SUPPORTING INFORMATION APPENDIX

A monoclonal antibody targeting the β-barrel assembly machine of *Escherichia coli* is bactericidal

Storek et al.

SI MATERIALS AND METHODS

Growth conditions

Mueller Hinton II cation-adjusted broth (MHB II, BBL 212322) supplemented with 0.002% Tween-80 was prepared according to manufacturer's instructions. Bacterial cultures were grown at 37°C. When appropriate, media was supplemented with kanamycin (50 μg/mL), carbenicillin (50 μg/mL), chloramphenicol (12.5 μg/mL), hygromycin (200 μg/mL), gentamicin (10 μg/mL), or arabinose (0.2% wt/vol).

Bacterial strains and plasmids

Bacterial strains and relevant primers are listed in **Table S2**. A kanamycin deletion-insertion mutation of *waaD* was obtained from the Keio collection(1). Mutant strains were created using λ Red recombination(2). Briefly, pKD4 or pKD3 was amplified with primers containing 50 bp nucleotide homology extensions (**Table S2**) to the gene of interest. The linear product was transformed into the appropriate background strain containing pSIM18(3), recovered for 4 hours, and selected on media containing 50 μg/mL kanamycin or 12.5 μg/mL chloramphenicol, as appropriate. Mutations were confirmed by PCR and sequencing. To make sequential mutants, the antibiotic marker was flipped out using pCP20(2).

The conditional *bamA* strain, Δ*bamA*::P_{BAD}-*bamA*, was created by inserting P_{BAD}-*bamA* at the *attB* site in MG1655 followed by deletion of the native copy of *bamA*(2, 4). Briefly, *bamA* was cloned into pBAD24 using standard methods. P_{BAD}-*bamA* was amplified from pBAD24-*bamA* and sub-cloned into pLDR9. pLDR9-P_{BAD}-*bamA* was digested with NotI, ligated, and transformed into MG1655 expressing pLDR8. PCR and DNA sequencing confirmed insertion of P_{BAD}-*bamA* at the *attB* site. After integration of P_{BAD}-*bamA*, the native copy of *bamA* was deleted using λ Red recombination as described above. In the absence of arabinose, the conditional Δ*bamA*::P_{BAD}-*bamA* strain did not grow.

BamA barrel swap chimeras were created in a Δ*ompT* mutant strain obtained from the Keio collection(1). The kanamycin-resistance cassette was removed using pCP20 and the Δ*waaD*::chloramphenicol-resistance-cassette (Cm) deletion was introduced by λ Red recombination(2). Plasmid pD884SR-*ompT* was generated by amplifying *ompT* from *E. coli* BW25113 genomic DNA and inserted into pD884-SR (DNA2.0™) via Electra-Cloning per manufacturer's instructions. Linear DNA constructs were created containing the *bamA* gene from the species of interest transcriptionally fused to a downstream gentamicin resistance cassette and flanked on both ends by homology to the *bamA* locus in the *E. coli* chromosome and inserted into *E. coli* BW25113 Δ*ompT*,

Δ*waaD*::Cm pD884SR-OmpT by λ Red recombination as described above. Replacement of the native *E. coli* β-barrel sequence was confirmed by sequencing.

Construction of pBla_BamA, BamA loop variants, and BamA ΔL6 was as follows. pBla_short BamA was created by first replacing the rhamnose-inducible promoter in pD884-SR with a constitutively active P_{bla} promoter(5) via PIPE cloning(6) to generate the plasmid pBla. Next, the *bamA*::Gent construct, described above, was introduced into pBla to yield pBla_BamA. PIPE cloning with primers BamA_del676-700_F / BamA_del676-700_R used to remove amino acids 676 through 700, yielding pBla_BamAΔL6 (P676_C700del). Site-directed mutants were made using QuickChange II XL Site-Directed Mutagenesis Kit (Agilent) per manufacturer's instructions. Mutations were confirmed by sequencing. Each *bamA* mutant plasmid was transformed into the conditional Δ*bamA*::P_{BAD}-*bamA,* Δ*waaD*::Cm.

The σ^E reporter p*rpoHP3-lacZ* pGNE18 was constructed based on previously validated σ^E reporter strains(7). Briefly, the *rpoH*P3 promoter was fused to *lacZ* (amplified from MG1655) and cloned into a low copy vector, pACYC184, using standard cloning techniques.

p*lpxM* was created by amplifying *lpxM* by PCR and cloning into pLMG18 using Gibson Assembly (NEB) according to the manufacture's protocol. IPTG was not needed to induce sufficient expression of *lpxM* and was not included in the described experiments.

GNE4297 used in fluorescence microscopy was created based on previously described methods(8, 9). pGFP (cytoplasm) was created by cloning sfGFP into pBla_Short using PIPE cloning(6). pdsbA(SS)-mCherry (periplasm) was constructed based on a previously described periplasmic mCherry reporter and cloned into pLMG18 using Gibson Assembly (NEB) according to the manufacture's protocol.

Antibody activity assay

Mice and rats were immunized with combinations of sub-lethal injections of *E. coli* bacteria and purified *E. coli* BamA protein. Hybridoma fusions were sorted and BamA-ELISA+ supernatants were screened for activity in the growth inhibition assay (below). 7 antibodies with greater than 90% inhibition of *E. coli* Δ*waaD* growth were all competitive by ELISA. MAB1 was selected for characterization as a representative from these mAbs. MAB2 did not affect *E. coli* Δ*waaD* growth but was BamA-ELISA+ and both MAB1 and MAB2 bound to *E. coli* Δ*waaD* cells (**Fig. S1**).

Strains were grown to log phase in MHB II supplemented with 0.002% Tween-80, diluted to a final OD_{600} 0.01 in sterile round-bottom 96-well plates (Costar). Antibodies were added at indicated concentrations, plates were incubated statically at 37°C, and bacterial viability was monitored via CFU counts. Bacterial inhibition activity was measured after 4 hours by optical density (OD_{600}) after 25 seconds of shaking in a plate reader. Bacterial strains containing plasmids were grown overnight and to log phase in selective media, but were tested for antibody activity in the absence of antibiotic selection. The conditional *bamA* strain (Δ*bamA*::P_{BAD}-*bamA*, Δ*waaD*::Cm) expressing

bamA site-directed mutants was grown in the absence of arabinose both overnight, to log phase, and during the experiment to ensure the only BamA produced was the copy provided on the pBla plasmid. Biological triplicates were run for each experiment.

Fluorescent-activated cell-sorting (FACS) based binding assay

Bacterial strains were grown to log phase in MHB II supplemented with 0.002% Tween-80. Cells were harvested and re-suspended to an OD_{600} 0.5 in wash buffer (PBS supplemented with 1% BSA). Primary antibodies were added at 1 µg/mL and incubated at room temperature for 1h. Cells were washed and incubated with FITC-conjugated secondary antibodies (1:200) for 1h at room temperature. Cells were washed and fixed in 2% paraformaldehyde (PFA) in PBS for 10 min prior to analysis on a FACSAria (BD) using FACSDiva software (BD). 10,000 events were captured for analysis and quantified for mean fluorescence intensity (MFI). Biological triplicates were run for each experiment. Bacterial strains containing plasmids or expressing *bamA* site-directed mutants were grown as described above.

