SUPPLEMENTARY FIGURES AND TABLES.

Figure S1. Quantifications of pull-downs in Fig. 1 based on three independent replicates. (A) *in vivo* Strep-BamA pull-down, and **(B)** *in vitro* His-BamA pull-down. Graphs represent the quantification of corresponding bands normalized to the level of the bait protein, BamA, and additionally compared to that of the *bamE(WT)* strain in (A). Graphs represent mean +/- SD. Statistical analysis was performed by using one-way ANOVA in comparison with the WT control. n.s. = P ≥ 0.05, **P <0.01, ****P < 0.0001. N/A stands for "not applicable".

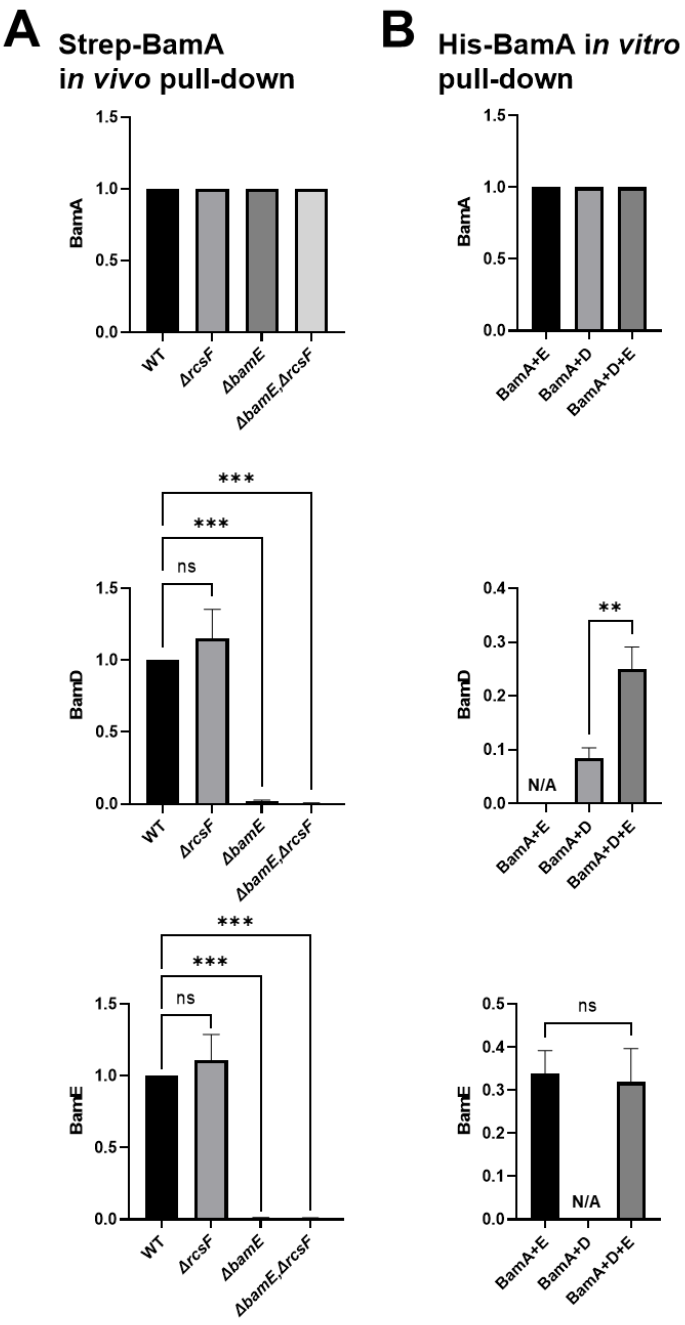


Figure S2. Structural prediction of BamE/BamA and BamE/BamD complexes from representative species of α-, β-, γ-Proteobacteria compared to *E. coli*. Protein sequences of mature (devoid of their signal sequences) BamE, BamA, and BamD were analyzed using ColabFold (MMseqs2 with AlphaFold2-multimer)(Mirdita *et al.*, 2022). The interaction interface based on the top-scoring models is shown. The residues establishing polar contacts were identified using the PDBePISA(Krissinel & Henrick, 2007), and are listed in Table S1 and S2. The protein sequence alignment is shown in Fig.S3. Predicted structures are shown in comparison to the published structures of the *E. coli* Bam complex (PDB: 5ekq (Bakelar *et al.*, 2016) and 5d0o (Gu *et al.*, 2016)).

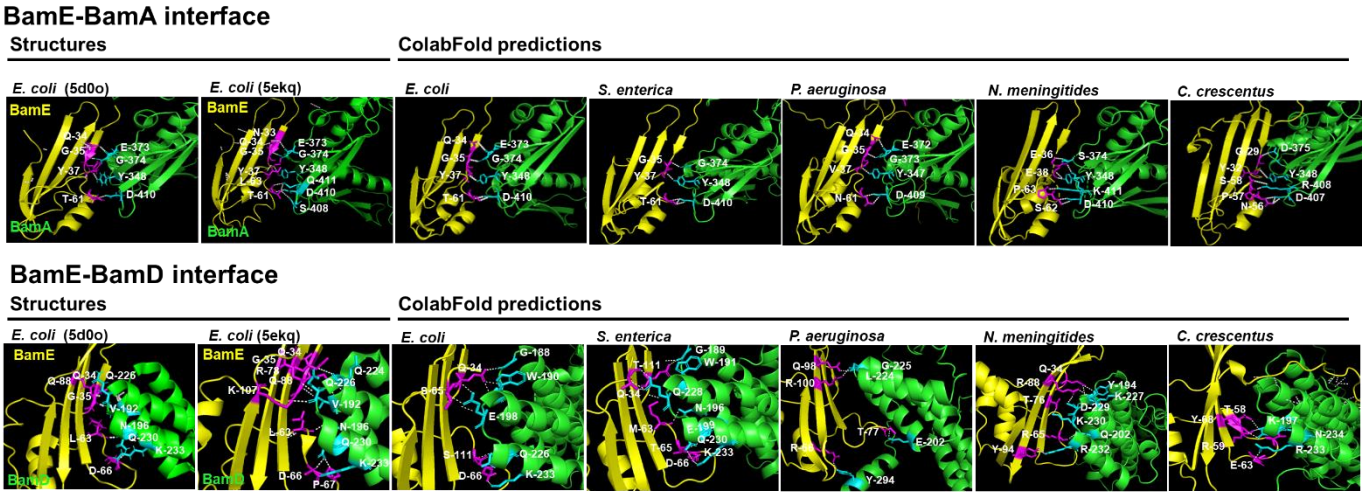
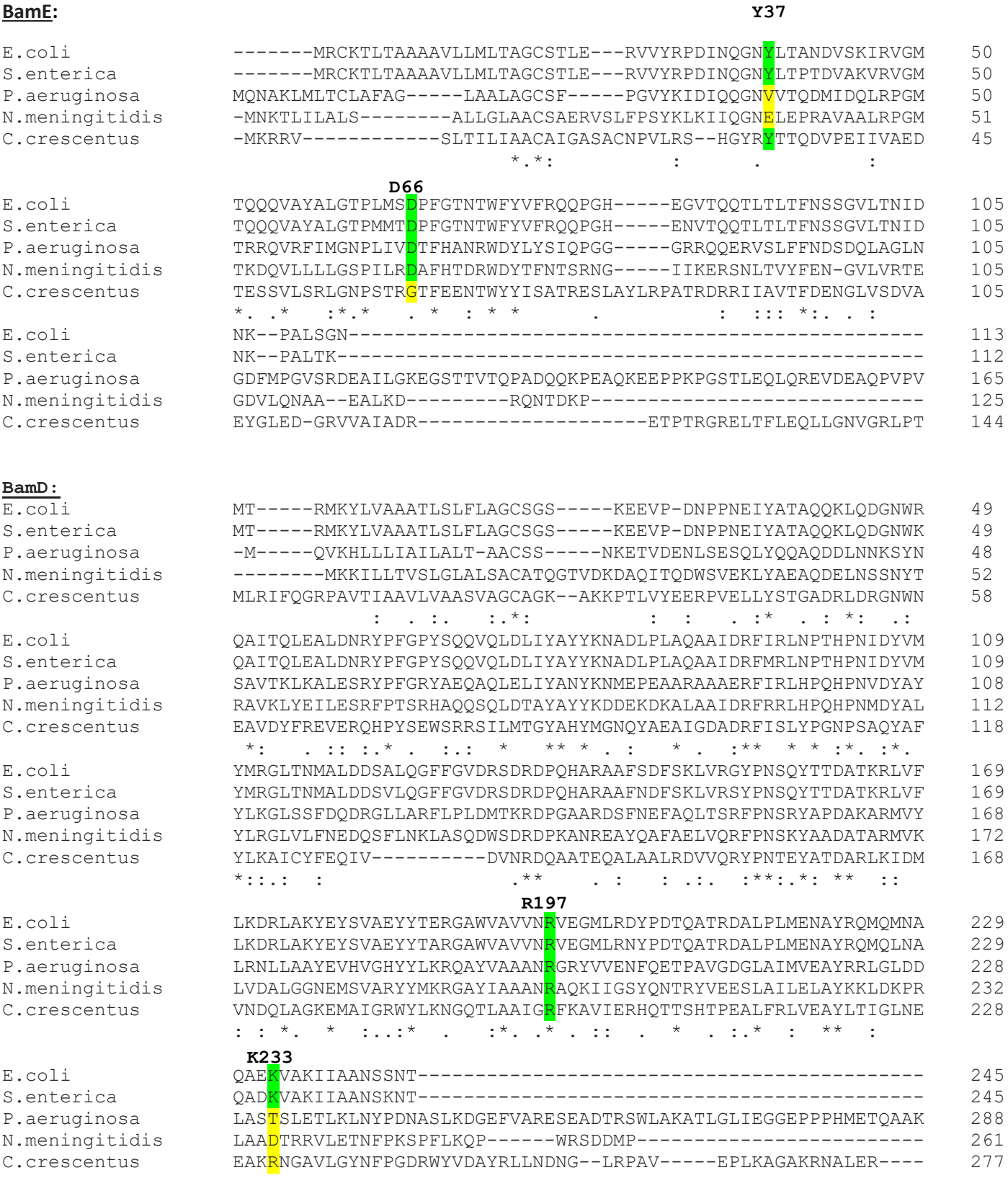


