Supplemental material for:

Functions of the BamBCDE lipoproteins revealed by bypass mutations in BamA

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Table S1: Linkage disruption analysis with *bamB*::kan *xyz*::cam*.

Strain	Linkage frequency (%) *bamB*::kan *xyz*::cam	Standard deviation
MC4100	45%	9.2%
Δ*bamC*Δ*bamE* Tn7att::*bamA*	0%	0%
Δ*bamC*Δ*bamE* Tn7att::$bamA_{E470K}$	12.7%	4%
Δ*bamC*Δ*bamE* Tn7att::$bamA_{A496P}$	0%	0%
Δ*bamC*Δ*bamE* Tn7att::$bamA_{A499S}$	0%	0%
Δ*bamC*Δ*bamE* Δ*rcsF* Tn7att::*bamA*	0%	0%
Δ*bamC*Δ*bamE* Δ*rcsF* Tn7att::$bamA_{E470K}$	13.7%	7.1%
Δ*bamC*Δ*bamE* Δ*rcsF* Tn7att::$bamA_{A496P}$	10.9%	6.3%
Δ*bamC*Δ*bamE* Δ*rcsF* Tn7att::$bamA_{499S}$	4.5%	3.3%

*P1 phage carrying *bamB*::kan *xyz*::cam was used to infect the indicated strains. Linkage frequency represents the number of kanamycin resistant transductants over the total chloramphenicol resistant transductants. Transductions were performed in biological triplicate.

Table S2: Linkage disruption analysis with *bamD*::kan *nadB*::Tn10*.

Strain	Linkage frequency (%) *bamD*::kan *nadB*::Tn10	Standard deviation
bamD^{+} λ_{att} P_{BAD} *bamD* (+ara)	26.7%	4.7%
Tn7att::*bamA*	0%	0%
Tn7att::$bamA_{A496P}$	14%	7.2%
Tn7att::$bamA_{A499S}$	0%	0%
Δ*rcsF* Tn7att::*bamA*	0%	0%
Δ*rcsB* Tn7att::*bamA*	0%	0%
Δ*rcsF* Tn7att::$bamA_{A496P}$	12.3%	5.5%
Δ*rcsF* Tn7att::$bamA_{A499S}$	17.9%	1.4%
Δ*rcsB* Tn7att::$bamA_{A499S}$	0%	0%
Δ*bamC bamE*::cam Tn7att::*bamA*	0%	0%
Δ*bamC bamE*::cam Tn7att::$bamA_{E470K}$	10%	1.7%
Δ*bamC bamE*::cam Tn7att::$bamA_{A496P}$	8.8%	2.5%
Δ*bamC bamE*::cam Tn7att::$bamA_{A499S}$	0%	0%
Δ*bamC bamE*::cam Δ*rcsF* Tn7att::*bamA*	0%	0%
Δ*bamC bamE*::cam Δ*rcsF* Tn7att::$bamA_{E470K}$	4.9%	3.6%
Δ*bamC bamE*::cam Δ*rcsF* Tn7att::$bamA_{A496P}$	8.8%	2.3%
Δ*bamC bamE*::cam Δ*rcsF* Tn7att::$bamA_{A499S}$	14.9%	2.6%

*****Linkage disruption was performed by infecting the indicated strains with P1 phage carrying *bamD*::kan *nadB*::Tn10. The number of kanamycin resistant colonies over the total number of tetracycline resistant colonies was reported as linkage frequency.

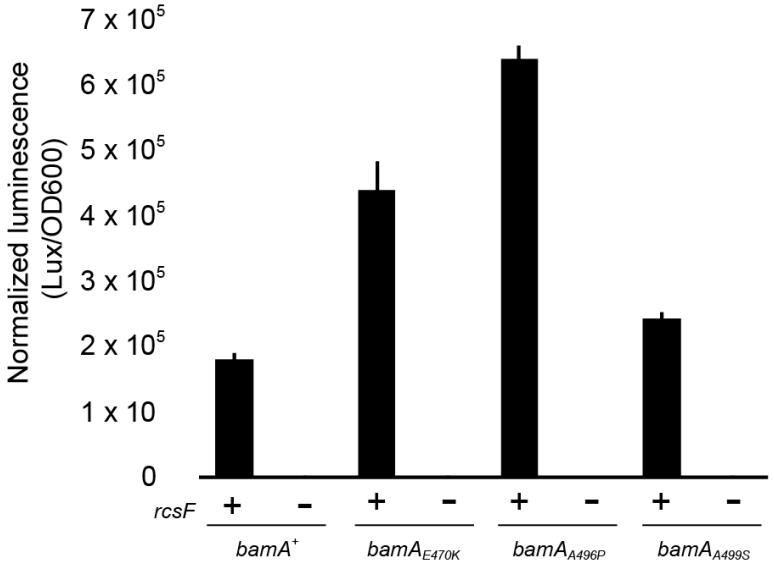

Figure S1: RcsF/OMP assembly in the *bamA* suppressor alleles as measured P_{rprA}-*lux* activity. The labeled strains carrying pCS26(cam)::P_{rprA}-*lux* were grown to mid-log at 30°C. Lux activity was normalized by OD600 and data represents the average of three biological replicates with errors bars being SEM.

