Supporting Information

Membrane curvature and the Tol-Pal complex determine polar localization of the chemoreceptor Tar in *E. coli*

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Content:

Supporting Table S1: *E. coli* strains used in this study. kan: kanamycin, mGFP: monomeric green fluorescent protein.

strain	relevant genotype	reference
BW25113	F-, *Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(1)
TSS688	*Δtsr-7028 Δ(tar-tap)5201 Δtrg-100 Δaer-1*	(2)
JW0731-1	BW25113 *Δpal-790::kan*	(1)
JW0729-3	BW25113 *ΔtolA788::kan*	(1)
JW1877-1	BW25113 *ΔcheA741::kan*	(1)
JW1875-5	BW25113 Δtar-739::kan	(1)
TSE24	JW1875-5 / pTNV148	this work
TSE25	JW1875-5 / pTNV149	this work
TSE29	BW25113 / pTNV149	this work
TSE31	JW0731-1 / pTNV149	this work
TSE32	JW0729-3 / pTNV149	this work
TSE38	JW1877-1 /pTNV149	this work
TSE41	BW25113 / pTNV154	this work
TSE42	BW25113 / pTNV153	this work
TSE48	BW25113 / pTNV155	this work
TSE67	BW25113 / pTNV162	this work
TSE68	JW0731-1 / pTNV153	this work
TSE69	JW0731-1 / pTNV154	this work
TSE71	JW0731-1 / pTNV162	this work
TSE79	TSS688 / pTNV149	this work
TSE80	TSS688 / pTNV153	this work
TSE81	TSS688 / pTNV154	this work

Supporting Table S2: Plasmids used in this study. *gfp*: green fluorescent protein, *mgfp*: monomeric GFP, *camR*: chloramphenicol acetyltransferase, *ampR*: β-lactamase TEM-1.

Name	Relevant feature	reference
pBAD24-tar-GFP	pBAD-*tar-gfp-ampR*	(3)
pBAD24-tar-mGFP	pBAD-*tar-mgfp-ampR*	this work
p29SEN	Rep101 pSC101 ori *ampR*	(4)
pSAV57	pTrcDown, p15Aori camR	(5)
pTNV100	*amyE3'*-spec-PxylR-*mgfp*-mcs-*amyE5'* ampR	this work
pTNV107	pBAD-*tar-mgfp-ampR* pSC101 ori araC	this work
pTNV148	P*tar-tar-mgfp-ampR*-pSC101 ori	this work
pTNV149	Ptrc-*tar-mgfp-ampR*-pSC101 ori	this work
pTNV153	Ptrc-*tar*(G248G-D249G-L250G)-*mgfp-ampR* pSC101 ori	this work
pTNV154	Ptrc-*tar*(N379R)-*mgfp-ampR* pSC101 ori	this work
pTNV155	Ptrc-*mgfp-ftsN- ampR* pSC101	this work
pTNV162	Ptrc-*mgfp-glpT-ampR*-pSC101	this work

Supporting Table S3: Primers used in this study.