SDS-PAGE, Western immunoblotting, and antibodies

Bacterial were grown to log phase, normalized to OD_{600}, and pelleted. Samples were re-suspended in 1x LDS sample buffer (ThermoFisher Scientific) and boiled for 5 min prior to loading on a 4-12% Bis-Tris SDS-PAGE gel. Proteins were transferred onto cellulose membranes using the iBlot 2 Dry Blotting System (ThermoFisher Scientific). Membranes were blocked for 1h in Blocking Buffer (TBS containing 5% nonfat milk and 0.05% Tween-20), washed, and then incubated either overnight at 4°C or room temperature for 1 h with the following primary Abs: human α-LptD 3D11 (1 µg/mL, Genentech), mouse α-BamA (1 μg/mL, Genentech), rabbit α-Lpp (1 μg/mL, Genentech(10)), rabbit α-MsbA YZ35555 (1 µg/mL, Genentech), rabbit α-GroEL (1: 25,000, Enzo), rabbit α-OmpA (1:1,000, Biorbyt), or rabbit α-OmpC (1: 1,000, Biorbyt). Appropriate HRP-linked secondary antibodies (GE Healthcare) were diluted 1:20,000 in TBST and incubated with the membrane for 1h at RT. Blots were developed using ECL Prime Western Blotting Detection Reagent (Amersham). α-BamA antibody MAB1 is a rat antibody (Genentech) and the non-inhibitory control α-BamA mAb is a mouse antibody (Genentech).

Cell fractionation

Untreated bacterial cells (100 mL) were grown at 37°C, shaking to OD_{600} 1.0 in MHB. Cells were harvested by centrifugation at 4°C, resuspended in 10 mL 25mM Hepes, pH 7.4, 1X cOmplete, mini, EDTA-free Protease Inhibitor Cocktail (Roche), and lysed by passaging the 2X through the LVI Microfluidizer Homogenizer (Microfluidics). Cell debris were collected by centrifuging 4,000 x g for 10 min at 4°C. The supernatant (6 mL, whole cell lysate) was centrifuged at 250,000 x g for 1h at 4°C. The pellet containing the membranes was washed in buffer and re-centrifuged. The membrane pellet was resuspended in 6 mL 25mM Hepes, pH 7.4, 2% sodium lauroyl-sarcosinate (Sarkosyl, Sigma) and rocked at RT for 30 min. A 1 mL sample was removed for total membrane fraction analysis. The remaining sample was centrifuged at 250,000 x g for 1h at RT. The pellet containing the OM fraction was resuspended in an equal volume to the input volume unless specified. Protein samples were diluted in LDS sample buffer, heated to

95°C for 5 min, separated using a 10% Bis-Tris NuPAGE gel (Invitrogen), and stained with InstantBlue protein stain (Sigma-Aldrich). Experiments with MAB1 treatment were modified to preserve antibody stocks. Here, 150 mL bacterial cultures (OD_{600} 0.05) were incubated with 10 nM MAB1 for 1.5h then cells were harvested and fractionated as described above, but the OMs were concentrated 20X to improve visualization.

σ^E activity

Overnight cultures of bacteria possessing pGNE18 (pACAY184 + *rpoH*P3-*lacZ*) were back-diluted to OD_{600} 0.01 allowed to grow to OD_{600} 0.2 and back-diluted again to OD_{600} 0.01 to start the assay. Antibodies were added to 10 nM and at each desired time point cells were analyzed for β-galactosidase production using Beta-Glo Assay (Promega) and normalized to the number of viable bacterial cells as measured using BacTiter-Glo Microbial Cell Viability Assay (Promega). Both assays were carried out according to the manufacture's protocols. Biological triplicates were analyzed.

Ethidium bromide accumulation assay

Ethidium bromide (EtBr) accumulation was measured as previously described(11). Bacterial strains were grown to log phase, washed in PBS and re-suspended to OD_{600} 0.1. 180 µl of cells was added to a 96-well black flat-bottom plate (Costar). 20µl of EtBr (100 µM) was added to the cells and PBS controls to a final concentration of 10 µM. The plate was incubated at 37°C for 2 hours and then fluorescence was read using a plate reader (excitation at 515 nm, emission at 600 nm). Biological triplicates were analyzed.

Time-lapse Microscopy

Bacterial cells were grown to log phase in the presence of 1 mM IPTG and appropriate antibiotics, diluted to OD_{600} of 0.1, treated with antibodies for 90 min. Cells were washed in antibody-free media and spotted onto 1% agarose pads containing fresh media. Imaging was performed on a Nikon Eclipse TE inverted fluorescence microscope with a 100x (NA 1.30) oil-immersion objective (Nikon Instruments). Images were collected using an Andor DR electron-multiplying CCD camera (Andor Technology) using NIS-Elements software (Nikon Instruments). Cells were maintained at 37 °C during imaging with a temperature-controlled environmental chamber (World Precision Instruments).

Membrane fluidity

Membrane fluidity was measured using the Membrane Fluidity Kit (Markergene/Abcam) in which, a lipophilic pyrene probe was incorporated into the membranes of specific bacterial strains. Upon membrane incorporation and spatial interaction, the monomeric pyrene probe undergoes excimer formation dramatically shifting the emission spectrum of the pyrene probe to a longer red wavelength. By measuring the ratio of excimer (emission at 470 nm) to monomer (emission at 405 nm) fluorescence, a quantitative monitoring of the membrane fluidity can be achieved(12). The ratio depends on the amount of incorporation making comparison of conditions where there were differences in incorporation complicated (**Extended Figure 8**). Bacterial strains were grown to log phase, washed in PBS supplemented with EDTA (4 mM depending on the strains and conditions), labeled with labeling mix (10 µM pyrenedecanoic acid (PDA), 0.08% F-127,

supplemented with EDTA in PBS) in the dark for 30 min rocking at room temperature, washed 2x in PBS, and measured for fluorescence at two wavelengths (excitation at 350 nm, emission at 405 nm and 470 nm). To confirm membrane incorporation, emission spectra were monitored from 380 nm to 600 nm and compared to unlabeled cells. Biological triplicates were analyzed.