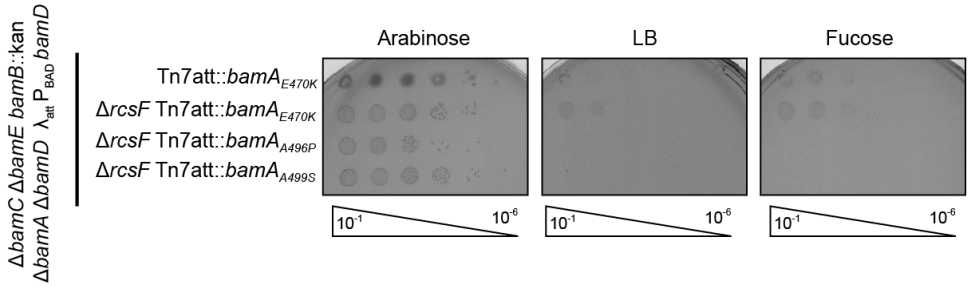

Figure S2: **Testing minimal BAM complexes.** Δ*bamB*Δ*bamC*Δ*bamE* mutants expressing an arabinose-inducible copy of *bamD* were grown overnight at 30°C in media containing arabinose. Cells were normalized by OD600, serially diluted, and spotted onto media containing arabinose to induce expression of *bamD* or fucose to prevent expression.

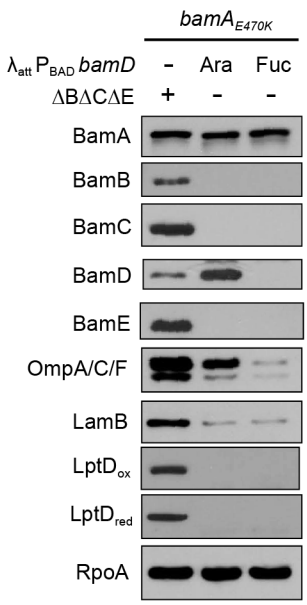

Figure S3: OMP assembly is impaired in Δ*bamB*Δ*bamC*Δ*bamE bamA*$_{E470K}$ BamD-depleted cells. Immunoblot analysis of BamD-depleted cells from the depletion shown in Figure 4D. Stationary phase samples were electrophoresed and probed for BamABCDE and several OMPs. RpoA served as a loading control.