name	sequence	description
TerS327	CAGGAAGCGGCTCAGGATCCTGAGTAAACTTGGTCTGACAGT	Forward primer to linearize pBAD24-tar-mGFP
TerS328	CACCGTCATCACCGAAACGCGCGA	Reverse primer to linearize pBAD24-tar-mGFP
TerS362	GGATCCTGAGCCGCTTCCTGA	Reverse primer to amplify *mgfp* from pTNV100
TerS412	CGCATGGGGAGACCCCACACTACCA	Reverse sequencing primer for pBAD vectors
TerS418	CATGGTGAATTCCTCCTGCTAGCCCA	Reverse primer to amplify pBAD24 including its RBS and start codon
TerS425	GCGTTTCGGTGATGACGGTGCGGCGGCACCTCGCTAACGGA	Forward primer to amplify origin of replication of p29SEN (pSC101 Ori)
TerS426	GGATCCTGAGCCGCTTCCTGCTTGCGCGCACCGCCCGAACACCA	Reverse primer to amplify origin of replication of p29SEN (pSC101 Ori)
TerS457	GCCGCGGTTGAAGCCGCGCGTGCGGGT	Forward primer to amplify tar in pTNV149
TerS506	GTGTGAAATTGTTATCCGCTCACA	Reverse primer to amplify Ptrcdown promoter with lacI from pSAV057
TerS507	GAGCGGATAACAATTTCACACACCCGTTTTTTTGGGCTAGCAGGAGGA	Forward primer to insert Ptrc-down promoter into pTNV107
TerS508	CGGCTTGACGGAGTAGCATAGGGT	Forward primer to insert *tar* native promoter into pTNV107
TerS509	TATGCTACTCCGTCAAGCCGGGCGCTGTTAGATAGCGCGGCGTCAGAAGTGGCGTAA	Reverse primer to insert *tar* native promoter into pTNV107
TerS510	GCCGCCTGGGCGCTGCGACTGGCA	Reverse primer to amplify inside *tar*
TerS515	CGCAGTGAAATGGGCGGAGGA[1]GCGCAGAGCGT	Forward primer to design Tar(G248G D249G L250G) in pTNV149
TerS516	CTGCGCTCCTCC[1]GCCCATTTCACTGCGCCCGTCA	Reverse primer to design Tar(G248G D249G L250G) in pTNV149
TerS517	CGCGCGGCTTCAACCGCGGCTCT[1]CAGCGCGAGGATATTAGTCT	Mutagenesis primer to introduce N379R in Tar in pTNV149
TerS520	CCGGGCAGGCCATGTCTGCCAGCGGCCGCGACTCTAGAATTCG	Forward primer to linearize pTNV149
TerS521	CTAGCAGGAGGAATTCACCATGAGCAAAGGAGAAGAACTTTTCACT	Forward primer to amplify *mgfp* from pTNV100
TerS523	GGCAGACATGGCCTGCCCGGGGGGGGGATTTTGAGGGTTTCA	Reverse primer to amplify ftsN to design Pspac-msfGFP-4GS-FtsN
TerS541	CAGGAAGCGGCTCAGGATCCGCACAACGAGATTATGTACGCCGCA	Forward primer to create Pspac-msfGFP-4GS-FtsN
TerS544	GGCAGACATGGCCTGCCCGGGCCATTAGCCTCCGTTGCGTTCTTGCA	Reverse primer to amplify *glpT*
TerS545	CAGGAAGCGGCTCAGGATCCATGTTGAGTATTTTTAAACCAGCGCCA	Forward primer to amplify *glpT*
GFP(A206K)-for	CCTGTCGACACAATCTAAACTTTCGAAAGATCCC	Forward primer to introduce A206K in GFP in pBAD24-tar-GFP
GFP(A206K)-rev	GGGATCTTTCGAAAGTTTAGATTGTGTCGACAGG	Reverse primer to introduce A206K in GFP in pBAD24-tar-GFP

1: In red codons used to introduce mutations in amino acid sequences.