ELISA

Antibodies were screened by capture ELISA. Briefly, 50 μl of biotinylated BamA protein, diluted in assay buffer (PBS supplemented with 1.5% octyl-β-glucoside (βOG) and 0.5% BSA) was added to streptavidin coated 384-well plates (Thermo Scientific USA, Cat # 15504) and incubated at RT for 1h with shaking. The plates were washed 3x with wash buffer (PBS supplemented with 1.5% βOG). 50 μL of supernatants or purified antibody, neat or diluted in assay buffer, were added to the wells and incubated at RT for 1h with shaking. The plates were washed 3x with wash buffer. The captured antibody was detected with goat α-rat HRP secondary antibody (50 μL per well diluted at 1:10,000 in assay buffer, Bethyl Laboratories USA, Cat # A110-236P). The plates were incubated at RT for 1h, washed 3x with wash buffer, and 50 μl of substrate (TMB solution, Surmodics, USA Cat # TMBW-1000) was added to each well. The plates were incubated for 5 min at RT followed by addition of 50 μL of TMB stop solution (Surmodics, USA Cat # LPSP-1000). Plates were read at 630 nm in a plate reader.

Hydroxyl radical footprinting (HRF)

HRF labeling was performed using the fast photochemical oxidation of proteins (FPOP) methodology previously described(13). Labeling of both conditions was performed using "equal-weight" strategy where the total protein concentration of each sample was 0.5 mg/mL in the same labeling buffer (25mM Tris, pH 8.0, 100mM NaCl, 1.5% n-octylglucoside) after purification over a HiPrep 16/60 Sephacryl S-200 HR column (GE Healthcare Bio-Sciences, Pittsburgh, PA)(14). 15 µL/min of BamA solutions were mixed with 30mM $H_2 0_2$ at a rate of 7.5 µL/min through a micro-tee mixer (Cobert Associates Lab, St. Louis, MO) prior to light exposure. Oxidation was initiated by pulsing a focused 248 nm KrF excimer laser (GAM Laser Inc., Orlando, FL) at 70 mJ/pulse at a rate of 7 Hz through a 3.0 mm exposure window of 150 μm i.d. fused-silica tubing (Polymicro Technologies, Phoenix, AZ). Oxidized samples were quenched with 40 mM methionine (Sigma Aldrich) and 1 μM catalase (Sigma Aldrich) final concentrations. Samples were frozen and stored at -80°C before proteolysis and mass spectrometry. An unexposed sample for each state was collected to monitor background oxidation.

Samples were reduced (10 mM DTT, 25 mM Tris pH 8.0) and denatured (6M guanidinium hydrochloride, 1.0 mM EDTA, 360mM Tris pH 8.6) at 45°C for 10 minutes. Reduced cysteines were alkylated with 25mM sodium iodoacetate in denaturation buffer for 5 minutes at 45°C and quenched with 50 mM DTT at room temperature for 5 minutes. Samples were desalted prior to proteolysis over illustra NAP-5 column (GE Healthcare Bio-Sciences, Pittsburgh, PA) equilibrated in 25 mM Tris pH 8.0, and the eluate was digested with 5 µg Trypsin (Roche, Indianapolis, IN) at 37°C for 1 hour and quenched with formic acid (FA) to a final percentage of 3.5 for liquid chromatography.

10 μg of tryptic peptides of BamA were resolved with Waters ACQUITY UPLC using a CSH C18 (130 Å,1.7 μm, 2.1 x 150 mm) column heated to 77°C. Peptides were eluted with a gradient of 99% A (water, 0.1% FA) to 50% B (acetonitrile, 0.1% FA) over 40 minutes at 0.3 mL/min. To prevent carry-over, the column was washed with 95% B for 4 minutes and re-equilibrated to 99% A for 20 minutes between injections. Peptide spectra were collected on a Thermo Fisher Q-Exactive hybrid quadrupole-Orbitrap mass spectrometer. Data dependent acquisition was used to collect MS-MS spectra of the ten most abundant peptides for the oxidized and unoxidized samples. Unmodified and oxidized species for each tryptic peptide were identified from the fragmentation spectra using Byonic™ (Protein Metrics, San Carlos, CA). Percent oxidation was calculated as previously described using the extracted mass of each identified species from the precursor scans (350 – 2000 m/z) with Byologic Footprint® (Protein Metrics, San Carlos, CA)(13-15). The triplicate samples were averaged for the final percent oxidation and error bars represent the standard deviation of three measurements.

Resistant mutant whole genome sequencing and variant analysis

Resistance frequency was determined by P_0 method(16). Genomic DNA was isolated from MAB1 resistant bacterial cultures as well as the parent Δ*waaD* strain as specified for Gram-negative bacteria using the Blood and Cell Culture DNA Maxi kit (Qiagen) and sequenced by HiSeq 2000 (Illumina) to generate 75 bp paired-end data. Reads from each isolate were mapped onto the reference genome (*E. coli* BW25113, accession number NZ_CP009273), using GSNAP version 2013-10-10(17) with default parameter settings. Candidate variants were identified using the Genentech in-house bioinformatics pipeline, which utilizes the following R and Bioconductor packages: GenomicRanges(18), GenomicAlignments(18), VariantTools(19), and gmapR(20). Only base-calls with a Q-score ≥ 30 were tallied for variant calling.

Statistics

All experiments examining membrane fluidity, EtBr uptake, σ^E activation, HRF, and bacterial survival were analyzed via the unpaired Student's *t* test using Prism 6.0 (GraphPad Software). Data presented on log axes were log transformed prior to analyses. For MAB1 growth inhibition experiments, IC50 values (the concentration of inhibitor required to reduce bacterial growth by 50%) were calculated using non-linear regression analysis (sigmoidal, 4PL, X is log(concentration)) and compared via the unpaired Student's *t* test using Prism 6.0 (GraphPad Software). The Bonferroni correction was applied to control for multiple comparisons. For curves that did not have an IC50 value or did not reduce the OD_{600} >50% compared to no treatment, the highest antibody concentration tested was used for statistical comparison. In Figure 5H, the IC50 of the 50 mM NaCl curve was manually calculated using a linear curve. IC50 values are presented as mean ± standard error of the mean (SEM). The IC50 values and statistical analyses for data in Figure 5 are shown in **Table S3**.

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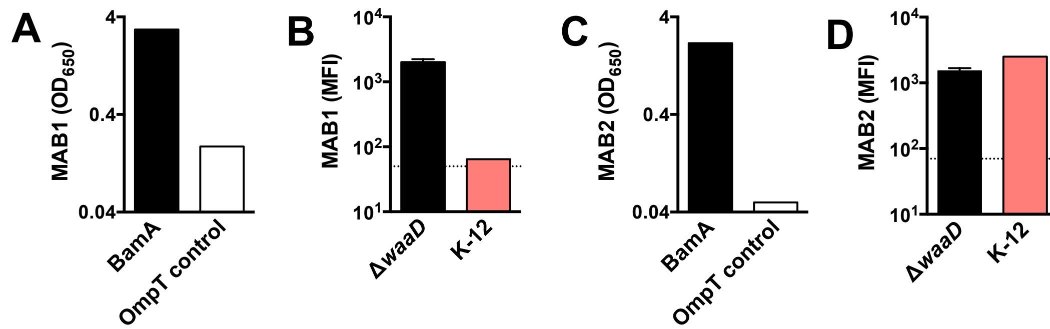
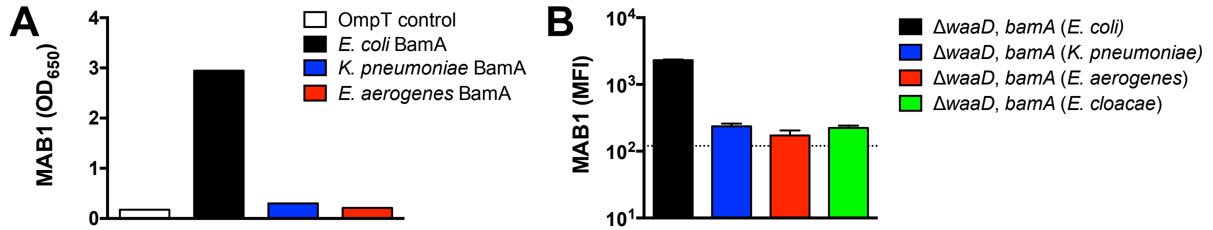
SI FIGURES

