation of the width of EAEC cells (in SIM medium) is shown on the top (lower and upper boundaries of the box plot correspond to the 25% and 75% percentiles, respectively; the vertical bar represents the median value and the whiskers represent the 10% and 90% percentiles. The mean width and its standard deviation (0.87 ± 0.11) is indicated on top (n = 96, from five biological replicates).



Supplementary Figure 6 | Distribution of the sheath residence time.
Percentage of sheaths that contracts at the indicated residence time (in s) in wild-type (WT, **a**), Δ*tagA* (**b**) and Δ*tagA tagA*$^{+}$ cells (**c**) (n = 111, n = 132 and n= 62, respectively, from three biological replicates). The mean of residence time (in s), the standard deviation and the statistical significance relative to WT (NS, non-significant; ****, $p < 0.0001$; two-sided Student's t-test) are indicated on top.



Supplementary Figure 7 | Uncropped whole blots of Figures 2b and d and Supplementary Figure 1d. Uncropped blots corresponding to each panel in Fig. 2b, 2d and Supplementary Fig. 1d are shown, including molecular weight markers lanes. Blots are labeled as shown in the corresponding Figure. Red rectangles correspond to the cropped portions of the blots used in the corresponding Figure. The antibodies used for each immuno-detection is indicated. For Fig. 2b, the position of the IgG beads are indicated by the arrows. Molecular weight markers (in kDa) are indicated on right (SeeBlue® pre-stained, Invitrogen).