

## Supplemental Material

Soltes *et al.*, Distinctive roles for periplasmic proteases in the maintenance of essential outer membrane protein assembly

### Supplemental Material References:

1. **Ruiz N, Falcone B, Kahne D, Silhavy TJ.** 2005. Chemical conditionality: a genetic strategy to probe organelle assembly. *Cell* **121**:307–17.
2. **Chimalakonda G, Ruiz N, Chng S-S, Garner RA, Kahne D, Silhavy TJ.** 2011. Lipoprotein LptE is required for the assembly of LptD by the beta-barrel assembly machine in the outer membrane of *Escherichia coli*. *Proc Natl Acad Sci USA* **108**:2492–7.
3. **Rigel NW, Schwalm J, Ricci DP, Silhavy TJ.** 2012. BamE modulates the *Escherichia coli* beta-barrel assembly machine component BamA. *J Bacteriol* **194**:1002–8.
4. **Schwalm J, Mahoney TF, Soltes GR, Silhavy TJ.** 2013. A role for Skp in LptD assembly in *Escherichia coli*. *J Bacteriol* **195**:3734–3742.
5. **Wu T, McCandlish AC, Gronenberg LS, Chng S-S, Silhavy TJ, Kahne D.** 2006. Identification of a protein complex that assembles lipopolysaccharide in the outer membrane of *Escherichia coli*. *Proc Natl Acad Sci USA* **103**:11754–11759.
6. **Ricci DP, Hagan CL, Kahne D, Silhavy TJ.** 2012. Activation of the *Escherichia coli*  $\beta$ -barrel assembly machine (Bam) is required for essential components to interact properly with substrate. *Proc Natl Acad Sci USA* **109**:3487–91.
7. **Ruiz, N., Gronenberg, L. S., Kahne, D. & Silhavy, T. J.** 2008. Identification of two inner- membrane proteins required for the transport of lipopolysaccharide to the outer membrane of *Escherichia coli*. *Proc Natl Acad Sci USA* **105**, 5537–5542.
8. **Sutterlin, H.,** 2016. In preparation

**Table S1: Strains and Plasmids**

<i>E. coli</i> K-12		
strains	Genotype and relevant features	Reference
NR754	MC4100 <i>ara+ / r</i>	(7)
NR698	MC4100 <i>lptD4213</i>	(1)
HC340	$\Delta$ <i>lptD</i> pLptD	(8)
HC1036	$\Delta$ <i>lptD</i> pLptD Y721D	(8)
GS583	NR754 <i>degP::kan</i>	This study
GS584	NR754 <i>degQ::kan</i>	This study
GS585	NR754 <i>ompT::kan</i>	This study
GS586	NR754 <i>ptrA::kan</i>	This study
GS587	NR754 <i>tsp::kan</i>	This study

GS588	NR754 <i>ycaL::kan</i>	This study
GS589	NR754 <i>ydgD::kan</i>	This study
GS590	NR754 <i>yggG::kan</i>	This study
GS603	NR754 <i>yfgC::kan</i>	This study
GS605	NR754 <i>yhiJ::kan</i>	This study
GS600	NR698 <i>degP::kan</i>	This study
GS601	NR698 <i>degQ::kan</i>	This study
GS602	NR698 <i>ompT::kan</i>	This study
GS604	NR698 <i>yfgC::kan</i>	This study
GS606	NR698 <i>yhiJ::kan</i>	This study
GS607	NR698 <i>ycaL::kan</i>	This study
GS608	NR698 <i>yggG::kan</i>	This study
GS609	NR698 <i>ydgD::kan</i>	This study
GS610	NR698 <i>ptrA::kan</i>	This study
GS615	HC340 <i>degP::kan</i>	This study
GS616	HC1036 <i>degP::kan</i>	This study
GS617	HC340 <i>degQ::kan</i>	This study
GS618	HC1036 <i>degQ::kan</i>	This study
GS619	HC340 <i>ompT::kan</i>	This study
GS620	HC1036 <i>ompT::kan</i>	This study
GS621	HC340 <i>ptrA::kan</i>	This study
GS622	HC1036 <i>ptrA::kan</i>	This study
GS623	HC340 <i>tsp::kan</i>	This study
GS624	HC1036 <i>tsp::kan</i>	This study
GS625	HC340 <i>ycaL::kan</i>	This study
GS626	HC1036 <i>ycaL::kan</i>	This study
GS627	HC340 <i>ydgD::kan</i>	This study
GS628	HC1036 <i>ydgD::kan</i>	This study
GS629	HC340 <i>yggG::kan</i>	This study
GS630	HC1036 <i>yggG::kan</i>	This study
GS631	HC340 <i>yfgC::kan</i>	This study
GS632	HC1036 <i>yfgC::kan</i>	This study
GS633	HC340 <i>yhiJ::kan</i>	This study
GS634	HC1036 <i>yhiJ::kan</i>	This study
HC1289	$\Delta$ <i>lptD</i> <i>bamA</i> F494L <i>yaeH::cam</i> pLptD	(8)
HC1290	$\Delta$ <i>lptD</i> <i>bamA</i> F494L <i>yaeH::cam</i> pLptD Y721D	(8)
GC169	$\Delta$ <i>lptE</i> pLptE	(2)
GC192	$\Delta$ <i>lptE</i> pLptE6	(2)
GS669	GC169 <i>degP::kan</i>	This study
GS670	GC192 <i>degP::kan</i>	This study
GS671	GC169 <i>tsp::kan</i>	This study
GS672	GC192 <i>tsp::kan</i>	This study
GS673	GC169 <i>ycaL::kan</i>	This study