Fig. S1



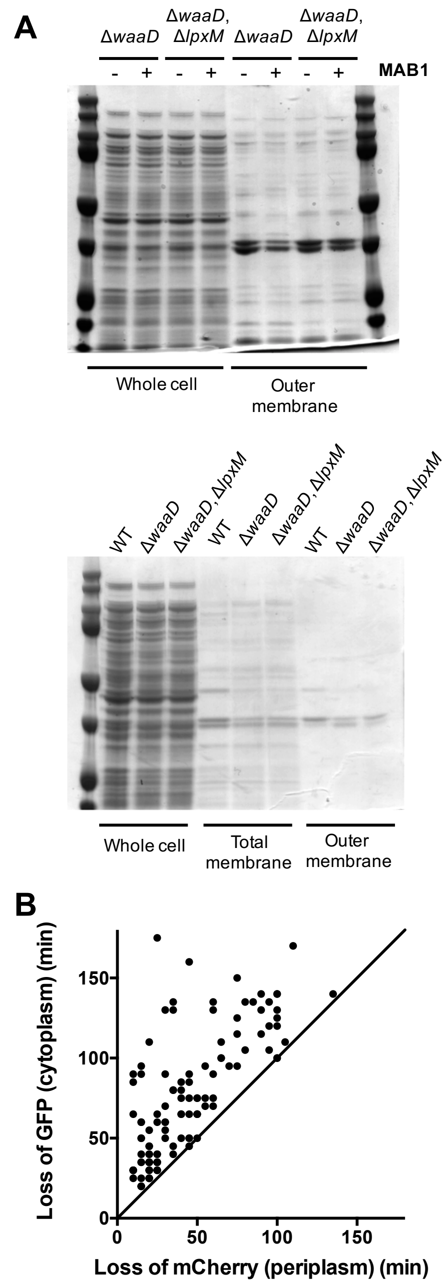
MAB1 binds BamA on the surface of *E. coli* Δ*waaD*. *In vitro* ELISA assay monitoring binding of (**A**) MAB1 and (**C**) MAB2 to purified BamA protein. Non-specific binding was determined using an unrelated *E. coli* β-barrel protein, OmpT. Whole cell binding by (**B**) MAB1 and (**D**) MAB2 to *E. coli* Δ*waaD* and K-12 strains measured by FACS. Mean fluorescence intensities (MFIs) are plotted. The dotted line is the background control with no primary antibody.

Fig. S2



MAB1 does not bind to BamA from closely related Enterobacteriaceae species. (**A**) *In vitro* ELISA assay monitoring binding of MAB1 to purified BamA protein from *E. coli*, *K. pneumoniae*, or *E. aerogenes*. The control for mAb binding was an unrelated *E. coli* β-barrel protein, OmpT. (**B**) Quantification of FACS mean fluorescent intensities (MFIs) from biological triplicate samples, a representative of which is shown in Figure 1c. Means and standard deviations are shown for *E. coli* Δ*waaD* producing BamA with the *E. coli*, *K. pneumoniae*, *E. aerogenes*, or *E. cloacae* BamA β-barrel. The dotted line is the background control is in absence of a primary mAb.

Fig. S3



(**A**) Coomassie-stained SDS-PAGE of whole-cell lysates, total membrane and OMs isolated from *E. coli* (top) treated with 10 nM MAB1 for 1.5h (top) or untreated bacterial cells (bottom) as indicated. The OM preparations were concentrated 20-fold to improve visualization. (**B**) Scatter plot of time points at which single cells lost mCherry (periplasm) and GFP (cytoplasm) visualized by fluorescence microscopy of *E. coli* Δ*waaD* (n=118) after 90 minutes of treatment with 13 nM MAB. Cells losing mCherry (periplasm) before imaging or that still contained GFP (cytoplasm) at the end of imaging were excluded.

Fig. S4

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                       L1                             L2
Ecoli       rntgsfnfgigygtesgvsfqagvqqdnwlgtgyavgingtkndyqtyaelsvtnpyftv 480
Kpneumo     rntgsfnfgigygtesgvsfqagvqqdnwlgtgyavgingtkndyqtytelsvtnpyftv 480
Eaeroge     rntgslnfgigygtesgvsfqagvqqdnwlgtgysvgingtkndyqtyaelsvtnpyftv 480
Ecloaca     rntgsfnfgvgygtesgvsfqvgvqqdnwlgtgysvgingtkndyqtysefsvtnpyftv 480
            *****:***:***********.************:*************:*:*********

                          L3                                         L4
Ecoli       dgvslggrlfyndfqaddadlsdytnksygtdvtlgfpineynslraglgyvhnslsnmq 540
Kpneumo     dgvslggrvfyndfdandadlsdytnksygtditlgfpvneyntlragvgyvhnslsnmq 540
Eaeroge     dgvslggrifyndfdandadlsdytnksygtdvtlgfpineynslraglgyvhnslsnmq 540
Ecloaca     dgvslggrifyndfkaddadlssytnksygvdgtlgfpvneyntlraglgyvhndlsnmq 540
            ********:*****.*:*****.*******.* *****:****:****:*****.*****
                                                          --------------
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                          495

Ecoli       pqvamwrylysmgehpstsdqdnsfktddftfnygwtynkldrgyfptdgsrvnltgkvt 600
Kpneumo     pqvamwrylnsmgqypdntndrnsfsandftfnygwtynkldrgffptegsrvnlngkvt 600
Eaeroge     pqvamwrylnsmgqypdttndnnsfsaddftfnygwtynrldrgyfptegsrvnlngkvt 600
Ecloaca     pqvamwryldsigqsastssdnngfaaddftfnygwtynrldrgyfptegsrvnlngkvt 600
            ********* *:*:  ..:.: *.* ::***********:****:***:******.****
            ------- ------------------
                     ^   ^^    ^^^   ^^
                     550 554   560   566
            L5                                      L6
Ecoli       ipgsdneyykvtldtatyvpidddhkwvvlgrtrwgygdglggkempfyenfyaggsstv 660
Kpneumo     ipgsdneyykatldtatyvpidndhqwvvlgrtrfgygdgiggkempfyenfyaggsstv 660
Eaeroge     ipgsdneyykvtldtatyvpidddhqwvvlgrtrfgygdglggkempfyenfyaggsstv 660
Ecloaca     ipgsdnefykltldtasyfpidddhkwvvlgrtrwgygdglggkempfyenfyaggsstv 660
            *******:** *****:*.***:**:********:*****:*******************