Fig. S1

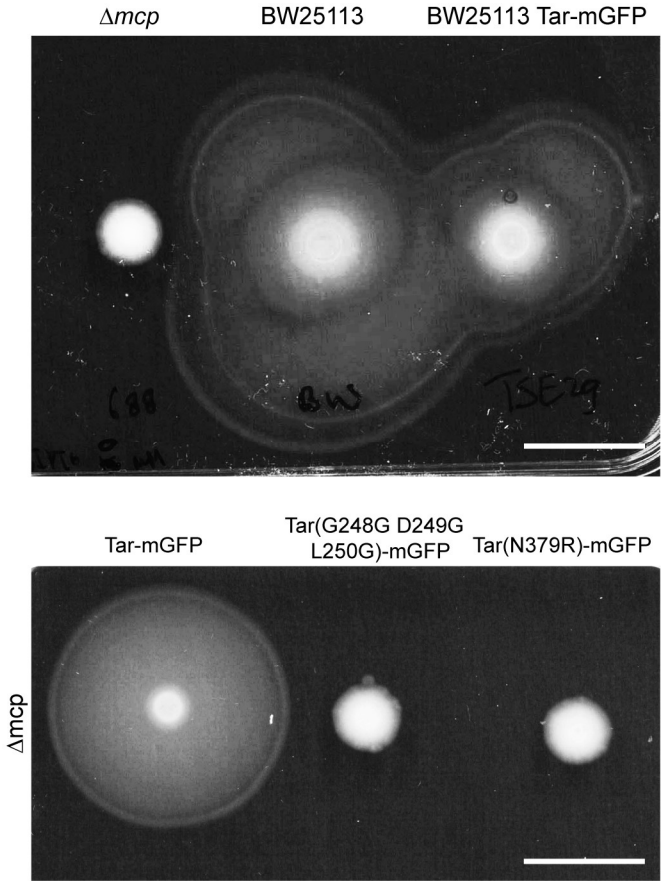

Fig. S1. Swarming motility assay

Swarming motility assay to test functionality of Tar-mGFP fusions. Representative image of colonies of *E. coli* Δmcp, *E. coli* BW25113, *E. coli* BW25113 + Tar-mGFP, *E. coli* Δmcp + Tar-mGFP, *E. coli* Δmcp + Tar(G488G D249G L250G)-mGFP and *E. coli* Δmcp Tar(N379R)-mGFP after 18 hours of growth at 30 °C on swarming motility agar. Fusion proteins were induced with 15 μM IPTG. Scale bars are 2 cm. Strain used are TSS668, BW25113, TSE29, TSE79, TSE80, and TSE81.

Fig. S2

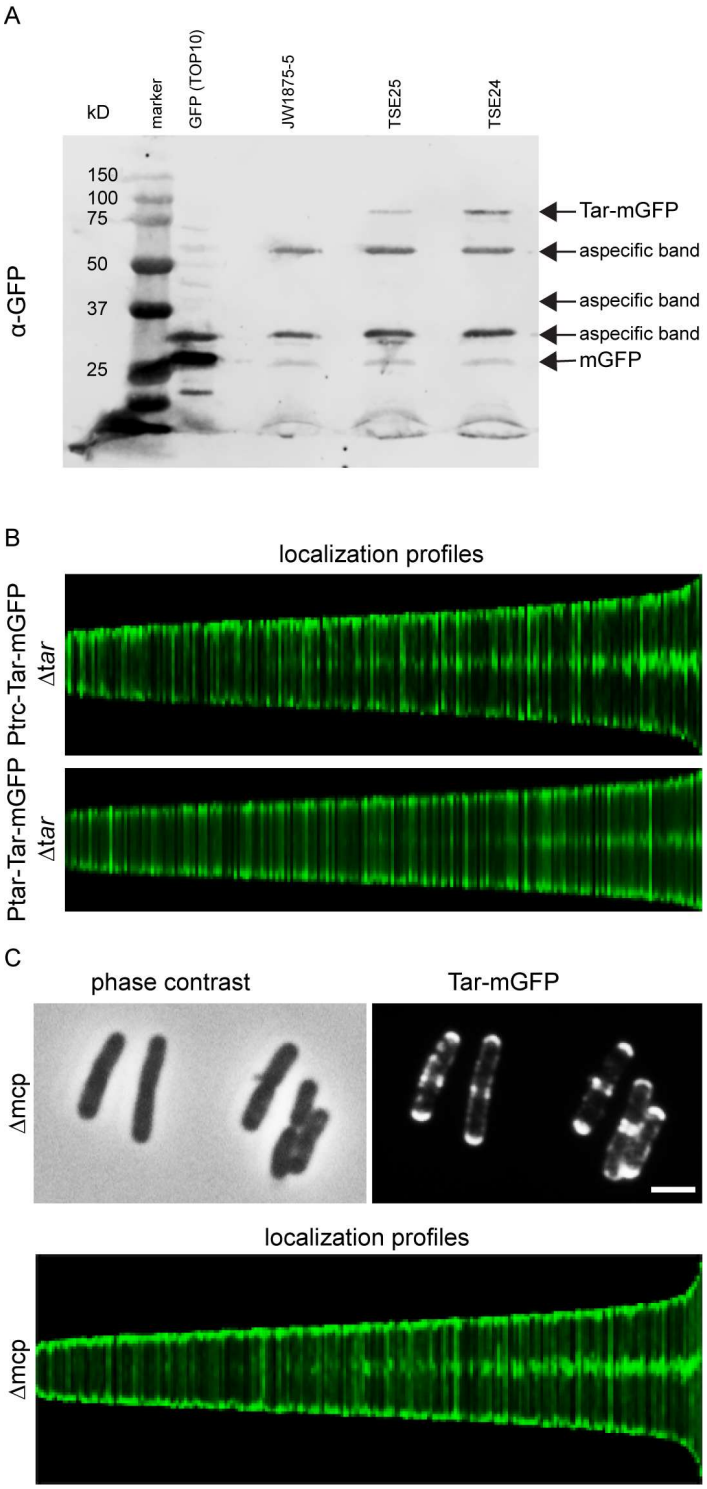

Fig. S2. Tar-mGFP expressed from native promoter and IPTG-inducible promoter.

