Supplemental Information

EXPERIMENTAL PROCEDURES

Materials

The bacteria used in this work were obtained from the American Type Culture Collection (MRSA ATCC 700787, *Escherichia coli* ATCC 11775, *Bacillus subtilis* ATCC 6051 and *S. aureus* ATCC 25923). MRSA MW2 and MRSA MW2 *ΔtarO* (referenced in Campbell, et al., 2011)[1] were a generous gift from Dr. Suzanne Walker. Mammalian cell lines (HeLa, HCT116, HEK292, and PCS-400-010) were obtained from ATCC. Chemicals from Sigma-Aldrich (DMSO, low molecular-weight branched polyethylenimine, and growth media) were used as purchased. Antibiotics (oxacillin, ampicillin, amoxicillin, and vancomycin) were purchased from Gold Biotechnology.

In Vitro Efficacy Checkerboard Assay

Checkerboard assays were used to determine synergy of BPEI with oxacillin against bacteria. Stock solutions of oxacillin were made in DMSO and added to pre-sterilized 96-well plates with cation-adjusted Mueller Hinton-Broth so that the final DMSO concentration was <1%. Bacteria was added to each well on the plate so that the final cell density was ~5 x 10^5 cells/mL. Optical density readings were made immediately after inoculation using a Tecan Infinite M20 plate reader with a wavelength of 600 nm. The plates were incubated for 20 hours in a humidified incubator at 37°C and a final OD_{600} reading was recorded. The change in OD_{600} was calculated by subtracting the initial OD_{600} from the final OD_{600} reading. A change in OD_{600} greater than 0.050 was considered to be positive growth. The minimum inhibitory concentration (MIC) was assigned as the lowest concentration of antibiotic or BPEI that did not allow growth. The fractional inhibitory concentration (FIC) was calculated using the previously-established

equation.[2] An FIC lower than 0.5 indicates synergy, between 0.5 and 1 represents additivity, and greater than 1 shows antagonism. Each assay was done in triplicate and reported as the modal FIC value. Minimum bactericidal concentrations (MBC) were determined by counting colonies on agar plates inoculated with the contents of the 96-well plates after the final OD_{600} readings were taken.

Mammalian Cell Viability and Nephrotoxicity Assays

A CellTiter-Blue (Promega) assay was performed as described by Burgett, *et al.* [3] Briefly, cells were plated and grown at 37°C for 20-24 hrs. Compounds (dissolved in water) were delivered to the cells and grown for another 48 hrs. CellTiter-Blue was added and fluorescence (544 nm excitation; 590 nm emission) was measured. Growth relative to untreated cells was calculated. All assays were done in triplicate.

A Pierce Lactate Dehydrogenase (LDH) Cytotoxicity (Thermo) assay was performed with PCS-400-010 cells. Cells were plated (2,500 cells/well) and grown for 24 hrs. at 37 °C. The cells were treated and grown for another 24 hrs. Supernatant (50 μL) was transferred to the reaction mixture (50 μL) for each well and the plates were incubated further at room temperature for 30 minutes. Stop solution (50 μL) was added to each well after 30 minutes. Absorbance was measured at 490 nm and 680 nm. To determine LDH activity, the 680 nm absorbance value was subtracted from the 490 nm value. Percent cytotoxicity was calculated by subtracting the spontaneous LDH activity from the treatment LDH activity and comparing to the maximum LDH activity. The study was done in triplicate.

Growth and Time-Killing Curve

TSB growth media augmented with various amount of BPEI and/or oxacillin was inoculated at 0.5% from an overnight culture of MRSA 700787. During cell growth at 37 °C,

with shaking (200 rpm), the OD_{600} was monitored hourly for each sample. Aliquots were transferred to TSB agar plates and colony counts were taken at 0 hr., 4 hr., 8 hr., and 24 hr. Each study was done in triplicate.

Scanning Electron Microscopy

MRSA 700787 cells were inoculated 0.5% from an overnight culture and grown at 37 °C with shaking (with or without BPEI). The optical density was monitored, and growth was stopped when the bacteria reached late-lag phase. Aliquots were fixed with a mixture of 2% glutaraldehyde and 2% formaldehyde in 0.1 M cacodylate buffer for 30 minutes at room temperature. The cells were washed and fixed with 1% OsO_4 for 30 minutes at room temperature in the dark. Afterwards, the cells were washed three times with water. One drop of each sample was placed on poly-L-lysine coated coverslips. The samples immediately underwent a dehydration series by immersion in ethanol solutions (20%, 35%, 50%, 70%, 95%, and 100%) for 15 minutes each. The samples were dried with HMDS and then sputter-coated with AuPd. The samples were imaged on a Zeiss NEON SEM. Size analysis was performed on ImageJ, and ANOVA was used to establish statistical significance.

