Matusiak I . et al. supplemental material

Table S1. Constructs used in the study

Constructs	Relevant genotype	Source
Constructs used in *E. coli* BTH system		
pUT18C	pUC replicon, p_{lac} promoter, ampicillin resistance, IPTG-inducible, T18 fragment of *cyaA* gene (encoding 225 to 399 amino acids)	(Karimova et al., 1998)
pKT25	P15A replicon, p_{lac} promoter, kanamycin resistance, IPTG-inducible T25 fragment of *cyaA* gene (encoding 1- 224 amino acids)	(Karimova et al., 1998)
pKT25*parA*	pKT25 derivative containing *M. smegmatis parA* gene	(Ginda et al., 2013)
pKT25*parA*T3A	pKT25 derivative containing *M. smegmatis parA*T3A gene	(Pióro et al., 2019)
pKT25*parA*K44A	pKT25 derivative containing *M. smegmatis parA*K44A gene	(Pióro et al., 2019)
pKT25*parA*D68A	pKT25 derivative containing *M. smegmatis parA*D68A gene	(Pióro et al., 2019)
pKT25*parA*G40V	pKT25 derivative containing *M. smegmatis parA*G40V gene	(Pióro et al., 2019)
pKT25*parA*R219E	pKT25 derivative containing *M. smegmatis parA*R219E gene	(Pióro et al., 2019)
pKT25*parB*	pKT25 derivative containing *M. smegmatis parB* gene	(Ginda et al., 2013)
pUT18C*divIVA*	pUT18C derivative containing *M. smegmatis divIVA* gene	(Ginda et al., 2013)
pUT18Cd*ivIVA I-II*	pUT18C derivative containing a fragment of *M. smegmatis divIVA* gene used for the production of T18C-DivIVAI-II (amino acids 1-72)	(Ginda et al., 2013)
pUT18CdiIVA II-III	pUT18C derivative containing a fragment of *M. smegmatis divIVA* gene used for the production of T18C-DivIVAII - III (amino acids 1-143)	(Ginda et al., 2013)
pUT18C*divIVA III-IV*	pUT18C derivative containing a fragment of *M. smegmatis divIVA* gene used for the production of T18C-DivIVAIII - IV (amino acids 68-272)	(Ginda et al., 2013)
pUT18C*divIVA IV*	pUT18C derivative containing a fragment of *M. smegmatis divIVA* gene used for the production of T18C-DivIVAIV (amino acids 139-272)	(Ginda et al., 2013)
pKT25*divIVA*	pKT25 derivative containing *M. smegmatis divIVA* gene	(Ginda et al., 2013)
pUT18C*msmeg_5597*	pUT18C derivative containing *M. smegmatis msmeg_5597* (*papM*) gene	This study
pKT25*msmeg_5597*	pKT25 derivative containing *M. smegmatis* (*msmeg_*5597) *papM* gene	This study
Constructs used for protein purification		
pGEX-6P-2	pGEX-6P-2 ampicillin resistance, GST-tag vector	Lab stock, University of Wroclaw

pGEX-6P-2*parA*	pGEX-6P-2 derivative containing *M. smegmatis parA* gene	(Jakimowicz et al., 2007)
pET28a	pET28a kanamycin resistance, His-tag vector	Lab stock, University of Wroclaw
pET28a*papM*	pET28a derivative containing *M. smegmatis papM* gene	This study
Constructs used for colocalization microscopy in *E. coli*		
pETDuet-1	ampicillin resistance vector, two multiple cloning sites, the pBR322-derived ColE1 replicon, *lacI* gene	Lab stock, University of Wroclaw
pJP108*divIVA*$_{Ms}$	pJP108 containing *icsA507-620-mcherry* fused with *M. smegmatis divIVA* gene	(Pióro et al., 2019)
pETDuet-*pknB*$_{KD}$	pETDuet-1 derivative containing fragment of *M. smegmatis pknB* gene encoding kinase domain (11-274 aa)	This study
pETDuet-*mcherry-divIVA*	pETDuet-1 derivative containing *icsA507-620-mcherry* fused with *M. smegmatis divIVA* gene	This study
pETDuet-*pknB*$_{KD}$ *mcherry-divIVA*	pETDuet-1 derivative containing fragment of *M. smegmatis pknB* gene encoding kinase domain (11-274 aa) (MCS1) and *divIVA* gene fused with *mcherry* (MCS2)	This study
pETDuet-*pknB*$_{KD}$ *mcherry-divIVAT74A*	pETDuet-1 derivative containing fragment of *M. smegmatis pknB* gene encoding kinase domain (11-274 aa) (MCS1) and *divIVAT74A* gene fused with *mcherry* (MCS2)	This study
pETDuet-*ics-mcherry*	pETDuet-1 derivative containing *icsA507-620-mcherry*	This study
pACYC-Duet-1	chloramphenicol resistance vector, two multiple cloning sites, the P15A replicon, *lacI* gene	Lab stock, University of Wroclaw
pACYC-*egfp-parA*	pACYCDuet-1 derivative containing *egfp* gene fused with *M. smegmatis parA* gene	(Pióro et al., 2019)
pACYC-*papM-mT2*	pACYCDuet-1 derivative containing *M. smegmatis papM* gene fused with *mturquoise2*	This study
pACYC-*papM-mT2; egfp-parA*	pACYCDuet-1 derivative containing *M. smegmatis papM* gene fused with *mturquoise2* (MCS1) and *egfp* gene fused with *M. smegmatis parA* gene (MCS2)	This study
Constructs used for *M. smegmatis* modifications		
p2Nil	kanamycin resistance, *oriE*, suicide vector for allelic replacement	(Parish and Stoker, 2000)
pGOAL	ampicillin resistance, *oriE*, selective PacI cassette with *lacZ*, *sacB* and *kanR* genes	(Parish and Stoker, 2000)
p2Nil*ΔpapM*GOAL	p2Nil derivative for deletion of *papM* gene	This study
p2Nil*lsr2-mT2*GOAL	p2Nil containing *mTurquoise2* gene fused with *lsr2*	(Kołodziej et al., 2021)
pMV306p$_{ami}$	pMV306 containing acetamide inducible promoter p$_{ami}$	(Ginda et al., 2013)
pMV306p$_{ami}$*papM*	pMV306p$_{ami}$ derivative containing *M. smegmatis papM* gene	This study

pMVp$_{nat}$*egfp-parA*	pMV306p$_{ami}$ derivative containing *egfp* gene fused with *parA* gene	(Ginda et al., 2013)
pMVp$_{nat}$PA*mcherry-parA*	pMV306p$_{ami}$ containing PA*mcherry* gene fused with *parA* under its native promoter	(Pióro et al., 2019)
pKW08p$_{tet}$	pKW08 containing tetracycline inducible promoter p$_{tet}$	Lab stock, University of Wroclaw
pKW08p$_{tet}$*mcherry-divIVA*	pKW08p$_{tet}$ derivative containing *mcherry* gene fused with *divIVA*	This study

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