Figure S3 Multiple sequence alignment of protein sequences used for ColabFold protein complex modeling generated using Clustal Omega. Only BamA POTRA 5 domain is shown for clarity because it is the site for BamD and BamE interaction. Interacting residues that are discussed in this study are highlighted.



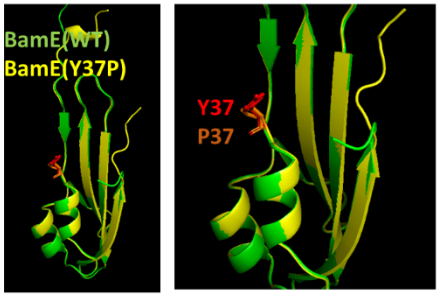
```
                 *      :  *
E.coli           ------------------------------------------------------   245
S.enterica       ------------------------------------------------------   245
P.aeruginosa     DVIKQYEDAEREIPAELKPENQDHSADDEKPESDDDEDSGRSWWSYMTFGLFD   341
N.meningitidis   ----------------------------------------WWRYWH-----   267
C.crescentus     -ILSKDKEATLAPPGERKAK------------------KG--LL--GPLGM--   305
```

BamA(POTRA5):

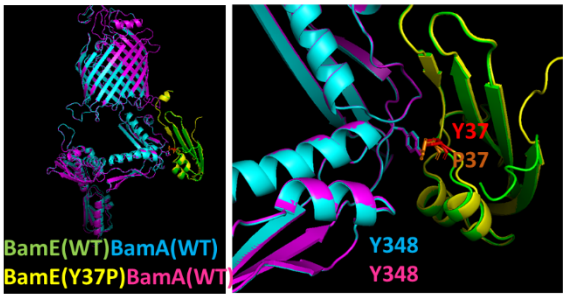
```
                                                                Y348
E.coli           GTKVTKMEDDIKKLLGRYGYAYPRVQSMPEINDADKTVKLRVNVDAGNRFYVRKIRFEGN   357
S.enterica       GTKVTKMEDDIKKLLGRYGYAYPRVQSQPEINDADKTVKLRVNVDAGNRFYVRKIRFEGN   357
P.aeruginosa     RKVMTTTSDLITRRLGNEGYTFANVNGVPEAHDDDKTVSVTFVVDPGKRAYVNRINFRGN   356
N.meningitidis   RQQMTAVLGEIQNRMGSAGYAYSEISVQPLPNAETKTVDFVLHIEPGRKIYVNEIHITGN   357
C.crescentus     DERIEQATDALTFAAGAAGFAFVDVRPRYVPNRETKTVDVVFQVREGPRVYVDRIDIVGN   355
                    :    . :    *  *:::  :      :   ***.. . :  * : ** .* : **
                          E373
E.coli           DTSKDAVLRREMRQMEGAWLGSDLVDQGKERLNRLGFFETVDTDTQRVPGSPDQVDVVYK   417
S.enterica       DTSKDSVLRREMRQMEGAWLGSDLVDQGKERLNRLGFFETVDTDTQRVPGSPDQVDVVYK   417
P.aeruginosa     TKTEDEVLRREMRQMEGGWASTYLIDQSKARLERLGYFKEVNVETPAVPGTDDQVDVNYS   416
N.meningitidis   NKTRDEVVRRELRQMESAPYDTSKLQRSKERVELLGYFDNVQFDAVPLAGTPDKVDLNMS   417
C.crescentus     TRTLDYVLRRELEVAEGDAYNRVLVDRSKNNMRRLGFFKEVEIEDAP-GSAPDRTSLRVK   414
                   : * *:***:.  *.   .   :::.* .:. **:*. *: :     .: *:..:  .
```

Figure S4. *In silico* mutagenesis of the BamE/BamA interfacing Y37 residue. (A) Y37P is not predicted to affect overall structure (A) but weakness BamE/BamA interface (B) by lowering a number of polar contacts (C). Protein sequences of mature (devoid of their signal sequences) BamE, or BamE and BamA, were analyzed using ColabFold (MMseqs2 with AlphaFold2-multimer). The interaction interface based on the top-scoring models is shown. The residues establishing polar contacts were identified using the PDBePISA.

A



B



C

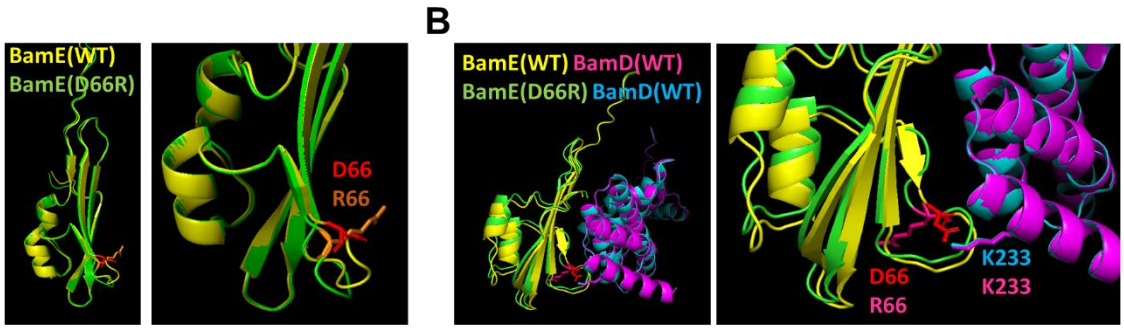
BamE(WT)/BamA polar contacts

#	BamE group	Dist. [Å]	BamA group
Hydrogen bonds			
1	TYR 28[N]	3.39	PRO 518[O]
2	ARG 29[NE]	3.24	ASP 481[OD2]
3	GLY 35[N]	2.75	GLY 374[O]
4	TYR 37[N]	3.23	TYR 348[OH]
5	THR 61[N]	2.44	ASP 410[OD2]
6	THR 61[OG1]	2.43	ASP 410[OD1]
7	THR 61[OG1]	3.83	SER 408[OG]
8	TYR 37[O]	3.49	TYR 348[OH]
9	TYR 37[O]	3.84	ARG 346[NH1]
10	LEU 59[O]	3.70	TYR 348[OH]