(A) Westernblot using anti-GFP serum of cell extracts from cells constitutively expressing GFP (*E. coli* TOP 10 / pTNV100), Δ*tar* cells (JW1875-5), and cells expressing Tar-mGFP (TSE25 and TSE24). Tar-mGFP was expressed from the native promotor of *tar* (strain TSE25) or from

the IPTG-inducible (15 μM IPTG) *Ptrc* promoter (strain TSE24). The expected size for the fusion proteins is 87 kDa. Expression from the pBAD promoter is approximately 2x that of the native promoter. (B) Comparison of Tar-mGFP localization when expressed from the Ptrc promoter (upper panel) or native promoter (lower panel). 4630 and 4111 cells were used to contruct the localization profiles, respectively. (C) Phase contrast and fluorescence microscopy images and localization profile of Tar-mGFP expressed in a Δ*mcp* mutant lacking all chemoreceptors (strain TSE79). 2089 cells were used to construct the localization profile. Scale bar is 2 μm.

Fig.S3

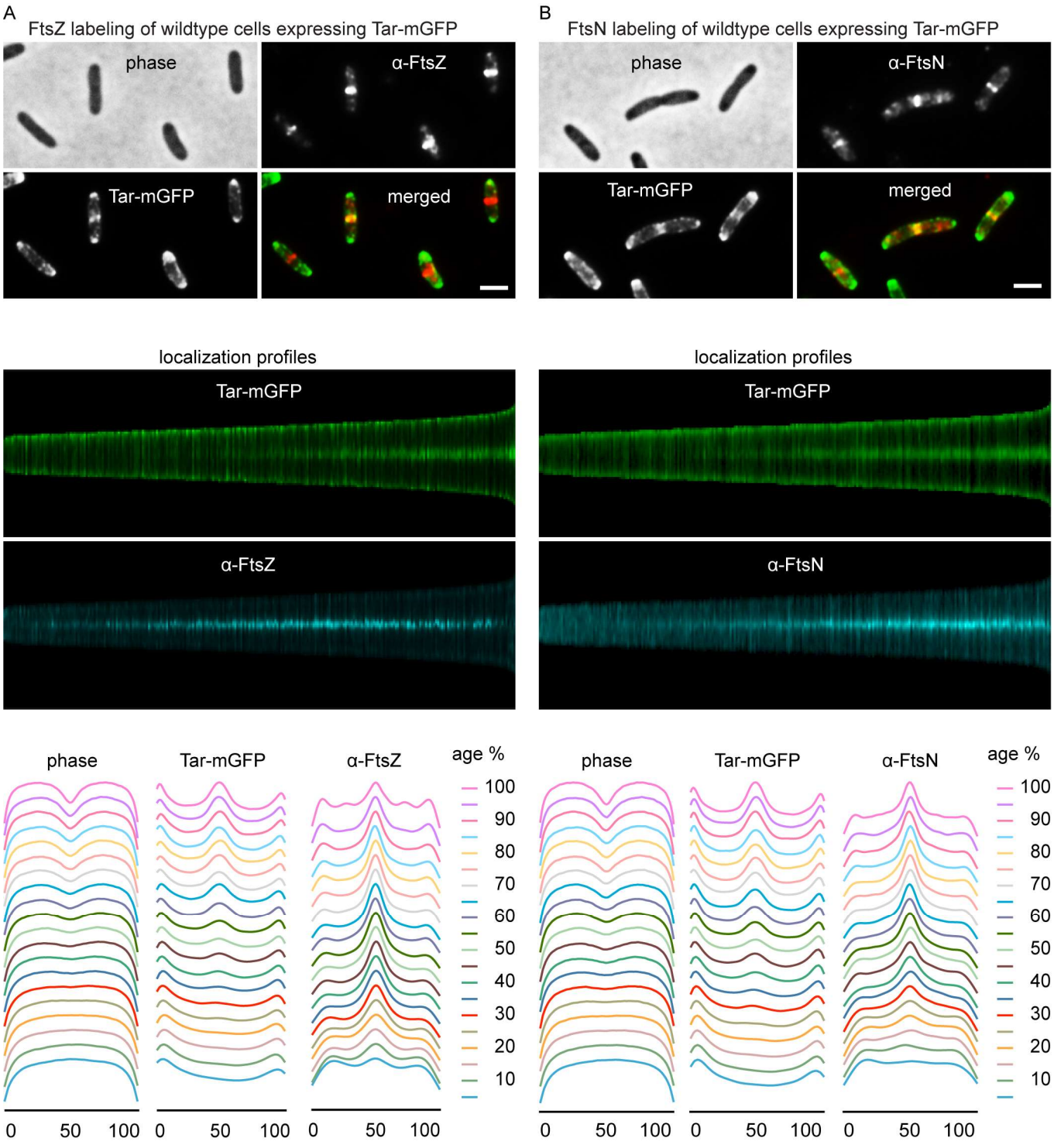

Fig. S3. Timing of Tar-mGFP midcell localization compared to FtsZ and FtsN. Tar-mGFP expressing cells were fixed and immune-labelled using FtsZ (A) and FtsN (B) antiserum. Top panel: fluorescence light microscopy images, middle panel: fluorescence localization profiles,

lower panel: graphical presentation of constriction (phase) and fluorescence signals during the cell cycle calculated from the localization profiles. Cell age refers to age classes divided in 5% (cell cycle) bins. 4816 and 3639 cells were used for FtsZ and FtsN labeling, respectively. Scale bars are 2 μm and the strain used is TSE29.

