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Supplementary Information for

Structure and mutagenic analysis of the lipid II flippase MurJ from *Escherichia coli*

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This PDF file includes:

Figs. S1 to S2
Tables S1 to S2

Other supplementary materials for this manuscript include the following:

Dataset S1

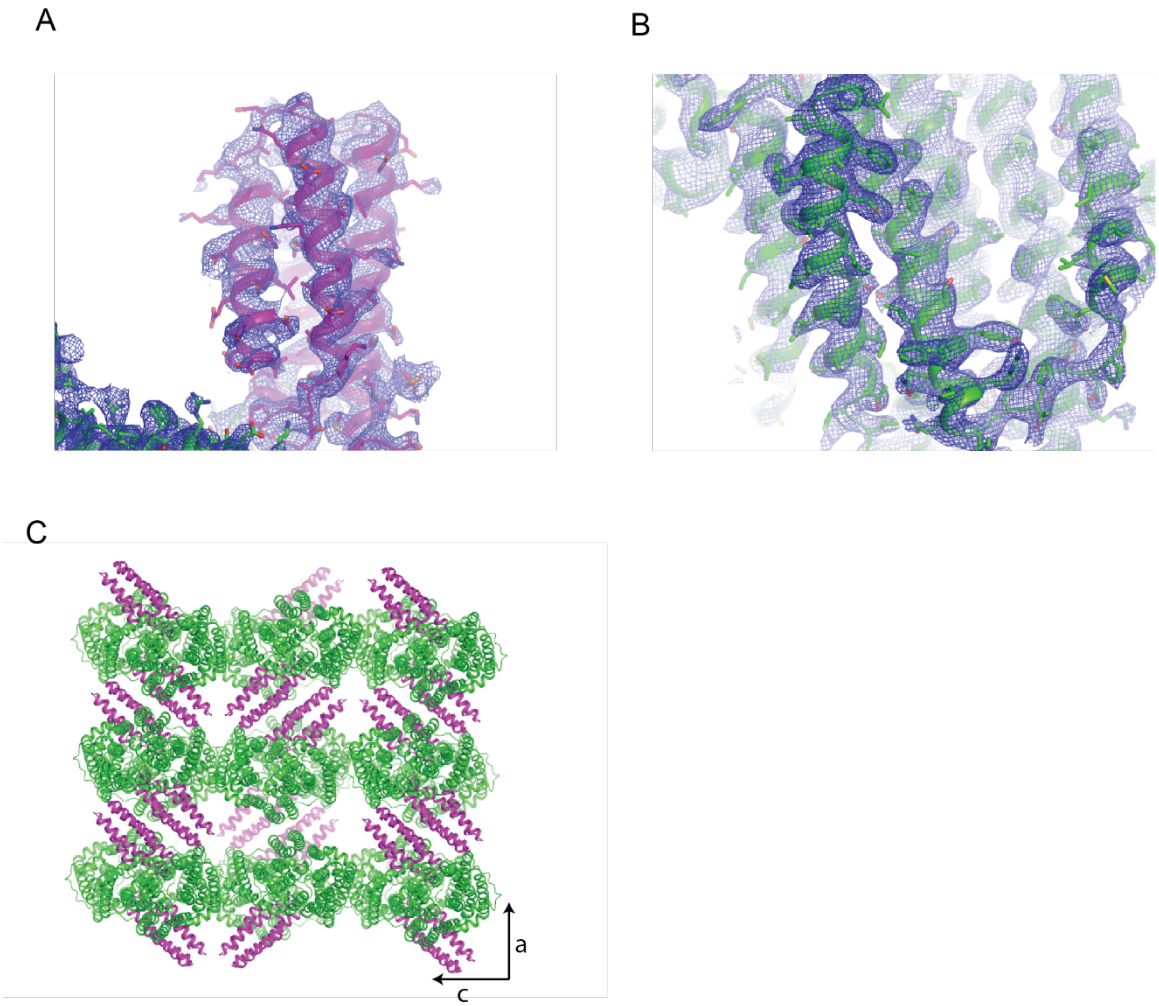

Fig. S1. Structure determination of *E. coli* MurJ. $2F_o$-F_c electron density map for BRIL (A) and MurJ (B) contoured at 1.0 σ. (C) The crystal packing of BRIL-MurJ fusion protein is shown. BRIL and MurJ are colored in magenta and green respectively.