BamE(Y37P)/BamA polar contacts

#	BamE group	Dist. [Å]	BamA group
Hydrogen bonds			
1	TYR 28[N]	3.51	PRO 518[O]
2	ARG 29[NE]	3.12	ASP 481[OD2]
3	GLY 35 N]	2.75	GLY 374[O]
4	THR 61[N]	2.53	ASP 410[OD2]
5	THR 61[OG1]	3.88	SER 408[OG]
6	THR 61[OG1]	2.39	ASP 410[OD1]
7	LEU 59[O]	3.47	TYR 348[OH]

Figure S5. *In silico* mutagenesis of the BamE/BamD interfacing D66 residue. (A) D66R is not predicted to affect overall structure (A) but weakness BamE/BamD interface (B) by lowering a number of polar contacts (C). Protein sequences of mature (devoid of their signal sequences) BamE, or BamE and BamD, were analyzed using ColabFold (MMseqs2 with AlphaFold2-multimer). The interaction interface based on the top-scoring models is shown. The residues establishing polar contacts were identified using the PDBePISA.



C

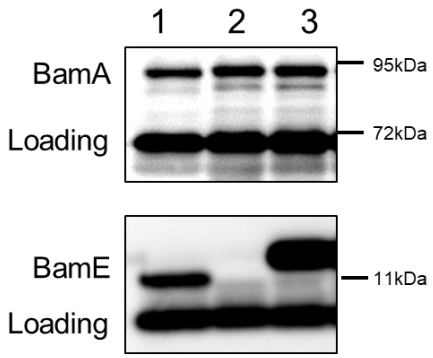
BamE(WT)/BamD polar contacts

#	BamE group	Dist. [Å]	BamD group
Hydrogen bonds			
1	GLN 34[NE2]	2.29	GLY 189[O]
2	ARG 78[NH1]	3.47	GLY 189[O]
3	SER 65[N]	3.74	GLU 199[OE1]
4	SER 65[N]	3.86	GLU 199[OE2]
5	GLN 88[NE2]	2.22	MET 225[O]
6	GLN 34[OE1]	2.15	TRP 191[N]
7	GLN 34[OE1]	2.54	VAL 192[N]
8	GLN 34[OE1]	3.40	ALA 193[N]
9	ASP 45[OD2]	3.02	LYS 233[NZ]
10	THR 49[OG1]	3.80	LYS 233[NZ]
Salt bridges			
1	ASP 66[OD2]	3.02	LYS 233[NZ]

BamE(D66R)/BamD polar contacts

#	BamE group	Dist. [Å]	BamD group
Hydrogen bonds			
1	ARG 78[NH1]	3.51	GLY 189[O]
2	PRO 67[N]	3.57	GLU 199[OE1]
3	ARG 66[N]	3.41	GLU 199[OE1]
4	GLN 88[NE2]	2.51	MET 225[O]
5	GLN 34[OE1]	3.10	VAL 192[N]

Figure S6. Immunoblot analysis of Strep-BamA and BamE-His protein levels expressed from pZS21 and pTrc99a plasmids relatively to the MC4100 parent strain. Note, addition of His8 tag to BamE increases its size by 1 kDa resulting in a size shift.



Lane	Strain#	Short genotype
1	MC4100	Wild-type
2	SK-80	*ΔbamE ΔbamA*//pZS21::*Strep-bamA* pTrc99a
3	SK-81	*ΔbamE ΔbamA*//pZS21::*Strep-bamA* pTrc99a::*bamE-His*

Figure S7. Immunoblot quantification of *in vivo* pull-down analysis presented in Fig. 3 based on three independent biological replicates. (A) BamE-His pull-down, and (B) Strep-BamA pull-down. Graphs represent the quantification of corresponding bands normalized to the level of the bait protein, BamE-His (A) and Strep-BamA (B), and compared to that of the *bamE(WT)* strain. Graphs represent mean +/- SD. Statistical analysis was performed by using one-way ANOVA in comparison with the WT control. n.s. = P ≥ 0.05, **P <0.01, ****P < 0.0001.

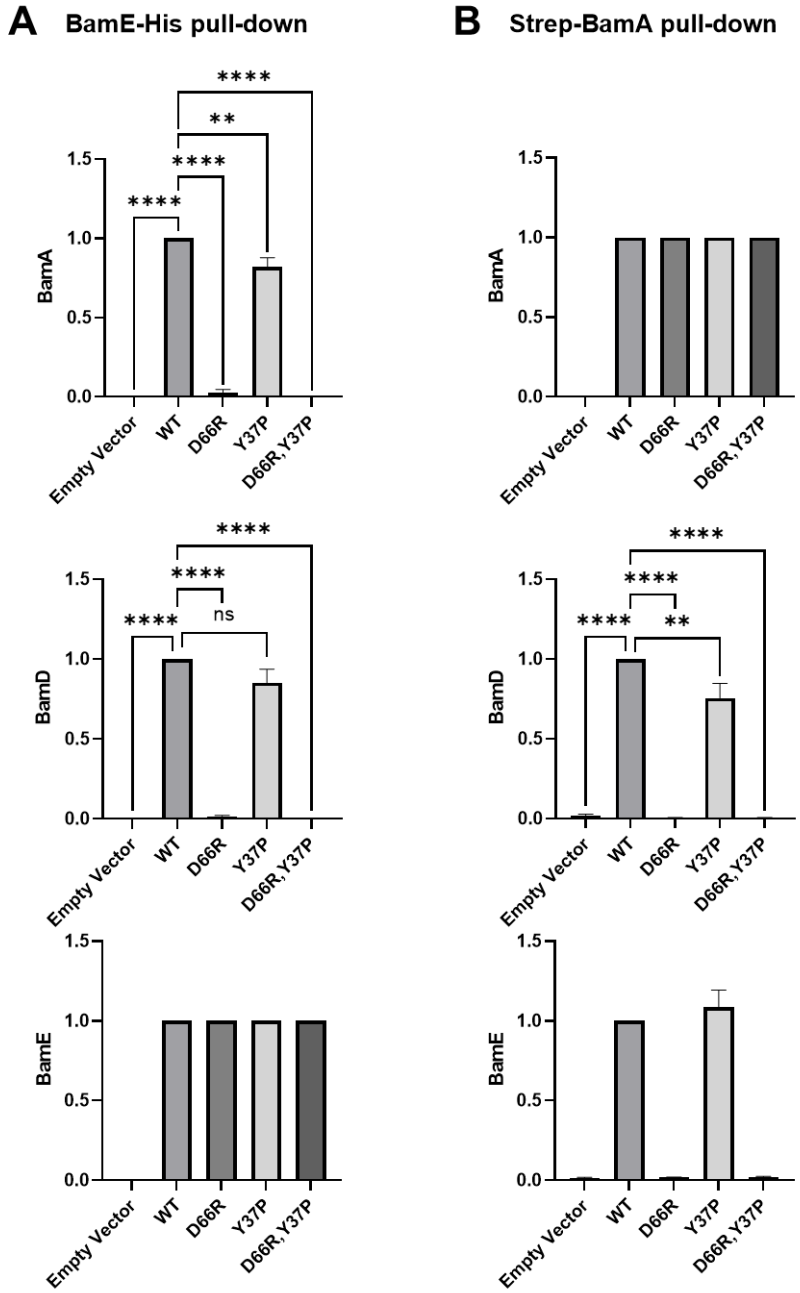


Figure S8. Phenotypic characterization of *bamE(Y37G)* mutant. (A, B) Effect of *bamE* mutations on BamA/BamD/BamE complex stability *in vivo* using Ni-NTA pull-down of BamE-His variants (A) or Streptactin pull-down of Strep-BamA in the presence of BamE-His variants (B). (C) Immunoblot analysis of *in vivo* formaldehyde cross-linked samples probed with α-BamA (Top) and α-RcsF antibodies (bottom). Quantification of RcsF/BamA and RcsF/OmpA cross-linking bands based on three independent biological replicates relative to the WT control, mean+/- SD. Statistical analysis was performed by using one-way ANOVA in comparison with the WT control. n.s. = P ≥ 0.05, **P <0.01, ****P < 0.0001. (**E)** Growth curve analysis of the AK-1255 [*ΔbamE bamB8 P_{BAD}-bamE*] derivative strains containing an empty vector and vectors with indicated *bamE* alleles in the absence of arabinose. Growth was monitored as described in Fig. 3.