Autolysis Assay

The autolysis protocol used was modified from Mani, *et al.*[4] MRSA 700787 cells were inoculated 0.5% from an overnight culture in TSB (some with the addition of BPEI) and grown to an OD_{600} of approximately 0.450. The cells were pelleted and washed once with cold water. The cells were then resuspended in 0.05 M Tris-HCl (pH of 7.5) with 0.05% Triton X-100, and incubated at 30 °C without shaking. The OD_{600} was monitored every 20 minutes with a single flip of the cuvette. The percentage drop in OD_{600} was calculated for each measurement. The study was done in triplicate, and values are reported as the average with standard deviation.

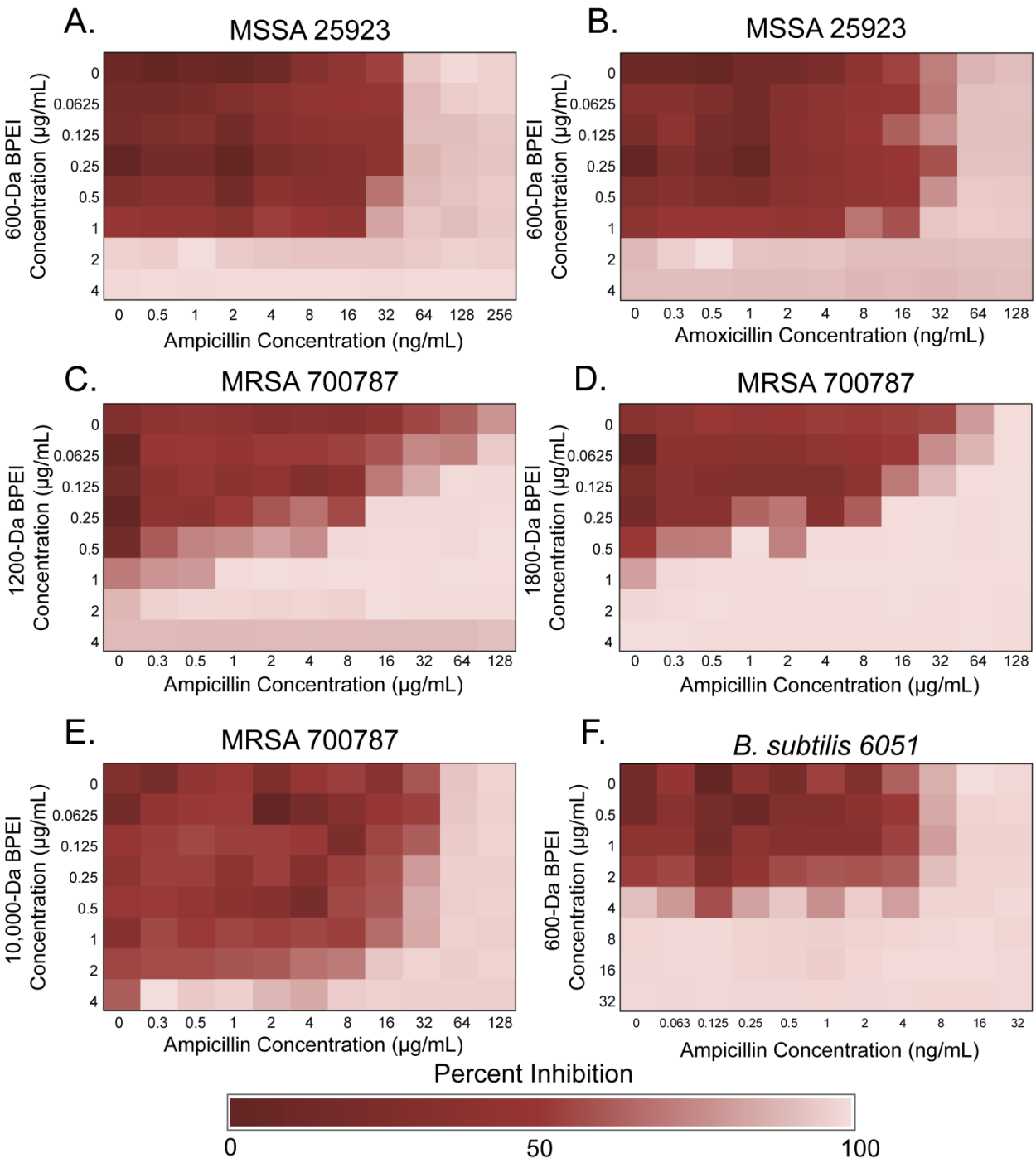

Figure S1. BPEI does not potentiates β-lactam activity against MSSA 25923 or *B. subtilis* 6051. Checkerboard assays show that BPEI not potentiates ampicillin (A) or amoxicillin (B) activity against MSSA 25923. 1,200-Da BPEI (C) and 1,800-Da BPEI (D) potentiate ampicillin acitivity against MRSA 700787 but 10,000-Da BPEI (E) does not. No synergy is seen between β-lactams and BPEI against *B. subtilis* 6051 (F). Each assay was performed as three separate trials and the presented data is shown as the average change in OD_{600}.