Ecoli       rgfqsntigpkavyfphqasny-dpdydyecatqdgakdlcksddavggnamavaslefi 719
Kpneumo     rgfqsntigpkavyfpassrhddddsydneckst--esapcksddavggnamavasleli 718
Eaeroge     rgfqsntigpkavylpsttsr---ddydtecast--esapcksddavggnamavasleli 715
Ecloaca     rgfqsnnigpkavyyggnde--------dncasr-dpkqvcssddavggnamavaslefi 711
            ******.*******               :* :    .  *.****************:*
                            ^^^ ^^  ^   ^   ^^^^^ ^^
                           ___________________________
                           676                      700
                                      L7
Ecoli       tptpfisdkyansvrtsffwdmgtvwdtnwdssq---ysgypdysdpsnirmsagialqw 776
Kpneumo     tptpfisdkyansvrtsvfwdmgtvwdthwdssa---yagypdysdpsnirmsagiavqw 775
Eaeroge     tptpfisdkyansvrtsvfmdmgtvwdtnwdstk---yggypdysdpsnirmsagiavqw 772
Ecloaca     tptpfisdkyansvrtsffwdagtvwdtnwentaqmraagvpdysdpgnirmsagialqw 771
            *****************.* * ******:*:.:     .* ******.*********:**
                                             ^    ^
                                             753  755
                            L8
Ecoli       msplgplvfsyaqpfkkydgdkaeqfqfnigktw 810
Kpneumo     msplgplvfsyaqpfkkydgdkaeqfqfnigktw 809
Eaeroge     msplgplvfsyaqpfkkydgdkaeqfqfnigktw 806
Ecloaca     msplgplvfsyaqpfkkydgdkseqfqfnigktw 805
            **********************:***********
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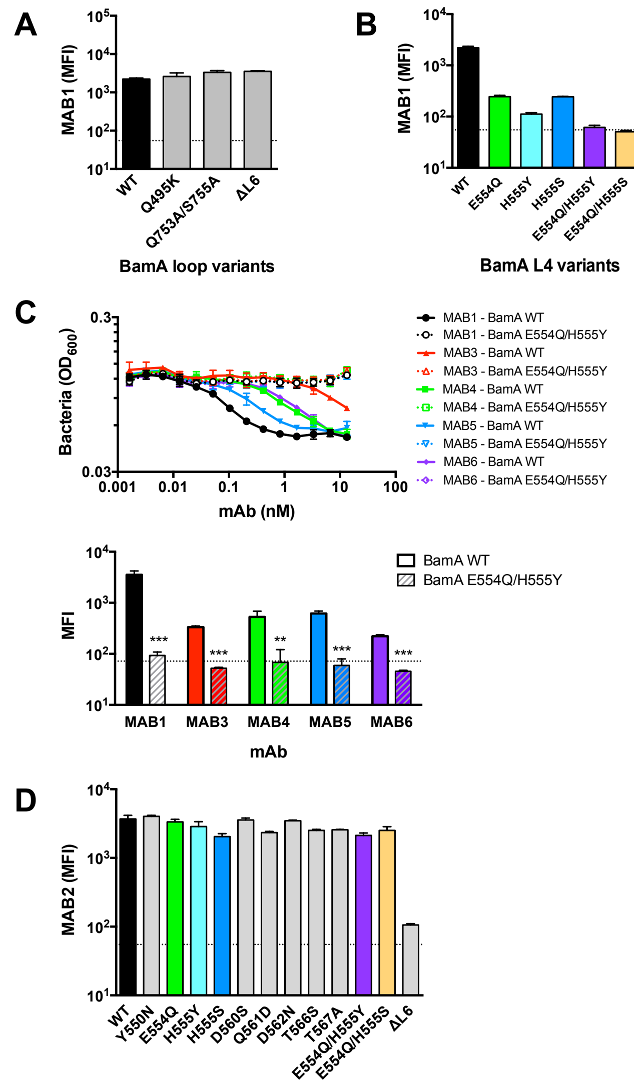
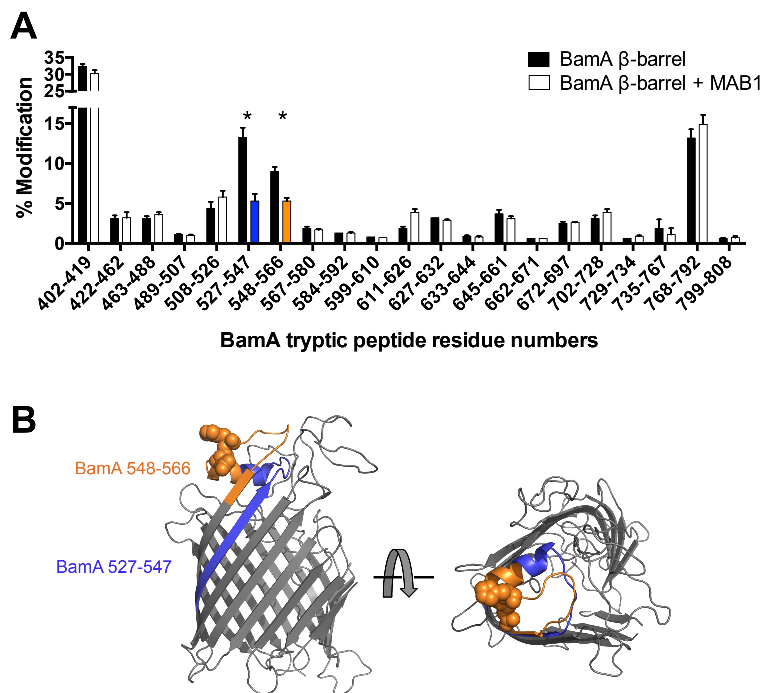
Amino acid sequence alignment of the BamA β-barrel region (residues 421-810) for *E. coli* (Ecoli), *K. pneumoniae* (Kpneumo), *E. aerogenes* (Eaeroge), and *E. cloacae* (Ecloaca). Approximate boundaries of the extracellular loops (L1-L8) are noted. Black highlighted residues with arrows below the sequence are loop positions unique to the *E. coli* BamA sequence. Positions with arrows below the sequence are unique to *E. coli* BamA and near a loop. The horizontal black line below L6 indicates extent of the L6 deletion tested in Fig. S5. Horizontal dashed lines indicate regions protected in the hydroxyl radical footprinting experiment (Fig. S6).