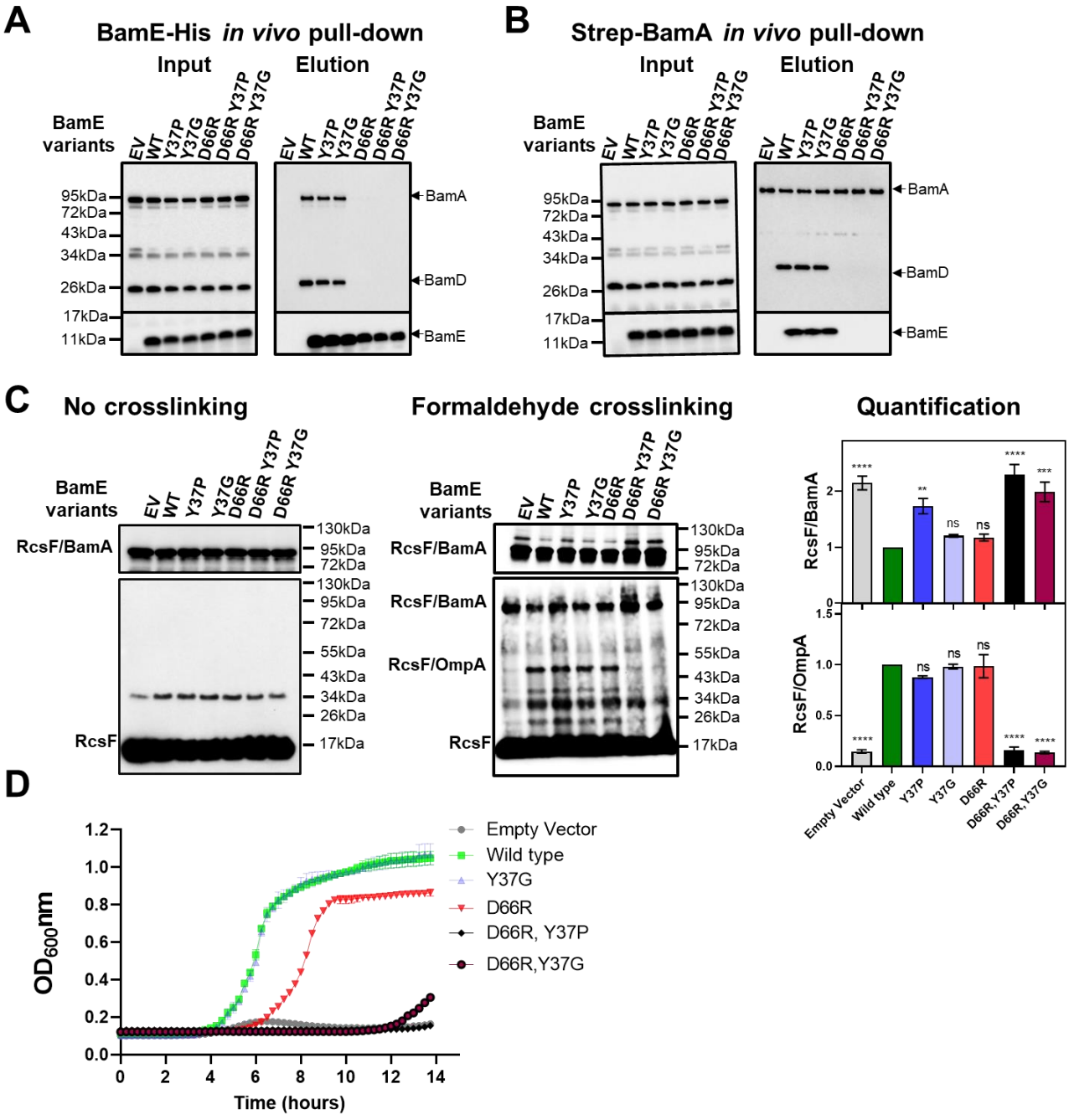
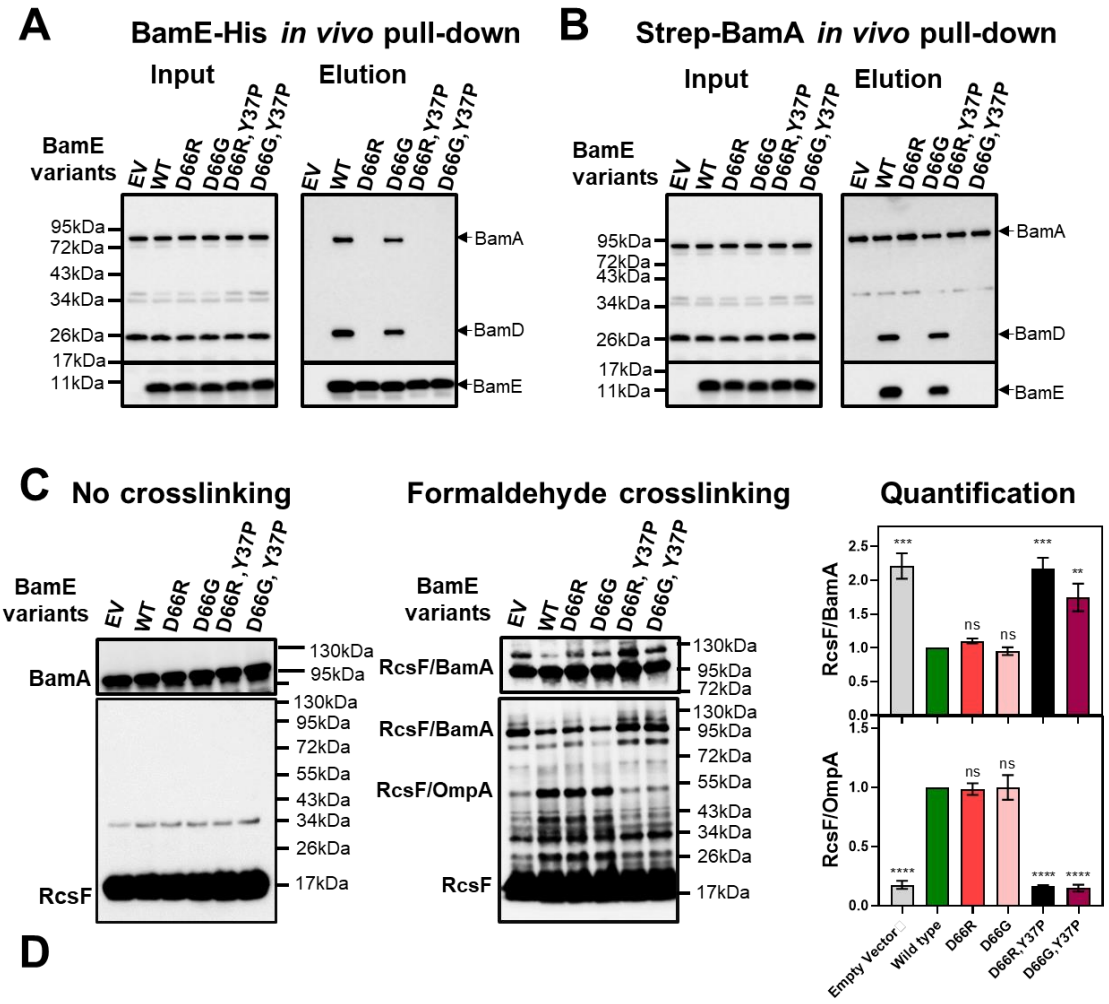