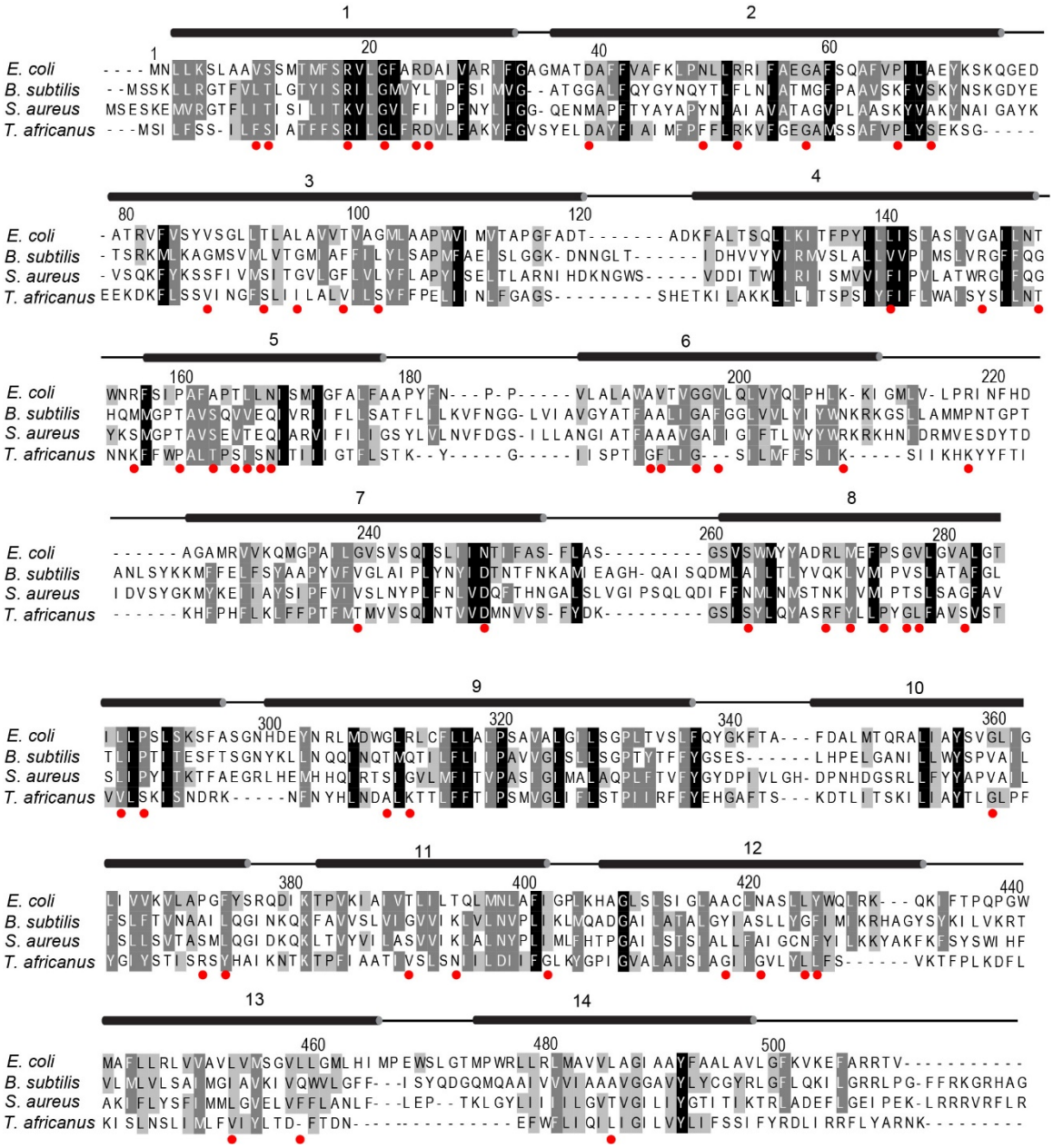

Fig. S2. Sequence alignment of MurJ from both Gram-positive and Gram-negative bacteria. 483 sequences retrieved from BLAST were used for alignment with *Escherichia coli, Bacillus subtilis, Staphylococcus aureus*, and *Thermosipho africanus* displayed here. Residues with more than 98%, 80% and 60% sequence similarity were shown in black, grey and light grey respectively. Secondary structure elements were shown above the alignment based on the crystal structure. The red circle below alignment denotes essential residues of MurJ in *E. coli* corresponding to **Figure 3**.

Table S1. Crystallographic statistics

Data collection*

Wavelength (Å)	1.033
Space group	$C222_1$
Number of crystals	5
Unit cell dimensions	
a, b, c (Å)	113.9,118.0,128.0
α, β, γ (°)	90, 90, 90
Resolution (Å)	50 - 3.5 (3.58 - 3.50)
Completeness (%)	95.5 (95.0)
$\langle I/\sigma(I) \rangle$	3.6 (0.7)
$CC_{1/2}$ (%)	95.3 (21.1)
Multiplicity	2.7 (2.4)

Refinement

Resolution (Å)	35.7 - 3.5 (3.85 - 3.5)
No. reflections	10640
R_{work}/R_{free} (%)	28.0 / 30.1
No. atoms	4608
Protein	4603
Solvent ions	5
B factors (Å^2)	74.8
RMS deviation	
Bond length (Å)	0.003
Bond angles (°)	0.740
Ramachandran statistics	
Favored	95.05%
Allowed	4.95%