Fig. S5



Effects of *E. coli*-specific BamA loop chimeras on MAB1 binding and inhibition activity. (**A**) MAB1 whole cell binding via FACS (MFI) to *E. coli ΔwaaD* cells producing indicated BamA loop variants. (**B**) MAB1 whole cell binding via FACS (MFI) to *E. coli ΔwaaD* expressing BamA L4 variants. Coloring is matched to Fig. 3, Fig. S5C, and Fig. S5D. (**C**) Growth inhibition was measured by *E. coli ΔwaaD* or *E. coli ΔwaaD* with BamA E554Q/H555Y density (OD_{600}) in the presence of MAB1 after 4 hours of treatment. (**D**) MAB2 whole cell binding to *E. coli ΔwaaD* expressing BamA L4 variants are plotted as FACS MFIs to show similar accessible BamA levels on the cell surface. Coloring is matched to Fig. 3, Fig. S5B, and Fig. S5D. Coloring is matched to Fig. 3, Fig. S5B and Fig. S5C. For all experiments means and standard deviations of biological triplicates are plotted. The dotted line is the background control with no primary antibody. Unpaired *t* test were used to compared BamA WT to BamA E554Q/H555Y for each antibody tested. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Fig. S6

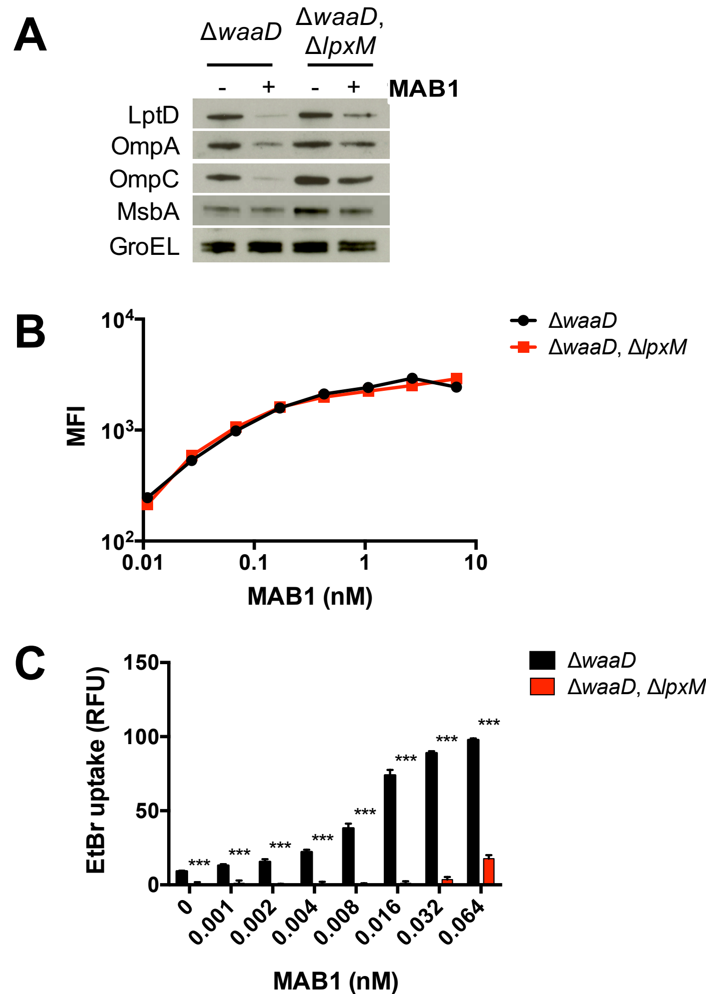


In vitro hydroxyl radical footprinting of the *E. coli* BamA β-barrel with MAB1. (**A**) Protection of the BamA β-barrel from hydroxyl radical modification in the MAB1 bound (open bars) versus unbound (black bars) state. Twenty-one unique trypsin fragments were resolved by LC-MS/MS following digestion of the BamA β-barrel after hydroxyl radical generation. The average and standard deviation for the percentage of modified peptides in triplicate reactions are shown. (**B**) Tryptic fragments spanning residues 527-547 (blue) and 548-566 (orange) showed decreased levels of modification in the BamA-MAB1 complex. Unpaired *t* test were used to compared each peptide with and without MAB1 for % modification. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Fig. S7

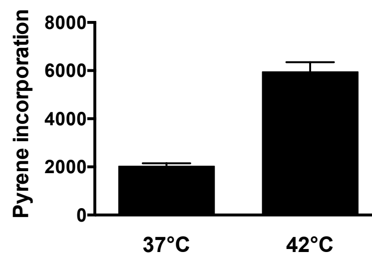
Cartoon of the *lpxM* gene identifying the location of distinct alleles selected for resistance to MAB1. Each mutation and resulting protein change are indicated above approximate positions in the gene. One allele was isolated twice.

Fig. S8



Effects of MAB1 on *E. coli* ∆*waaD*, ∆*lpxM* MAB1-resistant mutant. (**A**) Representative Western blot of OMPs and control proteins in the *E. coli* ∆*waaD* MAB1-sensitive and the *E. coli* ∆*waaD*, ∆*lpxM* MAB1-resistant strains. (**B**) FACS whole cell binding of different concentrations of MAB1 to the *E. coli* ∆*waaD* (black) and *E. coli* ∆*waaD*, ∆*lpxM* (red) strains. FACS voltage settings differed from those in Fig. 4. (**C**) Uptake of EtBr into the *E. coli* ∆*waaD* strain at increasing concentrations of MAB1 measured after 4 hours of treatment for biological triplicates. The means and standard deviations are plotted. Unpaired *t* test were used to compared EtBr uptake for ∆*waaD* and ∆*waaD*, ∆*lpxM* at each MAB1 concentration. *p<0.05, **p<0.01, ***p<0.001.

Fig. S9



Lipophilic PDA dye incorporation (excitation at 350 nm, emission at 405 nm) into the membranes of the *E. coli* Δ*waaD* strain was measured and compared for cells grown at 37°C and 42°C. Data is shown as average RFUs for triplicate samples.

SI TABLES

Table S1. Antibiotic potentiation by sub-inhibitory concentration of MAB1.

	Δ*waaD* MIC (μg/mL)	
Antibiotic	**No mAb1**	**MAB1**
Rifampicin	0.064	0.008
Gentamicin	0.23	0.23
Colistin	0.014	0.007

Table S2. Strains, plasmids, and primers used in this study.