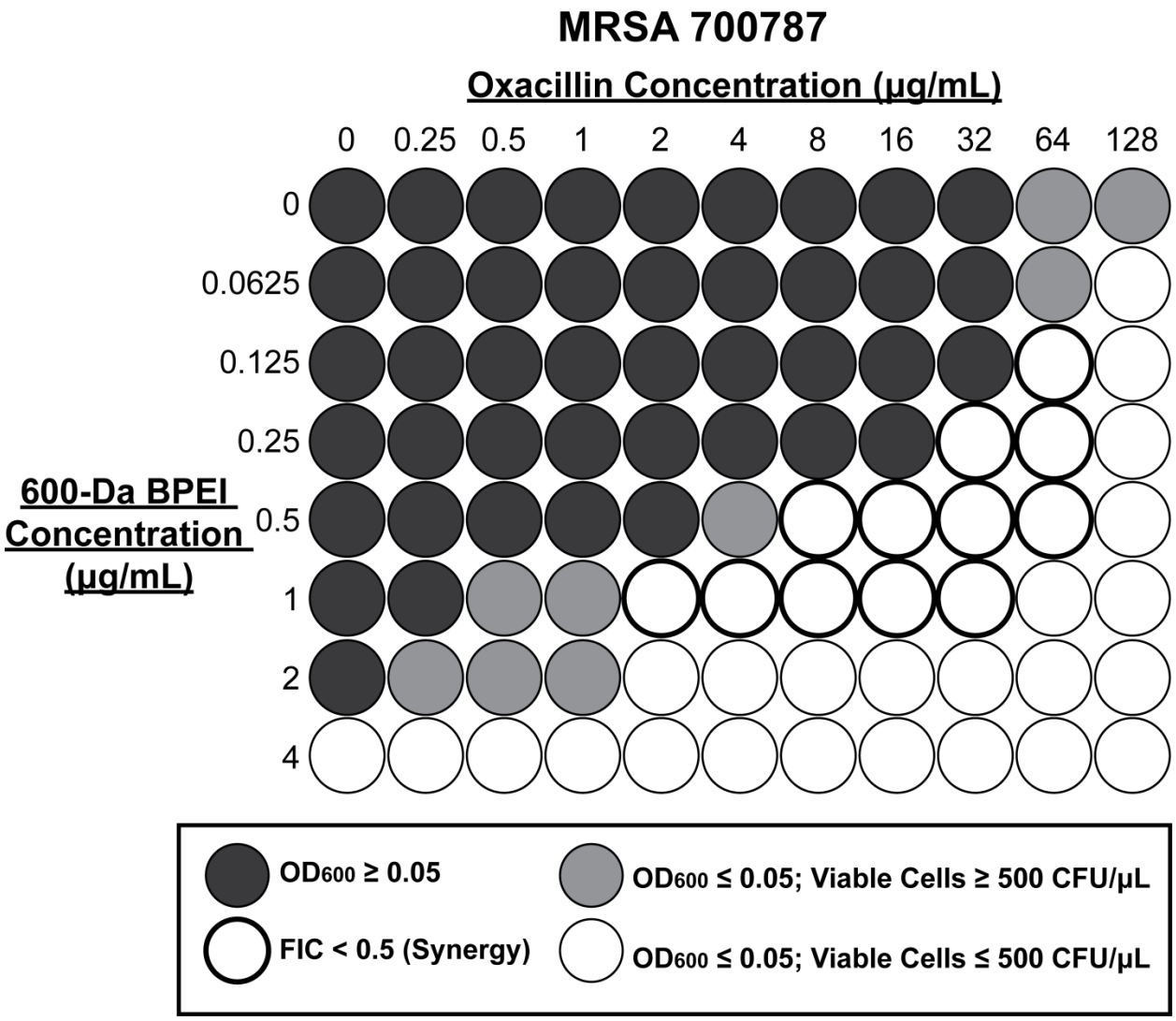

Figure S2. BPEI: oxacillin combination is bactericidal against MRSA. Dark shaded circles indicate wells where the bacteria grew as determined by an OD_{600} greater than 0.05. Lighter shaded circles show where cells were not killed but did not reach an OD_{600} of 0.05. Unshaded circles had less than 500 cells/µl. Bolded circles show an FIC less than 0.5.

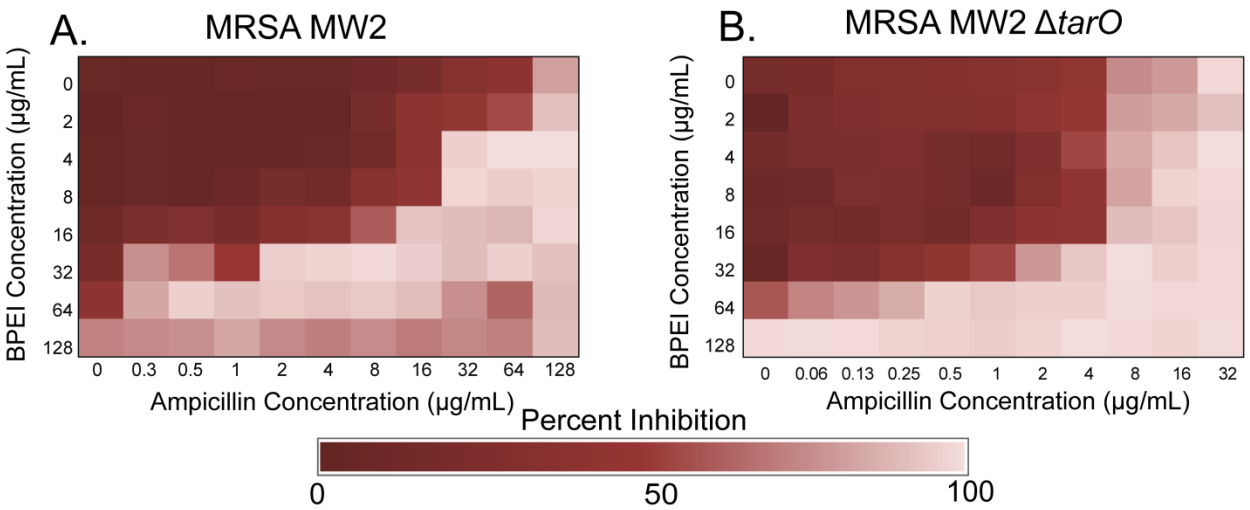

Figure S3. WTA is required for synergy between ampicillin and 600-Da BPEI. BPEI and ampicillin are synergistic (FIC=0.188) on the wild-type MRSA MW2 (A). MRSA cells lacking WTA through deletion of tarO (B) do not have synergy (FIC=1.0). Each assay was performed as three separate trials and the presented data is shown as the average.