GS674	GC192 <i>ycaL::kan</i>	This study
GS675	GC169 <i>ydgD::kan</i>	This study
GS676	GC192 <i>ydgD::kan</i>	This study
GS677	GC169 <i>yfgC::kan</i>	This study
GS678	GC192 <i>yfgC::kan</i>	This study
JAS16	NR754 $\Delta$ <i>surA</i>	(3)
JAS420	NR754 $\Delta$ <i>skp</i> $\Delta$ <i>fkpA</i>	(4)
GS885	JAS16 <i>yfgC::kan</i>	This study
GS352	JAS16 <i>degP::kan</i>	This study
GS1036	JAS420 <i>yfgC::kan</i>	This study
GS1043	JAS420 <i>degP::kan</i>	This study
GS968	NR754 <i>bamB::kan</i>	This study
GS969	NR754 $\Delta$ <i>ycaL bamB::kan</i>	This study
GS970	NR754 <i>bamE::kan</i>	This study
GS971	NR754 $\Delta$ <i>ycaL bamE::kan</i>	This study
GS1003	NR754 $\Delta$ <i>lptD ycaL</i> pLptD	This study
GS1004	NR754 $\Delta$ <i>lptD ycaL</i> pLptD Y721D	This study
GS1013	NR754 $\Delta$ <i>lptD ycaL</i> pLptD pBamD	This study
GS1014	NR754 $\Delta$ <i>lptD ycaL</i> pLptD pBamD O/E	This study
GS1015	NR754 $\Delta$ <i>lptD ycaL</i> pLptD Y721D pBamD	This study
GS1016	NR754 $\Delta$ <i>lptD ycaL</i> pLptD Y721D pBamD O/E	This study
GS1056	NR754 $\Delta$ <i>lptD ddegP</i> pLptD	This study
GS1057	NR754 $\Delta$ <i>lptD ddegP</i> pLptD Y721D	This study
GS1058	NR754 $\Delta$ <i>lptD yfgC</i> pLptD	This study
GS1059	NR754 $\Delta$ <i>lptD yfgC</i> pLptD Y721D	This study

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<b>Plasmids</b>	<b>Description</b>	
pLptD	pET2342:: <i>LptD</i> WT Amp <sup>r</sup>	(5)
pLptE	<i>lptE</i> cloned into the inducible pBAD18 plasmid, Amp <sup>r</sup>	(2)
pZS21	Low-copy vector; Kan <sup>r</sup>	(6)
pBamD	<i>bamD</i> cloned into pZS21	(3)
pBamD O/E	Unknown mutation in pZS21 backbone that increases copy number	(3)

**Table S2:** Outer membrane permeability phenotypes of constructed *ycaL* mutants

Genotype	Background	
	$\Delta bamB$	$\Delta bamE$
	OM Phenotype	OM Phenotype
MC4100	+	++
<i>ycaL::kan</i>	+	++

**OM Phenotype:** ++++ denotes growth similar to WT in the presence of antibiotics, whereas - denotes no growth.