Strains

GNE ID	Strain	Plasmid	Antibiotic resistance	Origin
GNE 49	BW25113 Δ*waaD::Kan*		Kan-R	(1)
GNE 115	BW25113			
GNE 143	Δ*bamA*::P_{BAD}-*bamA*		Carb-R, Kan-R	in-house
GNE 1101	Δ*bamA*::P_{BAD}-*bamA,* Δ*waaD::Cm*		Carb-R, Kan-R, Cm-R	in-house
GNE 4125	Δ*bamA*::P_{BAD}-*bamA,* Δ*waaD::Cm, ΔlpxM::Gent*		Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4075	BW25113 Δ*ompT*, Δ*waaD::cm*	pD884SR-OmpT	Cm-R, Carb-R	in-house
GNE 4076	BW25113 Δ*ompT*, Δ*waaD::cm* BamA_barrel (UPEC)::Gent	pD884SR-OmpT	Cm-R, Gent-R, Carb-R	in-house
GNE 4077	BW25113 Δ*ompT*, Δ*waaD::cm* BamA_barrel (*K. pneumoniae*)::Gent	pD884SR-OmpT	Cm-R, Gent-R, Carb-R	in-house
GNE 4078	BW25113 Δ*ompT*, Δ*waaD::cm* BamA_barrel (*E. aerogenes*)::Gent	pD884SR-OmpT	Cm-R, Gent-R, Carb-R	in-house
GNE 4117	BW25113 Δ*ompT*, Δ*waaD::cm* BamA_barrel (*E. cloacae*)::Gent	pD884SR-OmpT	Cm-R, Gent-R, Carb-R	in-house
GNE 3944	Δ*bamA*::P_{BAD}-*bamA,* Δ*waaD::Cm*	p*bamAWT*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 3945	Δ*bamA*::P_{BAD}-*bamA,* Δ*waaD::Cm*	p*bamAΔL6* *(*P676_C700del)	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4150	Δ*bamA*::P_{BAD}-*bamA,* Δ*waaD::Cm*	p*bamA Q495K*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4151	Δ*bamA*::P_{BAD}-*bamA,* Δ*waaD::Cm*	p*bamA Y550N*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4152	Δ*bamA*::P_{BAD}-*bamA,* Δ*waaD::Cm*	p*bamA E554Q*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4153	Δ*bamA*::P_{BAD}-*bamA,*	p*bamA H555Y*	Carb-R,	in-house

	ΔwaaD::Cm		Kan-R, Cm-R, Gent-R	
GNE 4154	Δ*bamA*::P_{BAD}-*bamA, ΔwaaD::Cm*	p*bamA D560S*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4155	Δ*bamA*::P_{BAD}-*bamA, ΔwaaD::Cm*	p*bamA Q561D*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4156	Δ*bamA*::P_{BAD}-*bamA, ΔwaaD::Cm*	p*bamA D562N*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4157	Δ*bamA*::P_{BAD}-*bamA, ΔwaaD::Cm*	p*bamA T567A*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4158	Δ*bamA*::P_{BAD}-*bamA, ΔwaaD::Cm*	p*bamA T596N*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4159	Δ*bamA*::P_{BAD}-*bamA, ΔwaaD::Cm*	p*bamA S752T*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4160	Δ*bamA*::P_{BAD}-*bamA, ΔwaaD::Cm*	p*bamA E554Q, H555S*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4161	Δ*bamA*::P_{BAD}-*bamA, ΔwaaD::Cm*	p*bamA D560S, Q561D, D562N*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4162	Δ*bamA*::P_{BAD}-*bamA, ΔwaaD::Cm*	p*bamA K566A, T567A*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4187	Δ*bamA*::P_{BAD}-*bamA, ΔwaaD::Cm*	p*bamA T566S*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4188	Δ*bamA*::P_{BAD}-*bamA, ΔwaaD::Cm*	p*bamA E554Q, H5555Y*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4190	Δ*bamA*::P_{BAD}-*bamA, ΔwaaD::Cm*	p*bamA H555S*	Carb-R, Kan-R, Cm-R, Gent-R	in-house
GNE 4297	BW25113 Δ*waaD::Kan* GFP (cyto), mCherry (peri)	pGFP, p^{ss}dsbA-mCherry	Kan-R, Gent-R, Cm-R	in-house

Plasmids

Name	**Description**	**Antibiotic resitance**	**Origin**
p*bamAWT*	pBla_short bamA (*E. coli*)	Carb-R, Gent-R	in-house
p*bamAΔL6*	pBla_short bamA ΔL6	Carb-R, Gent-R	in-house

	(P676_C700del)		
pLMG18	pLMG18, IPTG-inducible expression vector	Cm-R	in-house
p*lpxM*	pLMG18 *lpxM*	Cm-R	in-house
pLDR9	*attB* integration vector	Kan-R, Amp-R	ATCC
pLDR8	temperature-sensitive lambda integrase plasmid	Kan-R	ATCC
pKD4	lambda-red recombination	Kan-R	Addgene
pKD3	lambda-red recombination	Cm-R	Addgene
pSIM18	Expresses Lambda Red recombinase	Hygro-R	(3)
pCP20	temperature-sensitive FLP expression plasmid	Carb-R, Cm-R	Coli Genetic Stock Center
pACYC184	pACYC184 low copy plasmid	Tet-R, Cm-R	NEB
rpoHp3-lacZ	pACYC184 rpoHP3-lacZ	Tet-R, Cm-R	in-house
pD884SR-OmpT	Expresses OmpT	Carb-R	In-house
pD884-SR	rhamnose-inducible expression plasmid	Amp-R	ATUM
pGFP	pBla_short GFP (cytoplasmic GFP)	Gent-R	in-house
p^{ss}dsbA-mCherry	pLMG18 ^{ss}dsbA-mCherry (periplasmic mCherry)	Cm-R	in-house

Primers

Name	Sequence
Deletion strains	
waaD KO F	GTCTGAGATTGTCTCTGACTCCATAATTCGAAGGTTACAGTTATGATCATCgtgtaggctggagctgcttc
waaD KO R	CCCAAGACGGGCCGATCACCAGTATTTTCATGCAGAGCTCTTATGCGTCGCGcatatgaatatcctccttagttcctattc
lpxM KO F	CGCTACACTATCACCAGATTGATTTTTGCCTTATCCGAAACTGGAAAAGCgtgtaggctggagctgcttc
lpxM KO R	CTGAAGCAAACTTGAACTTATCATCAGGCGAAGGCCTCTCCTCGCGAGAGcatatgaatatcctccttagttcctattc
***lpxM* complementation**	
pLpxM GA F	gtgagcggataacaatttGCTAGCAGGAGGAATTCACCATGGAAACGAAAAAAAATAATAGC
pLpxM GA R	taatctgtatcaggctgTTATTTGATGGGATAAAGATCTTTG
pLMG18 GA F	AAATTGTTATCCGCTCACAATTC
pLMG18 GA R	CAGCCTGATACAGATTAAATCAGAACGC