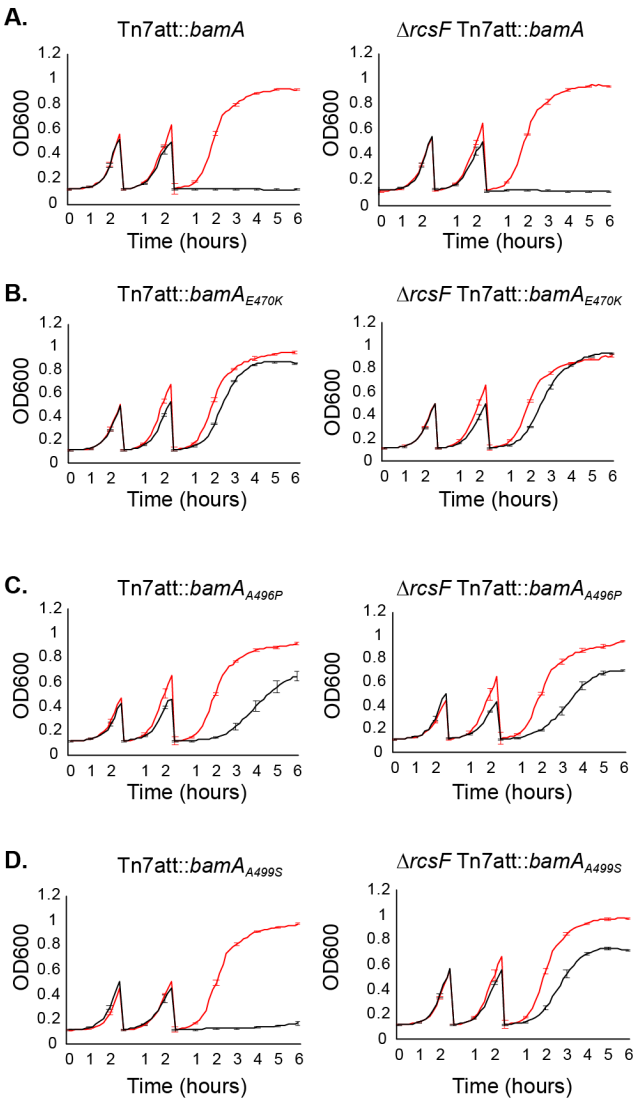

Figure S4: Cell viability upon liquid depletion of BamD. **(A-D)** Cells (+/- *rcsF*) expressing **(A)** BamA^{+}, **(B)** BamA^{E470K}, **(C)** BamA^{A496P}, or **(D)** BamA^{A499S} and carrying an arabinose inducible copy of *bamD* were grown overnight in arabinose. Cells were normalized by OD600 and inoculated into media supplemented with arabinose or fucose. Cultures were grown to and OD600 ~ 0.5 at 37°C and back-diluted into fresh media. The cycling was repeated once more. Depletions were performed at 37°C.

Table S3: Strains, plasmids, and oligonucleotides used in this study.

Strains	Genotype	Reference
MC4100	F-*araD139 (argF-lac)U169 rpsL150 relA1 flb5301 deoC1 ptsF25 thi*	(1) (2)
JCM158	MC4100 $ara^{r/-}$	(3)
JCM255	λ_{att} P_{BAD}::*bamD*	(4)
JCM500	Δ*bamA recA*::kan λ_{att} P_{BAD}::*bamA*	(4)
BH315	*bamB*::kan *xyz*::cam	This study
BH1417	Δ*bamA* Tn7att::*bamA*FRT	(4)
BH1418	Δ*bamA* Tn7att::$bamA_{E470K}$FRT	(4)
BH1779	Δ*bamC* Δ*bamE* Δ*bamA* Tn7att::*bamA*FRT	This study
BH1780	Δ*bamC* Δ*bamE* Δ*bamA* Tn7att::$bamA_{E470K}$FRT	This study
BH2076	Δ*bamA* Tn7att::$bamA_{A496P}$FRT	This study
BH2077	Δ*bamA* Tn7att::$bamA_{A499S}$FRT	This study
BH2084	Δ*bamC* Δ*bamE* Δ*bamA* Tn7att::$bamA_{A496P}$FRT	This study
BH2093	Δ*rcsF* Δ*bamA* Tn7att::*bamA*FRT	This study
BH2094	Δ*rcsF* Δ*bamA* Tn7att::$bamA_{E470K}$FRT	This study
BH2109	Δ*bamC* Δ*bamE* Δ*bamA* Tn7att::$bamA_{A499S}$FRT	This study
BH2142	Δ*rcsF* Δ*bamA* Tn7att::$bamA_{A499S}$FRT	This study
BH2222	Δ*bamC* Δ*bamE* Δ*rcsF* Δ*bamA* Tn7att::*bamA*FRT	This study
BH2223	Δ*bamC* Δ*bamE* Δ*rcsF* Δ*bamA* Tn7att::$bamA_{A499S}$FRT	This study
BH2259	Δ*bamC* Δ*bamE* Δ*rcsF* Δ*bamA* Tn7att::$bamA_{A496P}$FRT	This study
BH2305	*bamD*::kan *nadB*::Tn10 λ_{att} P_{BAD} *bamD*	(5)
BH2308	Δ*bamA* Tn7att::$bamA_{E470K}$FRT *bamD*::kan	(5)
BH2312	Δ*bamA* Tn7att::$bamA_{A496P}$FRT *bamD*::kan	This study
BH2316	Δ*rcsF* Δ*bamA* Tn7att::$bamA_{A499S}$FRT *bamD*::kan	This study
BH2320	Δ*bamC* Δ*bamE* Δ*bamA* Tn7att::$bamA_{E470K}$FRT *bamB*::kan	This study