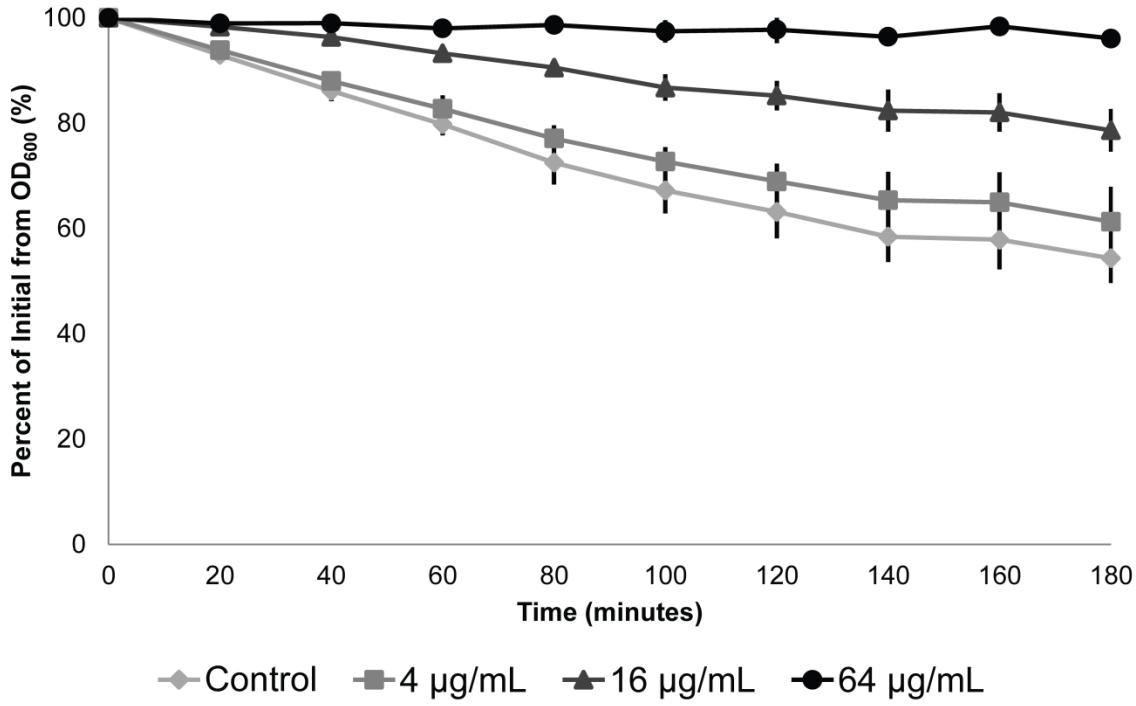

Figure S4. BPEI prevents Triton X-100 induced autolysis in MRSA 700787 in a concentration dependant manner. Data points are presented as the average of three trials and error bars denote the standard deviation.