σE reporter	
rpoHp3-lacZ F, BamH1	cgcggatccGCATTGAACTTGTGGATAAAATCACGGTCTGAAGGGAG AGGAGGTATATACATGACCATGATTACGGATTCACTG
rpoHp3-lacZ R, PstI	cgcctgcagTTATTTTTGACACCAGACCAACTG
Δ*bamA*::P_{BAD}-*bamA*	
bamA KO F	TGTTAATGGGGCTTGCACTTTTCAATGATTTCTCTCGGTTATGAGA GTTAGTTAGGAAGAACGCATAATAACGtgtgtaggctggagctgcttcg
bamA KO R	GGCGATCTTATATTGATCGCCTAAAGTCATCGCTACACTACCACT ACATTCCTTTGTGGAGAACACTTACCAcatatgaatatcctccttta
BamA NheI F	gcgcgctagcaggaggaattcaccATGGCGATGAAAAAACTGCTG
BamA XbaI R	cgcgtctagaTTACCAGGTTTTACCGATGTTG
pBAD-araC bamA SacI F	AAAAGAGCTcgatgcataatgtgcctgtc
pBAD-araC bamA SacI R	AAAAGAGCTcgttcaccgacaaacaacag
BamA species chimeras	
BamA_usF	ATGATTTCTCTCGGTTATGAGAGTTAGTTAGGAAGAACGCATAATAACG ATGGCGATGAAAAAGTTGCTC
Gent_BamA_ds_R	TTACCAGGTTTTACCAATGTTAAACTG
BamA_ds_Gent_F	CAGTTTAACATTGGTAAAACCTGGTAAGAGTAAGCTTAACATAAGGAG GAA
BamA_Gent_R	CGCTACACTACCACTACATTCCTTTGTGGAGAACACTTACCAGGTTTTA CCGATCTATTAGGTGGCGGTACTTGGGTCG
RseP_Int_F	GCATCTGCTGTTCCTTGCGATC
Skp_Int_R	CGTGTTAGAAACACCGGTTTTCTG
Kp_BamA_Int_F	GATAAAACCGTTAAGCTGCACGTT
Ea_BamA_Int_F	GATAAAACCGTGAAGCTGCATATC
pBla_BamA and mutants	
PBla_884SR_F	ATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATAG AGACCATCAAATTCTTTTAAGGAGGTAAAAAatg
PBla_884SR_R	TTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTAT TTAGAAAAAATAGATATCGCTCAATACTGACCAT
884SR_EcBamA_116us_F	GACCATCAAATTCTTTTAAGGAGGTAAAAAGTTCAAGACTTTTGTT ATCGCATTG
884SR_EcBamA_82ds_R	GGTGGCGGCCGCGAGACCCTAACCGTTCTTTGCGTGGCCCGGC
BamA_del676-700_new_F	TACTTCAAATCGGATGATGCTGTAGGCGGTAACG

BamA_del676-700_new_R	CGATTTGAAGTAAACTGCTTTCGGACCAATGGTATT
BamA QC Q495K F	CTATAATGACTTCAAGGCAGATGACGCCG
BamA QC Q495K R	CGGCGTCATCTGCCTTGAAGTCATTATAG
BamA QC Y550N F	GTGGCGTTATCTGAACTCTATGGGTG
BamA QC Y550N R	CACCCATAGAGTTCAGATAACGCCAC
BamA QC E554Q F	GTACTCTATGGGTcAACATCCGAGCAC
BamA QC E554Q R	GTGCTCGGATGTTgACCCATAGAGTAC
BamA QC H555Y F	CTCTATGGGTGAAtATCCGAGCACCTC
BamA QC H555Y R	GAGGTGCTCGGATaTTCACCCATAGAG
BamA QC D560S F	CCGAGCACCTCTagTCAGGATAACAGC
BamA QC D560S R	GCTGTTATCCTGActAGAGGTGCTCGG
BamA QC Q561D F	GAGCACCTCTGATgAtGATAACAGCTTC
BamA QC Q561D R	GAAGCTGTTATCaTcATCAGAGGTGCTC
BamA QC D562N F	CCTCTGATCAGaATAACAGCTTCAAAAC
BamA QC D562N R	GTTTTGAAGCTGTTATtCTGATCAGAGG
BamA QC K566S F	GATAACAGCTTCAgcACGGACGACTTC
BamA QC K566S R	GAAGTCGTCCGTgcTGAAGCTGTTATC
BamA QC T567A F	GATAACAGCTTCAAAgCGGACGACTTC
BamA QC T567A R	GAAGTCGTCCGcTTTGAAGCTGTTATC
BamA QC T596N F	GTGTCAACCTGAaCGGTAAAGTGACC
BamA QC T596N R	GGTCACTTTACCGtTCAGGTTGACAC
BamA QC S752T F	CTGGGATTCCAcCCAATATTCTGG
BamA QC S752T R	CCAGAATATTGGgTGGAATCCCAG

BamA QC E554Q, H555S F	GTACTCTATGGGTcAAagTCCGAGCACCTCTG
BamA QC E554Q, H555S R	CAGAGGTGCTCGGActTTgACCCATAGAGTAC
BamA QC L4 DQD560-2SDNF	GTGAACATCCGAGCACCTCTagTgAtaATAACAGCTTCAAAACGGACG
BamA QC L4 DQN560-2SDN R	CGTCCGTTTTGAAGCTGTTATtaTcActAGAGGTGCTCGGATGTTCAC
BamA QC K566A, T567A F	CAGGATAACAGCTTCgcAgCGGACGACTTCACGTTC
BamA QC K566A, T567A R	GAACGTGAAGTCGTCCGcTgcGAAGCTGTTATCCTG
BamA QC E554Q, H555Y F	GTACTCTATGGGTcAAtATCCGAGCACCTCTG
BamA QC E554Q, H555Y R	CAGAGGTGCTCGGATaTTgACCCATAGAGTAC
BamA QC H555S F	CTCTATGGGTGAAagTCCGAGCACCTCTG
BamA QC H555S R	CAGAGGTGCTCGGActTTCACCCATAGAG

Table S3. Analysis of IC50s for growth inhibition by MAB1 of *E. coli* Δ*waaD* in indicated strain and growth conditions (from Fig. 5).

Figure	Sample[1]	IC50 (nM)[2]	SEM (±)	*p* value[3]
5D	**Δ*waaD***	0.05	0.002	
5D	Δ*waaG*	>13.3		<0.001
5H	**0 mM NaCl**	0.086	0.01	
5H	10 mM NaCl	0.082	0.03	
5H	50 mM NaCl	0.185	0.03	
5H	100 mM NaCl	>13.3		<0.001
5H	150 mM NaCl	>13.3		<0.001
5H	171 mM NaCl	>13.3		<0.001
5L	**Δ*waaD*, 37°C**	0.078	0.0038	
5L	Δ*waaD*, 30°C	>13.3		<0.001
5L	Δ*waaD*, 42°C	0.0063	0.0002	<0.001
5L	Δ*waaD*, Δ*lpxM*, 37°C	>13.3		<0.001
5L	Δ*waaD*, Δ*lpxM*, 42°C	0.08	0.005	
5L	**Δ*waaD*, Δ*lpxM*, 37°C**	**>13.3**		
5L	Δ*waaD*, Δ*lpxM*, 42°C	0.08	0.005	<0.001

[1] IC50s in each figure were compared to the value in the **bolded** sample by unpaired *t* test.
[2] For samples that did not have a measurable IC50, the maximum tested concentration (13.3 nM) was used for statistical analyses. There were no SEMs for samples that lacked IC50s.
[3] No *p* value indicates no significant difference.