BH2323	Δ*bamC* Δ*bamE* Δ*rcsF* Δ*bamA* Tn7att::$bamA_{A496P}$FRT *bamB*::kan	This study
BH2327	Δ*bamC* Δ*bamE* Δ*rcsF* Δ*bamA* Tn7att::$bamA_{A499S}$FRT *bamB*::kan	This study
BH2339	Δ*rcsF* Δ*bamA* Tn7att::$bamA_{E470K}$FRT *bamD*::kan	(5)
BH2342	Δ*bamC* Δ*bamE* Δ*rcsF* Δ*bamA* Tn7att::$bamA_{E470K}$FRT *bamB*::kan	This study
BH2366	Δ*bamA* Δ*bamD* Tn7att::*bamA*FRT λ_{att} P_{BAD}::*bamD*	This study
BH2368	Δ*bamA* Δ*bamD* Tn7att::$bamA_{E470K}$FRT λ_{att} P_{BAD}::*bamD*	This study
BH2369	Δ*rcsF* Δ*bamA* Δ*bamD* Tn7att::$bamA_{E470K}$FRT λ_{att} P_{BAD}::*bamD*	This study
BH2370	Δ*bamA* Δ*bamD* Tn7att::$bamA_{A496P}$FRT λ_{att} P_{BAD}::*bamD*	This study
BH2372	Δ*bamA* Δ*bamD* Tn7att::$bamA_{A499S}$FRT λ_{att} P_{BAD}::*bamD*	This study
BH2373	Δ*rcsF* Δ*bamA* Δ*bamD* Tn7att::$bamA_{A499S}$FRT λ_{att} P_{BAD}::*bamD*	This study
BH2410	Δ*bamA* Δ*bamD* Tn7att::*bamA*FRT *bamB*::kan λ_{att} P_{BAD}::*bamD*	This study
BH2412	Δ*rcsF* Δ*bamA* Δ*bamD* Tn7att::$bamA_{E470K}$FRT *bamB*::kan λ_{att} P_{BAD}::*bamD*	This study
BH2413	Δ*bamA* Δ*bamD* Tn7att::$bamA_{E470K}$FRT *bamB*::kan λ_{att} P_{BAD}::*bamD*	This study
BH2414	Δ*bamA* Δ*bamD* Tn7att::$bamA_{A496P}$FRT *bamB*::kan λ_{att} P_{BAD}::*bamD*	This study
BH2416	Δ*bamA* Δ*bamD* Tn7att::$bamA_{A499S}$FRT *bamB*::kan λ_{att} P_{BAD}::*bamD*	This study
BH2417	Δ*rcsF* Δ*bamA* Δ*bamD* Tn7att::$bamA_{A499S}$FRT *bamB*::kan λ_{att} P_{BAD}::*bamD*	This study
BH2435	Δ*rcsB* Δ*bamA* Tn7att::$bamA_{A499S}$FRT	This study

BH2488	Δ*bamC* Δ*bamE* Δ*rcsF* Δ*bamA* Tn7att::$bamA_{E470K}$FRT	This study
BH2500	Δ*bamC* Δ*bamE* Δ*rcsF* Δ*bamA* Δ*bamD* Tn7att::$bamA_{E470K}$FRT λ_{att} P_{BAD}::*bamD bamB*::kan	This study
BH2513	Δ*bamC* Δ*bamE* Δ*rcsF* Δ*bamA* Δ*bamD* Tn7att::$bamA_{A499S}$FRT λ_{att} P_{BAD}::*bamD bamB*::kan	This study
BH2514	Δ*rcsF* Δ*bamA* Δ*bamD* Tn7att::*bamA*FRT λ_{att} P_{BAD}::*bamD*	This study
BH2515	Δ*rcsF* Δ*bamA* Δ*bamD* Tn7att::$bamA_{A496P}$FRT λ_{att} P_{BAD}::*bamD*	This study
BH2518	Δ*rcsF* Δ*bamA* Tn7att::$bamA_{A496P}$FRT	This study
BH2527	Δ*rcsF* Δ*bamA* Δ*bamD bamB*::kan Tn7att::*bamA*FRT λ_{att} P_{BAD}::*bamD*	This study
BH2534	Δ*rcsF* Δ*bamA* Tn7att::$bamA_{A496P}$FRT *bamD*::kan	This study
BH2543	Δ*bamC* Δ*bamE* Δ*bamA* Δ*bamD* Tn7att::$bamA_{E470K}$FRT λ_{att} P_{BAD}::*bamD bamB*::kan	This study
BH2546	Δ*bamC* Δ*bamE* Δ*rcsF* Δ*bamA* Δ*bamD* Tn7att::$bamA_{A496P}$FRT λ_{att} P_{BAD}::*bamD bamB*::kan	This study
BH2556	Δ*bamC* Δ*bamE* Δ*bamA* Δ*bamD* Tn7att::$bamA_{E470K}$FRT λ_{att} P_{BAD}::*bamD bamB*::kan (depleted in fucose)	This study
BH2559	Δ*bamC* Δ*bamE* Δ*bamA* Tn7att::$bamA_{E470K}$FRT *bamD*::kan	This study
BH2560	Δ*bamC* Δ*bamE* Δ*rcsF* Δ*bamA* Tn7att::$bamA_{E470K}$FRT *bamD*::kan	This study
BH2572	Δ*bamC* Δ*bamE* Δ*bamA* Tn7att::$bamA_{A496P}$FRT *bamD*::kan	This study
BH2573	Δ*bamC* Δ*bamE* Δ*rcsF* Δ*bamA* Tn7att::$bamA_{A496P}$FRT *bamD*::kan	This study
BH2574	Δ*bamC* Δ*bamE* Δ*rcsF* Δ*bamA* Tn7att::$bamA_{A499S}$FRT *bamD*::kan	This study
BH2588	Δ*bamC* Δ*bamA* Tn7att::*bamA*FRT *bamE*::cam	This study
BH2589	Δ*bamC* Δ*bamA* Tn7att::$bamA_{E470K}$FRT *bamE*::cam	This study