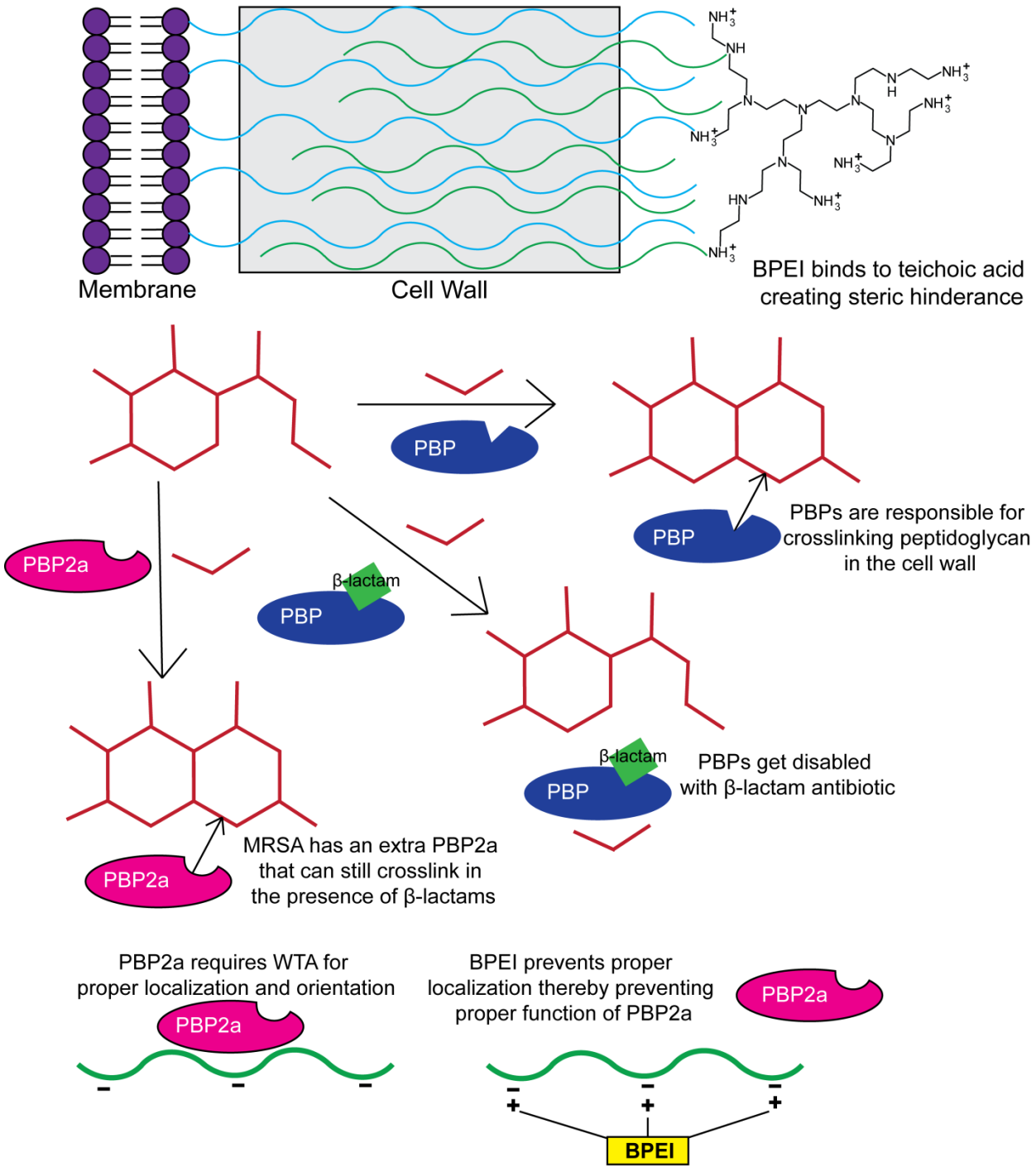

Figure S5. Our proposed mechanism of action is that BPEI electrostatically binds to WTA, which creates steric hindrance and prevents proper localization of PBP2a. Therefore, BPEI disables PBP2a through delocalization, and β-lactam antibiotics disable the other PBPs, which results in cells that are unable to crosslink the cell wall.

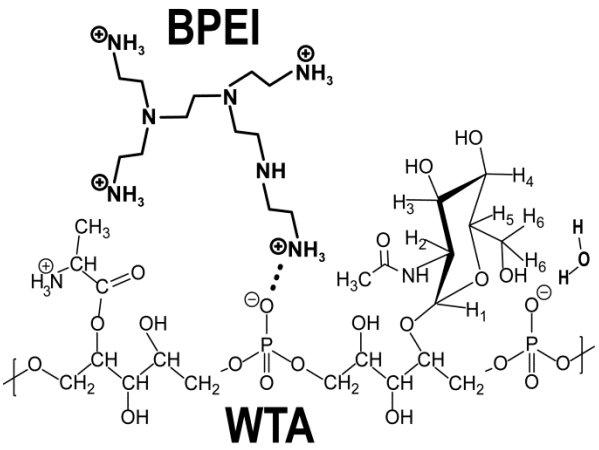

Figure S6. The cationic amine groups of BPEI electrostatically bind to the anionic phosphate groups of WTA.

References

1. Campbell, J.; Singh, A. K.; Santa Maria, J. P., Jr.; Kim, Y.; Brown, S.; Swoboda, J. G.; Mylonakis, E.; Wilkinson, B. J.; Walker, S., Synthetic lethal compound combinations reveal a fundamental connection between wall teichoic acid and peptidoglycan biosyntheses in Staphylococcus aureus. *Acs Chem Biol* **2011,** *6* (1), 106-16.
2. Orhan, G.; Bayram, A.; Zer, Y.; Balci, I., Synergy Tests by E Test and Checkerboard Methods of Antimicrobial Combinations against Brucella melitensis. *Journal of Clinical Microbiology* **2005,** *43* (1), 140-143.
3. Burgett, A. W. G.; Poulsen, T. B.; Wangkanont, K.; Anderson, D. R.; Kikuchi, C.; Shimada, K.; Okubo, S.; Fortner, K. C.; Mimaki, Y.; Kuroda, M.; Murphy, J. P.; Schwalb, D. J.; Petrella, E. C.; Cornella-Taracido, I.; Schirle, M.; Tallarico, J. A.; Shair, M. D., Natural products reveal cancer cell dependence on oxysterol-binding proteins. *Nature Chemical Biology* **2011,** *7* (9), 639-647.
4. Mani, N.; Tobin, P.; Jayaswal, R. K., Isolation and characterization of autolysis-defective mutants of Staphylococcus aureus created by Tn917-lacZ mutagenesis. *J. Bacteriol* **1993,** *175* (5), 1493-1499.