Figure S9. Phenotypic characterization of *bamE(D66G)* mutant. (A, B) Effect of *bamE* mutations on BamA/BamD/BamE complex stability *in vivo* using Ni-NTA pull-down of BamE-His variants (A) or Streptactin pull-down of Strep-BamA in the presence of BamE-His variants (B). Note that unlike *bamE(D66R)*, *bamE(D66G)* mutation alone does not destabilize the Bam complex. (C) Immunoblot analysis of *in vivo* formaldehyde cross-linked samples probed with α-BamA (Top) and α-RcsF antibodies (bottom). Quantification of RcsF/BamA and RcsF/OmpA cross-linking bands based on three independent biological replicates relative to the WT control, mean+/- SD. Statistical analysis was performed by using one-way ANOVA in comparison with the WT control. n.s. = P ≥ 0.05, ***P < 0.001. (**E)** Growth curve analysis of the AK-1255 [*ΔbamE bamB8* P_{BAD}*-bamE*] derivative strains containing an empty vector and vectors with indicated *bamE* alleles in the absence of arabinose. Growth was monitored as described in Fig. 3.



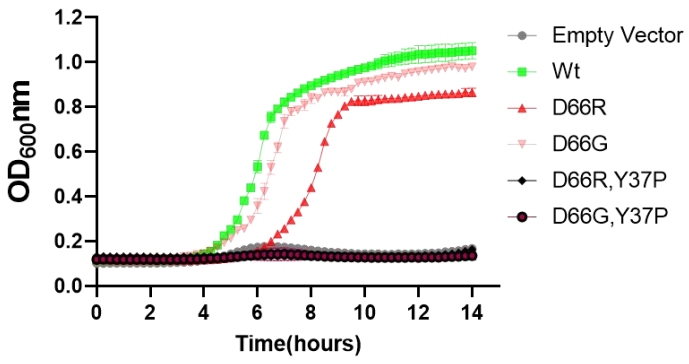


Figure S10. Plate growth phenotype of SK-130 [*ΔbamE ΔbamA* P_{BAD}*-bamA // pZS21:Strep-bamA(373A)*] and the *ΔrcsF* variant SK-155. Strains constructed and propagated in the presence of arabinose to allow expression of the chromosomal *bamA(WT)* allele. To test for genetic interaction, strains were streaked on indicated agar plates without arabinose; plates were incubated at 30°C or 37°C. Growth was assayed after 24 hours.

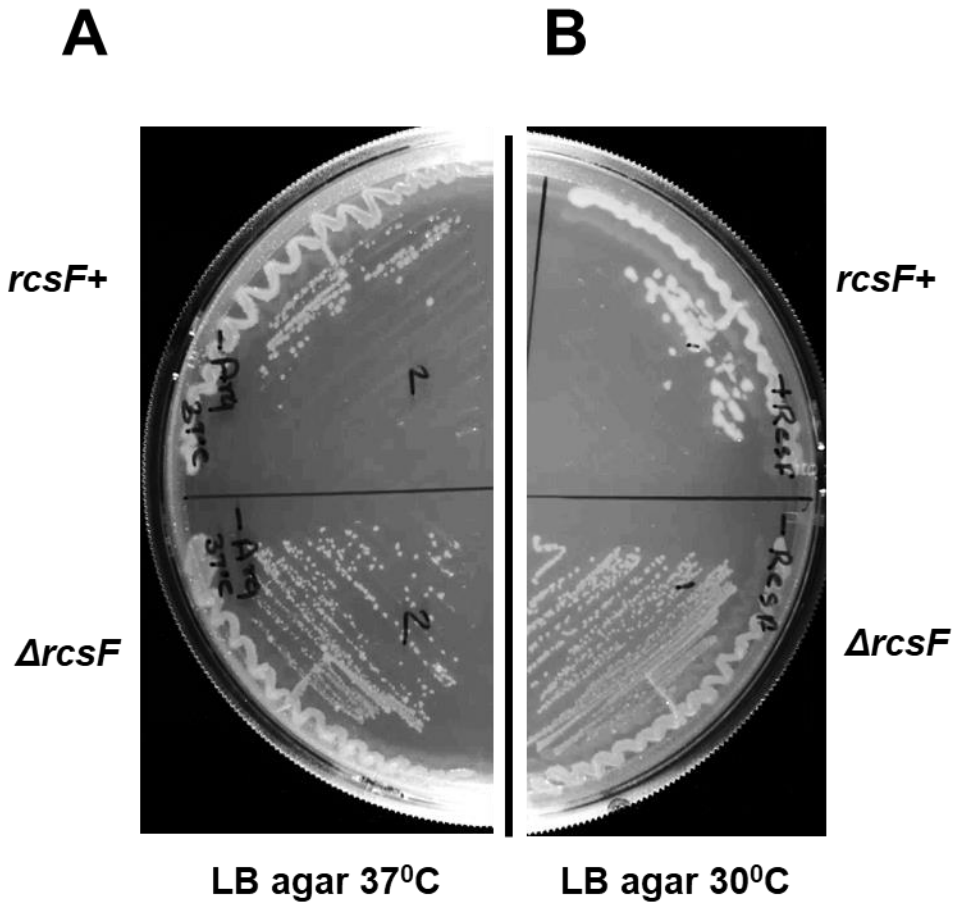


Figure S11. Plate growth phenotype of empty vector or pTrc99a::*bamE* variants in the SK-130 [*ΔbamE ΔbamA* P_{BAD}*-bamA // pZS21:Strep-bamA(373A)*] (A) and MT-171 [*bamE::cm bamD(R197L) nadB::Tn10*] (B) genetic backgrounds. Growth was assayed under permissive (M9 glucose minimal agar at 30°C, after 48 hrs) or non-permissive (LB agar at 37°C after 24 hrs) conditions.