BH2592	Δ*bamC* Δ*rcsF* Δ*bamA* Tn7att::*bamA*$_{E470K}$FRT *bamE*::cam	This study
BH2593	Δ*bamC* Δ*bamA* Tn7att::*bamA*$_{A496P}$FRT *bamE*::cam	This study
BH2600	Δ*bamC* Δ*rcsF* Δ*bamA* Tn7att::*bamA*FRT *bamE*::cam	This study
BH2601	Δ*bamC* Δ*bamA* Tn7att::*bamA*$_{A499S}$FRT *bamE*::cam	This study
BH2649	Δ*bamC* Δ*rcsF* Δ*bamA* Tn7att::*bamA*$_{A496P}$FRT *bamE*::cam	This study
BH2650	Δ*bamC* Δ*rcsF* Δ*bamA* Tn7att::*bamA*$_{A499S}$FRT *bamE*::cam	This study
BH2666	Δ*bamA* Tn7att::*bamA*FRT pCS26(cam):: P$_{rprA}$-*lux*	This study
BH2667	Δ*rcsF* Δ*bamA* Tn7att::*bamA*FRT pCS26(cam):: P$_{rprA}$-*lux*	This study
BH2669	Δ*bamA* Tn7att::*bamA*$_{E470K}$FRT pCS26(cam):: P$_{rprA}$-*lux*	This study
BH2668	Δ*rcsF* Δ*bamA* Tn7att::*bamA*$_{E470K}$FRT pCS26(cam):: P$_{rprA}$-*lux*	This study
BH2677	Δ*bamA* Tn7att::*bamA*$_{A496P}$FRT pCS26(cam):: P$_{rprA}$-*lux*	This study
BH2678	Δ*rcsF* Δ*bamA* Tn7att::*bamA*$_{A496P}$FRT pCS26(cam):: P$_{rprA}$-*lux*	This study
BH2680	Δ*bamA* Tn7att::*bamA*$_{A499S}$FRT pCS26(cam):: P$_{rprA}$-*lux*	This study
BH2681	Δ*rcsF* Δ*bamA* Tn7att::*bamA*$_{A499S}$FRT pCS26(cam):: P$_{rprA}$-*lux*	This study
BH2695	Δ*rcsF* Δ*bamA* Δ*bamD* Tn7att::*bamA*$_{A496P}$FRT *bamB*::kan λ_{att} P$_{BAD}$::*bamD*	This study
Plasmids	**Relevant features**	**Reference**
pGRG25Modular::*bamA*	*bamA* under control of the native promoter (defined as 1000bp upstream of ATG) for insertion at Tn7 attachment site. Kanamycin resistance cassette is located downstream and flanked by FRT sequences.	(4, 6) (5)
pGRG25Modular::*bamA*$_{A496P}$	*bamA*$_{A496P}$ on plasmid for Tn7 attachment site integration	This study

pGRG25Modular::*bamA*$_{A499S}$	*bamA*$_{A499S}$ on plasmid for Tn7 attachment site integration	This study
Oligonucleotides	**Sequence (5' → 3')**	**Information**
BH102-bamC-Fwd	GCGTCTGATGCCATTAC ACAAC	To amplify chromosomal *bamC*
BH103-bamC-Rev	CTTTTGCTGTCTGGTGTG CC	
BH310	ATGGCGATGAAAAAG TTG	To amplify *bamA* alleles from pZS21 plasmid for Gibson into pGRG25Modular::*bamA*
BH311	ATAGGAACTTCAAAA GGGCCTTACCAGGTTT TACCGATG	

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