Fig. S4

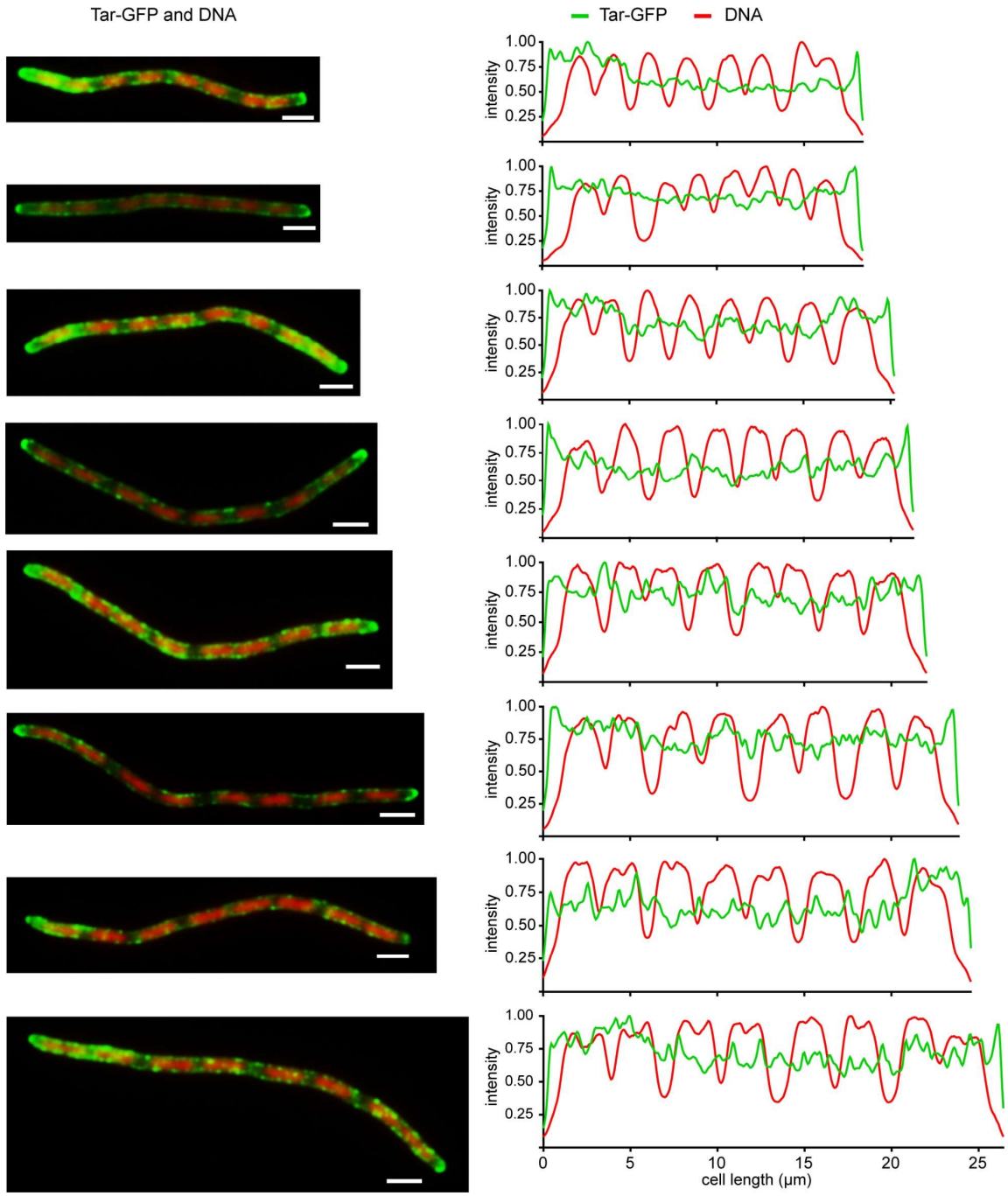

Fig. S4. Tar-GFP localization is unrelated to nucleoid position

Fluorescence microscopy images of cephalexin treated (4 h) cells expressing Tar-mGFP and stained with DAPI. Line scans of the GFP and DAPI signals are shown in the right panels. These images are more examples of cells shown in Fig. 2B in the main text. Scale bars are 2 μm. Strain used is TSE29.