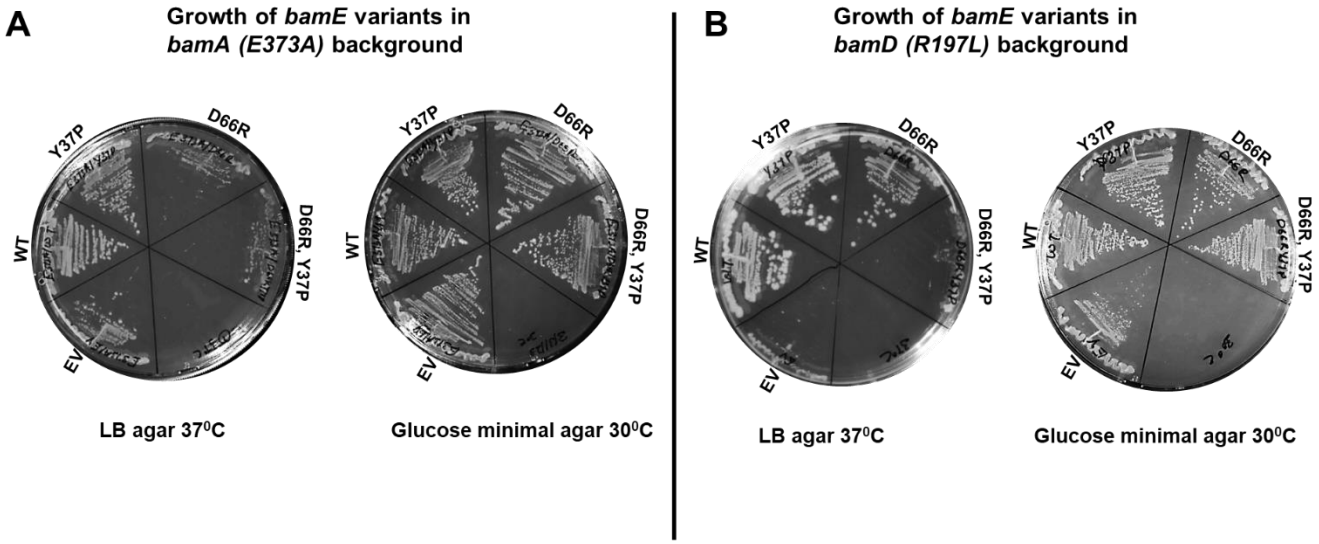


Figure S12. Immunoblot quantification of *in vivo* pull-down analysis presented in Fig. 4 based on three independent biological replicates. BamE-His (A) and Strep-BamA (B) pull-downs in the SK-130 [*ΔbamE ΔbamA* P_{BAD}*-bamA // pZS21:Strep-bamA(373A)*] genetic background; BamE-His (C) and Strep-BamA (D) pull-downs in the MT-171 [*bamE::cm bamD(R197L) nadB::Tn10*] (B) genetic background. Graphs represent the quantification of corresponding bands normalized to the level of the bait protein, BamE-His (A, C) and Strep-BamA (B, D), and compared to that of the *bamE(WT)* strain. Graphs represent mean +/- SD. Statistical analysis was performed by using one-way ANOVA in comparison with the WT control. n.s. = P ≥ 0.05, ****P < 0.0001.

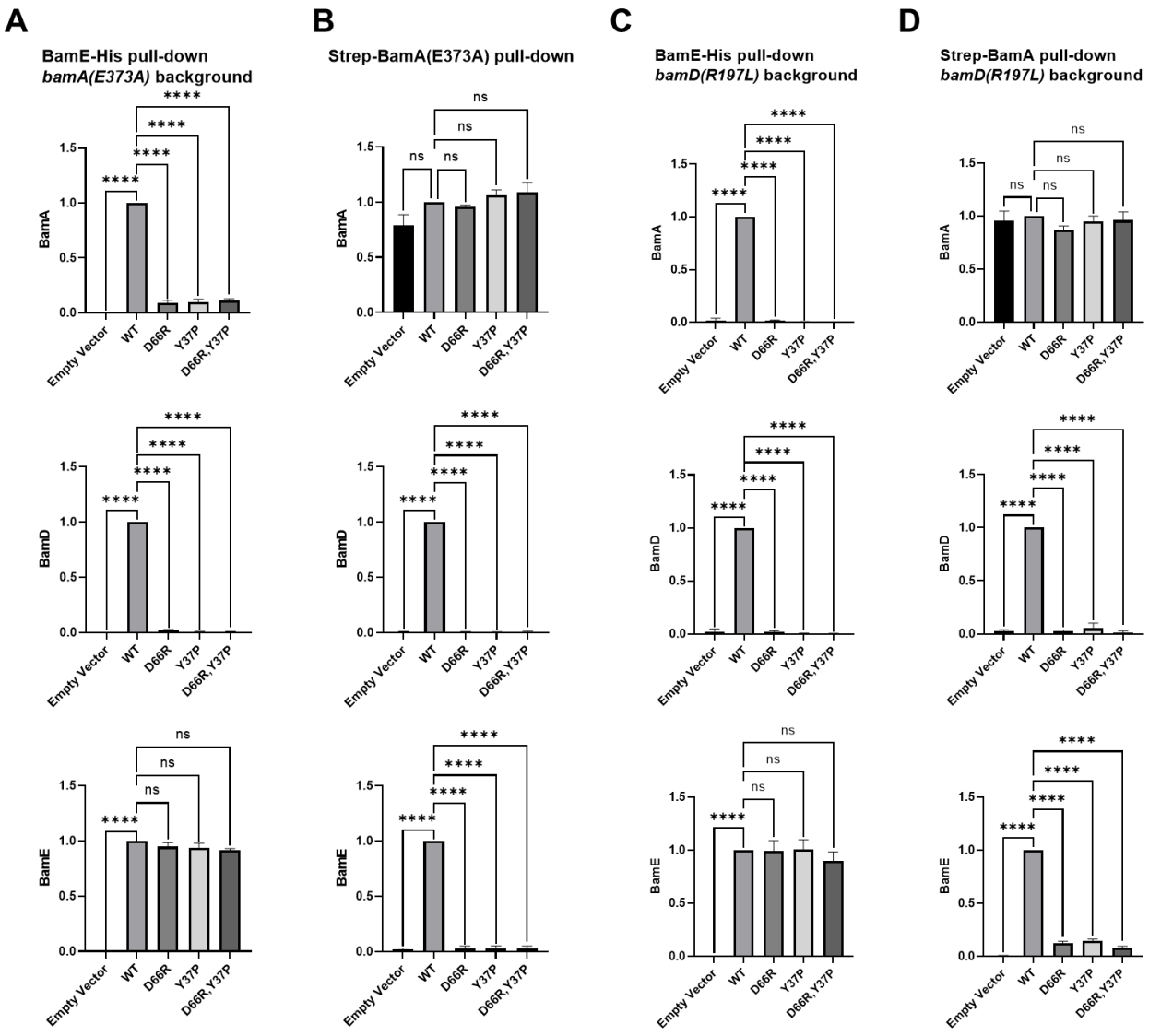


Table S1. Polar contact between BamE and BamA predicted based on the ColabFold protein complex modeling. Analysis of interacting interfaces was performed by using the PDBePISA server (Krissinel & Henrick, 2007).

Interaction of BamE/BamA interface in *E. coli*					
BamE	**Group**	**BamA**	**Group**	**Distance, A**	**interaction**
TYR37	[N]	TYR348	[OH]	3.11	H-bond
TYR37	[O]	TYR348	[OH]	3.53	H-bond
GLN34	[NE2]	GLU373	[O]	3.36	H-bond
GLY35	[N]	GLY374	[O]	2.78	H-bond
THR61	[N]	ASP410	[OD2]	3.00	H-bond
THR61	[OG1]	ASP410	[OD1]	2.93	H-bond
Interaction of BamE/BamA interface in *S. enterica*					
TYR37	[N]	TYR348	[OH]	3.28	H-bond
TYR37	[O]	TYR348	[OH]	3.57	H-bond
GLY35	[N]	GLY374	[O]	3.06	H-bond
THR61	[OG1]	ASP410	[OD1]	2.63	H-bond
THR61	[N]	ASP410	[OD2]	3.05	H-bond
Interaction of BamE/BamA interface in *P. aeruginosa*					
VAL37	[N]	TYR347	[OH]	2.66	H-bond
GLY35	[N]	GLY373	[O]	2.59	H-bond
ASN61	[N]	ASP409	[OD2]	2.75	H-bond
GLN34	[NE2]	GLU372	[O]	2.92	H-bond
PHE24	[O]	TYR518	[OH]	3.57	H-bond
TYR28	[N]	PRO519	[O]	3.43	H-bond
Interaction of BamE/BamA interface in *N. meningitides*					
GLU37	[N]	TYR348	[OH]	3.16	H-bond
GLY35	[N]	SER374	[O]	2.75	H-bond
SER62	[N]	ASP410	[OD2]	2.72	H-bond
SER27	[OG]	ASP481	[OD2]	2.83	H-bond
Interaction of BamE/BamA interface in *C. crescentus*					
TYR32	[N]	TYR346	[OH]	3.62	H-bond
GLY29	[N]	ASP33	[OD1]	2.84	H-bond
PRO57	[O]	ARG408	[NH2]	2.03	H-bond
SER58	[O]	ARG408	[NH1]	3.48	H-bond
ANS56	[ND2]	ASP407	[OD1]	2.37	H-bond

Table S2. Polar contact between BamE and BamD predicted based on the ColabFold protein complex modeling. Analysis of interacting interfaces was performed by using the PDBePISA server (Krissinel & Henrick, 2007).