Outliers	0

*Values in parentheses are for highest shell.

Table S2. Summary of deleterious mutations identified by Mut-Seq.

	Residue	Substitution		Residue	Substitution
Group I: Buried residues likely required for folding	V87	Asp	**Group II: Residues that compose the extracellular gate and stabilize the inward-open conformation**	D39	His
	T92	Arg		S263	Tyr
	L95	Arg			
	T99	Arg			
	G102	Arg			
	L140	Arg			
	G148	Arg			
	P160	Arg	**Group III**: **Residues that likely compose the intracellular gate and stabilize the outward-open conformation**	V10	Gly
	A163	Asp		S11	Arg
	T165	Lys, Arg		P66	Arg, Thr
	L166	Arg		A69	Pro
	L167	Arg		T153	Lys, Arg
	A192	Asp		R156	Pro
	V193	Asp		R218	Pro
	G196	Asp		I286	Phe, Asn
	V198	Asp		P289	Ala
	L208	Arg		P372	Arg
	G239	Asp	**Group IV: Solvent-exposed residues located in the central cavity or extended groove, likely involved in substrate binding**	R18	Leu, Pro
	N250	Lys		G21	Ala, Arg
	M272	Lys		R24	Gly, Gln
	P275	Arg		D25	Tyr, His
	V278	Glu		N49	Ile, Lys
	A282	Glu		R52	Cys, Pro
	L287	Trp		G58	Glu, Arg
	G310	Arg		N168	Lys
	G360	Arg		R270	Cys, Pro
	F374	Val, Ile		E273	Val, Lys
	I402	Ser		G277	Val, Asp, Arg
	A418	Pro		R312	Pro
	L425	Arg		T390	Lys, Arg
	Y426	Asp		T394	Lys, Arg
	L453	Arg		N421	Lys
	L459	Pro, Arg		L486	Arg