Fig.S5

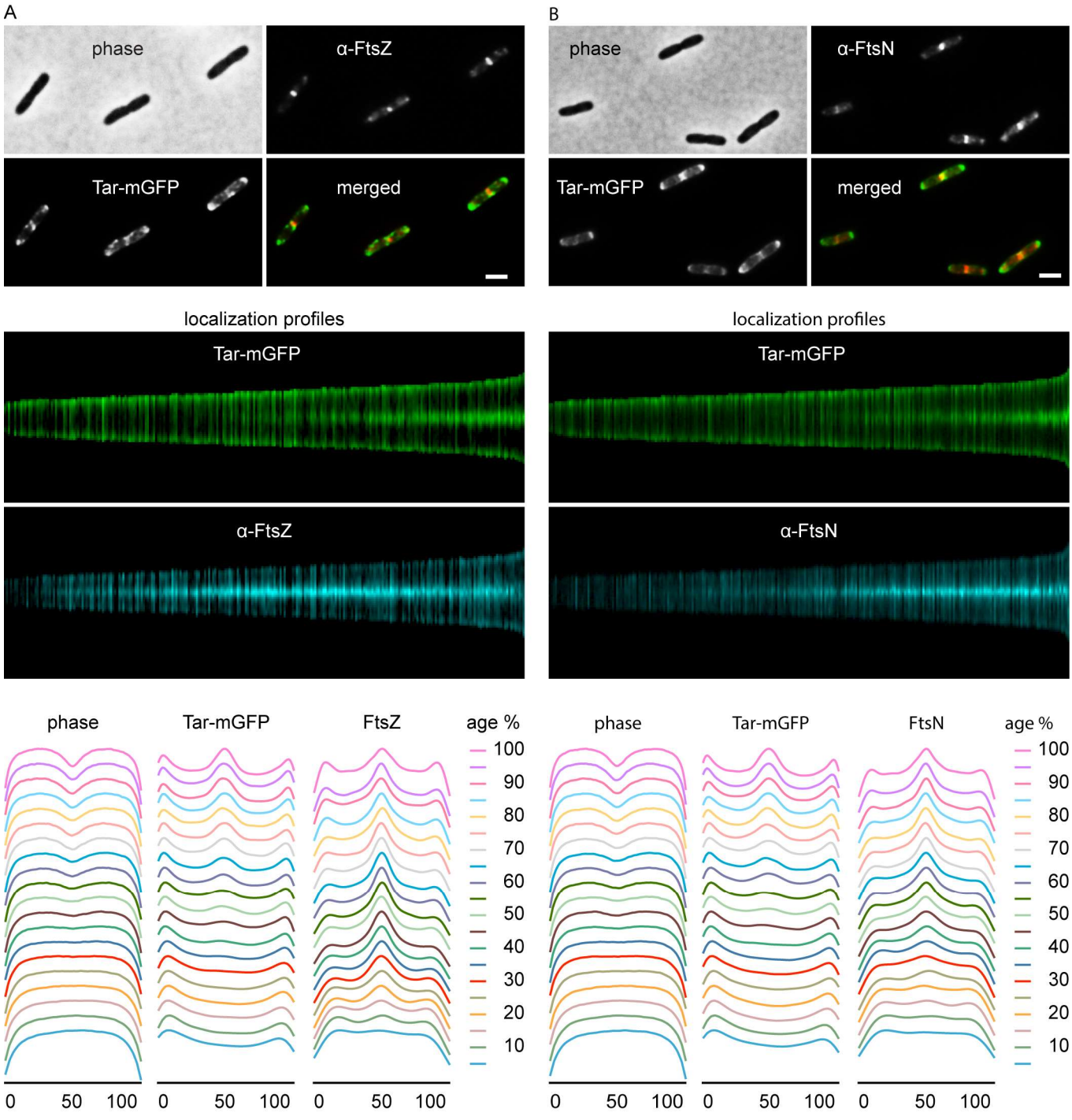

Fig. S5. Timing of Tar-mGFP midcell localization in Δ*cheA* compared to FtsZ and FtsN. Tar-mGFP expressing Δ*cheA* cells were fixed and immune-labelled using FtsZ (A) and FtsN (B) antiserum. Top panel: fluorescence light microscopy images, middle panel: fluorescence localization profiles, lower panel: graphical presentation of constriction (phase) and

fluorescence signals during the cell cycle calculated from the localization profiles. Cell age refers to age classes divided in 5% (cell cycle) bins. 2504 and 3187 cells were used for FtsZ and FtsN labeling, respectively. Scale bars are 2 μm and the strain used is TSE38.

Fig. S6

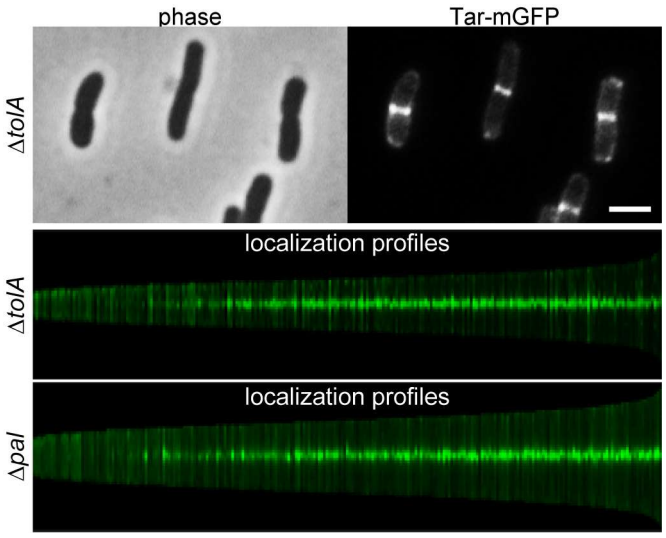