Interaction of BamE/BamD interface in *E. coli*					
BamE	**Group**	**BamD**	**Group**	**Distance, A**	**Interaction**
GLN34	[NE2]	GLY189	[O]	2.74	H-bond
GLN34	[OE1]	TRP191	[N]	2.63	H-bond
SER65	[OG]	GLU199	[OE1]	3.09	H-bond
SER111	[O]	GLN226	[NE2]	2.91	H-bond
SER111	[N]	GLN226	[OE1]	3.04	H-bond
ASP66	[OD2]	K233	[NZ]	2.81	Salt bridge
Interaction of BamE/BamD interface in *S. enterica*					
THR65	[OG1]	GLU199	[OE1]	3.13	H-bond
GLN34	[NE2]	GLY189	[O]	2.68	H-bond
GLN34	[OE1]	TRP191	[N]	2.63	H-bond
THR111	[OG1]	GLN228	[OE1]	2.84	H-bond
ASP66	[OD2]	GLN230	[NE2]	3.30	H-bond
ASP66	[OD2]	LYS233	[NZ]	2.96	Salt-bridge
Interaction of BamE/BamD interface in *P. aeruginosa*					
ARG66	[NH1]	TYR294	[OH]	2.77	H-bond
THR77	[OG1]	GLU202	[OE1]	3.02	H-bond
GLN98	[NE2]	L224	[O]	2.70	H-bond
ARG100	[NH1]	G225	[O]	2.99	H-bond
Interaction of BamE/BamD interface in *N. meningitides*					
GLN34	[OE1]	TYR193	[N]	3.23	H-bond
ARG65	[O]	GLN201	[NE2]	3.07	H-bond
TYR75	[O]	LYS229	[NZ]	3.81	H-bond
ARG88	[NH2]	LYS226	[O]	2.99	H-bond
ARG88	[NH1]	ASP229	[OD2]	3.26	Salt- bridge
TYR94	[OH]	ARG232	[NH1]	3.31	H-bond
Interaction of BamE/BamD interface in *C. crescentus*					
THR61	[OG1]	N234	[ND2]	3.39	H-bond
GLU63	[OE2]	ARG233	[NH]	2.34	Salt bridge
R59	[O]	LYS197	[NZ]	2.35	H-bond
T58	[OG1]	LYS197	[NZ]	2.96	H-bond

Table S3. Strains used in this study. Unless otherwise indicated, the host background is MC4100 (JCM158).

Strain	Genotype	Reference
Mach-1	F- *φ80(lacZ)ΔM15 ΔlacX74 hsdR(rK-mK+) ΔrecA1398 endA1 tonA*	Invitrogen
pET15b		Novagen
pTrc99a		(Amann *et al.*, 1988)
pZS21		(Lutz & Bujard, 1997)
JCM158	MC4100 *araR*/-	(Malinverni *et al.*, 2006)
JCM320	MC4100 *araR*/-; *ΔbamA λatt* (P_{BAD}-*bamA, bla')*	(Wu *et al.*, 2005)
BL21 (DE3)	F– *ompT hsdSB (rB– mB–) gal dcm (DE3)*	Novagen
SK05	BL21 (DE3)-pET15b::His-*bamA*	(Tata *et al.*, 2021)
SK28	BL21 (DE3)-pET15b::*bamE*-Strep	This study
SK29	BL21 (DE3)-pET15b::*bamD*-Strep	This study
SK35	BL21 (DE3)-pET15b::*bamD*-His	This study
SK36	BL21 (DE3)-pET15b::*bamE*-His	This study
SK110	BL21 (DE3)-pET15b::*bamE*-His(D66R)	This study
SK95	BL21 (DE3)-pET15b::*bamE*-Strep(Y37P)	This study
SK-129	*ΔbamA λatt* (P_{BAD}-*bamA, bla') Δ bamE* //pZS21-Twin-Strep-tag-*bamA*	This study
MT-428	*ΔbamA, ΔbamE, ΔrcsF nadB::Tn10* // pZS21-Twin-Strep-tag-*bamA*	(Tata *et al.*, 2021)
SK-77	MT-428//pTrc99a	This study
SK-78	MT-428//pTrc99a::*bamE-His8*	This study
BL21 (DE3)	F– *ompT hsdSB (rB– mB–) gal dcm (DE3)*	Novagen
SK05	BL21 (DE3)-pET15b::His-*bamA*	(Tata *et al.*, 2021)
SK28	BL21 (DE3)-pET15b::*bamE*-Strep	This study
SK29	BL21 (DE3)-pET15b::*bamD*-Strep	This study
SK35	BL21 (DE3)-pET15b::*bamD*-His	This study
SK36	BL21 (DE3)-pET15b::*bamE*-His	This study
SK110	BL21 (DE3)-pET15b::*bamE*-His(D66R)	This study
SK95	BL21 (DE3)-pET15b::*bamE*-Strep(Y37P)	This study
pTrc99a		(Amann *et al.*, 1988)
AK-790	*ΔbamE λatt (PrprA-lacZ)*	(Tata & Konovalova, 2019)
SK37	AK790//pTrc99a	This study
SK38	AK790// pTrc99a::*bamE-His8*	This study
SK43	AK790- pTrc99a::*bamE-His8* (D66R)	This study
SK44	AK790- pTrc99a:: *bamE-His8* (D66G)	This study
SK45	AK790- pTrc99a:: *bamE-His8* (Y37P)	This study
SK46	AK790- pTrc99a:: *bamE-His8* (D66R,Y37P)	This study
SK41	AK790- pTrc99a:: *bamE-His8* (Y37G)	This study
SK100	AK790- pTrc99a:: *bamE-His8* (D66R,Y37G)	This study
SK102	AK790- pTrc99a:: *bamE-His8* (D66G,Y37P)	This study
AK-1255	*ΔaraBAD ΔbamE λatt (P_{BAD}-bamE, bla') bamB8 yfhS::Tn10*	(Tata *et al.*, 2021)
SK49	AK1255-pTrc99a	This study
SK50	AK1255-pTrc99a::*bamE*-His8	This study
SK55	AK1255-pTrc99a::*bamE*-His8(D66R)	This study
SK56	AK1255-pTrc99a::*bamE*-His8(D66G)	This study

SK57	AK1255-pTrc99a::*bamE*-His8(Y37P)	This study
SK58	AK1255-pTrc99a::*bamE*-His8(D66R,Y37P)	This study
SK53	AK1255-pTrc99a::*bamE*-His8(Y37G)	This study
SK97	AK1255-pTrc99a::*bamE*-His8(D66R,Y37G)	This study
SK99	AK1255-pTrc99a::*bamE*-His8(D66G,Y37P)	This study
MT-567	*ΔbamA ΔbamE nadA::Tn10 λ att (PrprA-lacZ)// pZS21-Twin-Strep-tag-bamA*	(Tata *et al.*, 2021)
SK80	MT567-pTrc99a	This study
SK81	MT567-pTrc99a::*bamE*-His8	This study
SK84	MT567-pTrc99a::*bamE*-His8 (Y37G)	This study
SK86	MT567-pTrc99a::*bamE*-His8 (D66R)	This study
SK87	MT567-pTrc99a::*bamE*-His8 (D66G)	This study
SK88	MT567-pTrc99a::*bamE*-His8 (Y37P)	This study
SK89	MT567-pTrc99a::*bamE*-His8 (D66R,Y37P)	This study
SK103	MT567-pTrc99a::*bamE*-His8 (D66R,Y37G)	This study
SK105	MT567-pTrc99a::*bamE*-His8 (D66G, Y37P)	This study
SK-130	*ΔbamA λatt* (P_{BAD}-*bamA, bla') Δ bamE* //pZS21-Twin-Strep-tag-*bamA* (E373A)	This study
SK-131	SK-130//pTrc99a	This study
SK-132	SK-130// pTrc99a::*bamE*-His8	This study
SK-133	SK-130// pTrc99a::*bamE*-His8 (Y37P)	This study
SK-134	SK-130//pTrc99a::*bamE*-His8 (D66R)	This study
SK-135	SK-130//pTrc99a::*bamE*-His8 (D66R,Y37P)	This study
SK 155	SK-130 *ΔrcsF*	This study
MT-171	*bamD(R197L) nadB*::Tn10 *bamE::Cm λatt (PrprA-lacZ)*	(Tata & Konovalova, 2019)
AK-1233	MT-171 *ΔrcsF*	(Malinverni *et al.*, 2006)
SK-141	MT-171//pTrc99a	This study
SK-142	MT-171// pTrc99a::*bamE*-His8	This study
SK-143	MT-171// pTrc99a::*bamE*-His8 (Y37P)	This study
SK-144	MT-171//pTrc99a::*bamE*-His8 (D66R)	This study
SK-145	MT-171//pTrc99a::*bamE*-His8 (D66R,Y37P)	This study

Table S4. Primers used in this study:

Primer	Descriptions /Sequence	Used for
SK-8 BamE - Strep F	AGGACCCATGGGTTCCACTCTGGAGCGAGTGGTTTACCG	Cloning into pET15b
SK-9 BamE-Strep R	ATATCTCGAGTTATTTTTCGAACTGCGGGTGGCTCCA ACCACCACCACCAGAGTTACCACTCAGCGCAGGTTTGTTATC	
SK-10 BamD-Strep F	AGGACCATGGGTTCGGGGTCAAAGGAAGAAGTAC	Cloning into pET15b
SK-11 BamD-Strep R	ATATCTCGAGTTATTTTTCGAACTGCGGGTGGCTCCA ACCACCACCACCAGATGTATTGCTGCTGTTTGCG	
SK-20 BamE (His) F	CATCATCACTAACTCGAGATGGGTTCC	Cloning into pET15b
SK-21 BamE (His) R	ATGATGATGACCAGAGTTACCACTCAG	
AK-373 bamE F	ATCGCtctagaGAGGAAATACTATGCGCTGTAAAACGCTGACTGCT	Cloning into pTrc99a
AK-374 BamE-His8 R	ATCGCgtcgacTTAGTGGTGATGATGGTGGTGATGATGACCACCGTT ACCACTCAGCGCAGGTTTGTTATC	
SK-14 BamE (D66R) F	GCTGATGTCCCGTCCATTTGGTACGAATAC	Site-directed mutagenesis
SK-15 BamE (D66R) R	GGTGTACCCAATGCGTAC	
SK-16 BamE (D66G) F	GCTGATGTCCGGTCCATTTGGTA	Site-directed mutagenesis
SK-17 BamE (D66G) R	GGTGTACCCAATGCGTAC	
SK-18 BamE (Y37P) F	CCAGGGGAACCCTCTGACCGCTAAC	Site-directed mutagenesis
SK-19 BamE (Y37P) R	TTGATGTCAGGACGGTAAAC	
SK-34 BamE (Y37G) F	CCAGGGGAACGGTCTGACCGCTAAC	Site-directed mutagenesis
SK-35 BamE (Y37G) R	TTGATGTCAGGACGGTAAAC	

SUPPLEMENTAL MATERIALS METHODS

Bacterial strains and growth conditions: All the bacterial strains used in this study are listed in Table S3, and derived from MC4100 unless otherwise stated. Strains were grown at either 37°C or 30°C as indicated in Lysogeny broth (LB) (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) or M9 glucose minimal medium (26.1 mM Na2HPO4, 22 mM KH2PO4, 8.5 mM NaCl, 18.6 mM NH4Cl, 0.2% glucose, 1 mM MgSO4, 100 µg/ml thiamine and 100 µM β-NAD hydrate to support the growth of *nadA*::Tn*10* and *nadB*::Tn*10* strains. 0.4% w/v of L-arabinose (Goldbio) and antibiotics were added when appropriate at the following concentrations: chloramphenicol 20 µg/ml (Cam), kanamycin (Kan) 25 µg/ml, tetracycline (Tet) 10 µg/ml, ampicillin (Amp) 125 µg/mL.

Strains with pTrc99a derived plasmids were grown without IPTG. For *nadA*::Tn*10* and *nadB*::Tn*10* strains containing pZS21::Strep-BamA, media was supplemented with 2ng/ml anhydrotetracyline, (aTc) (Alfa Aesar) to derepress BamA expression.

Growth curve analysis was performed by growing 1 ml cultures in 24-well plates with Breathe-Easy ® sealing membrane (Sigma-Aldrich) at 37 °C with orbital shaking. The OD600 was monitored using BioTek Synergy H1 plate reader. All growth curves were performed in at least three independent biological replicates.

Cloning, expression, and purification of individual proteins.

Genes encoding tagged (His6 or Strep) versions of BamE and BamD without lipid modification were amplified using primers listed in Table S4 and Q5 high fidelity DNA polymerase. Gene products were cloned into the pET15b using standard molecular biology techniques, transformed into the *E. coli* Mach1 strain. Sequencing-confirmed clones were transformed in BL21(DE3) cells. Proteins were expressed by growing transformed bacteria in LB broth supplemented with 125 µg/mL of ampicillin at 37 °C. When the OD600nm of the culture reached 0.5, IPTG was added to a final concentration of 1 mM, and cultures were incubated for another 4 hours to allow expression. Cells were harvested by centrifugation, washed, and disrupted by Avestin Emulsiflex C3 at 30 psi in buffer A (25 mM Tris–HCl pH 8.0, 150 mM NaCl, 0.1mM PMSF, and 1x Protease Inhibitor Cocktail, 0.1 mg/mL lysozyme), and clarified by centrifugation at 15000g for 30 min at 4 C, and subjected to affinity chromatography using ÄKTA Pure 25 system (GE Healthcare Life Sciences).

For BamE-Strep and BamD-Strep purification, clarified supernatants were loaded onto StrepTrap™ HP column (GE Healthcare Life Sciences) pre-equilibrated in buffer B (25 mM Tris–HCl pH 8.0, 150 mM NaCl). The column was washed with buffer B, and bound proteins were eluted with buffer B containing 5 mM desthiobiotin.

For BamE-His purification, clarified supernatant was loaded onto HisTrap™ FF column (GE Healthcare Life Sciences) pre-equilibrated in buffer B (25 mM Tris–HCl pH 8.0, 150 mM NaCl). The column was washed with buffer B, and bound proteins were eluted with buffer B containing 250 mM imidazole.

Purification and refolding of His-BamA were described previously (Tata *et al.*, 2021).

After purification, all proteins were dialyzed in buffer B (25 mM Tris–HCl pH 8.0, 150 mM NaCl), and analyzed by 15% SDS polyacrylamide gel (SDS-PAGE).

In silico analysis:

All the protein sequences of BamA, BamD, and BamE of different bacterial species were retrieved from NCBI, and multiple sequence alignment of POTRA5 domain of BamA, BamD, and BamE of different bacterial species was done by using Clustal Omega (Sievers *et al.*, 2011).

ColabFold(Mirdita *et al.*, 2022) was used to predict the protein complexes of the corresponding protein pairs and mutant analysis. Amino acid sequences of the BamE, BamA or BamD proteins devoid of their signal sequence and the lipid-modified cysteine residue were analyzed using default settings (“protein structure prediction using "AlphaFold2-ptm" and complex prediction "AlphaFold-multimer-v2". For complexes "AlphaFold-multimer-v[1,2]" and "AlphaFold-ptm" can be used”) at the Google Collab interface. Top scoring high confidence PDB generated from ColabFold server was used for interacting interfaces analysis using the PDBePISA (Krissinel & Henrick, 2007).

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