Fig S6. Tar-mGFP localization in Δ*tolA* and Δ*pal* cells

Fluorescence microscopy image and localization profiles of Tar-mGFP expressed in a Δ*tolA* and Δ*pal* background. 4506 and 9044 cells were used to construct the localization profile for Δ*tolA* and Δ*pal*, respectively. The localization profile for Δ*pal* is the same as in Fig. 3B. Scale bar is 2 μm. The strains used are TSE31 and TSE32.

Fig. S7

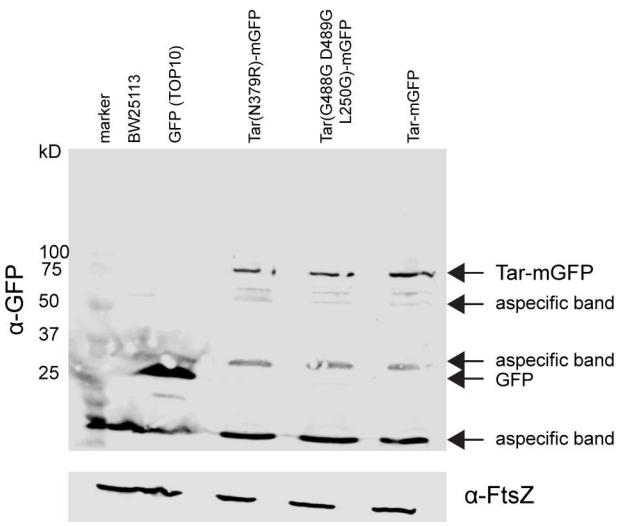

Fig. S7. Stability of Tar-mGFP mutants

Western blot analysis of Tar-mGFP mutants expressed in *E. coli*. The expected size for the fusion proteins is 87 kDa. Strains used are BW25113 (wild type), *E. coli* TOP10/pTNV100 (GFP) TSE29 (Tar-mGFP), TSE41 (Tar(G248G D249G L250G)-mGFP), and TSE42 (Tar(N379R)-mGFP).

Fig. S8

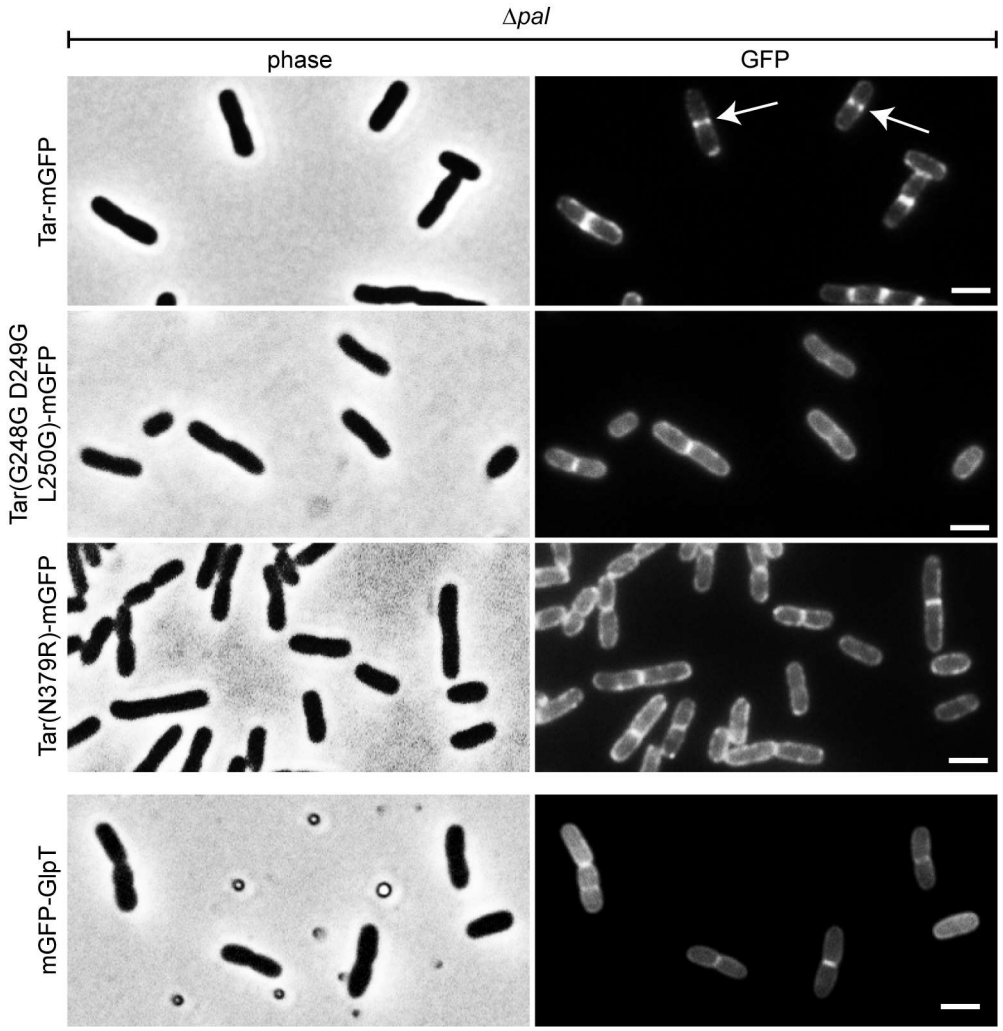

Fig. S8 Localization of Tar-mGFP mutants in Δ*pal* cells

Phase contrast and fluorescence images of Δ*pal* cells expressing Tar-mGFP, Tar-(G248G D249G L250G)-mGFP, Tar-(N379R)-mGFP and mGFP-GlpT. Wild type Tar-mGFP accumulates as two dots (white arrows) in ~47% of cells, while this accumulation was neither observed for the Tar-mGFP mutants proteins nor for the inner membrane marker mGFP-GlpT. Strain used are TSE31, TSE68, TSE69, and TSE71. The scale bars are 2 μm.

References

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