

Supporting information for:

Inhibition of *Streptococcus pneumoniae* growth by masarimycin.

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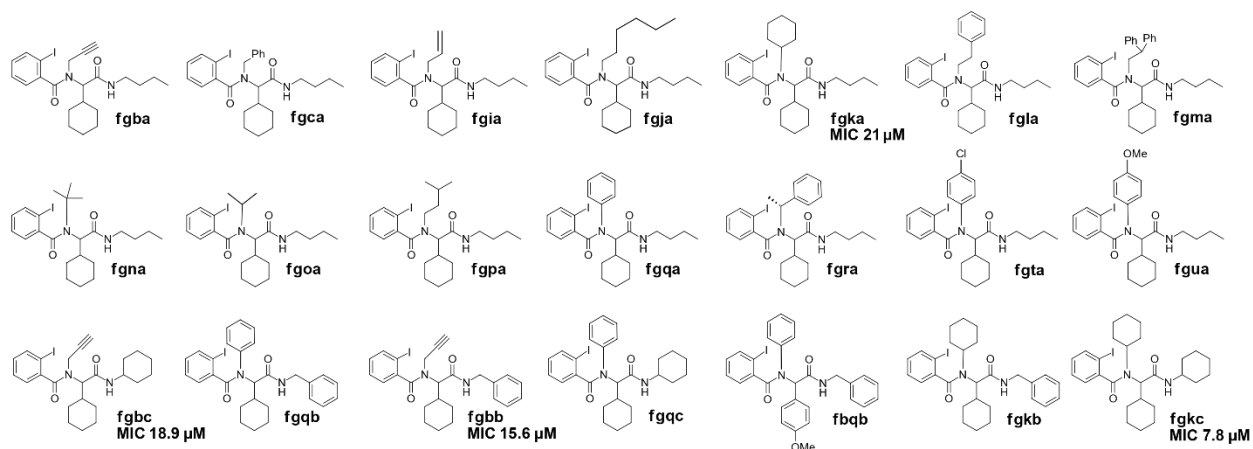


Figure S1. Structures of the diamide library screened against *Streptococcus pneumoniae*.

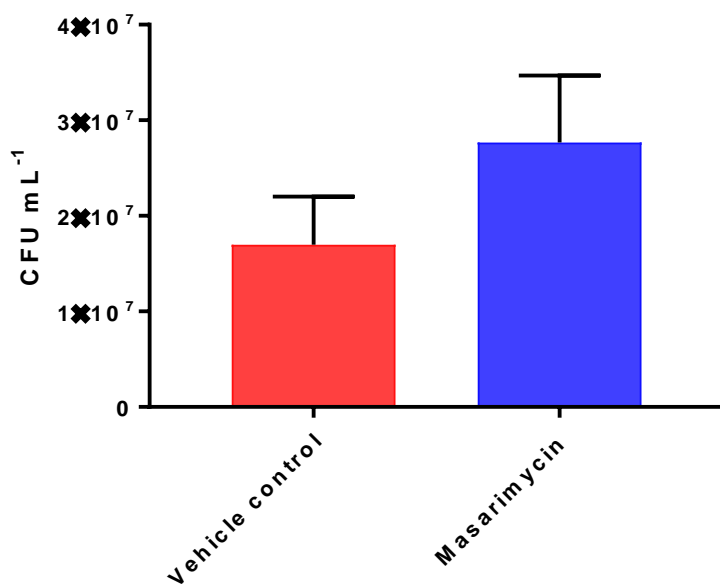


Figure S2. Bacteriostatic assay for masarimycin against *S. pneumoniae* R6

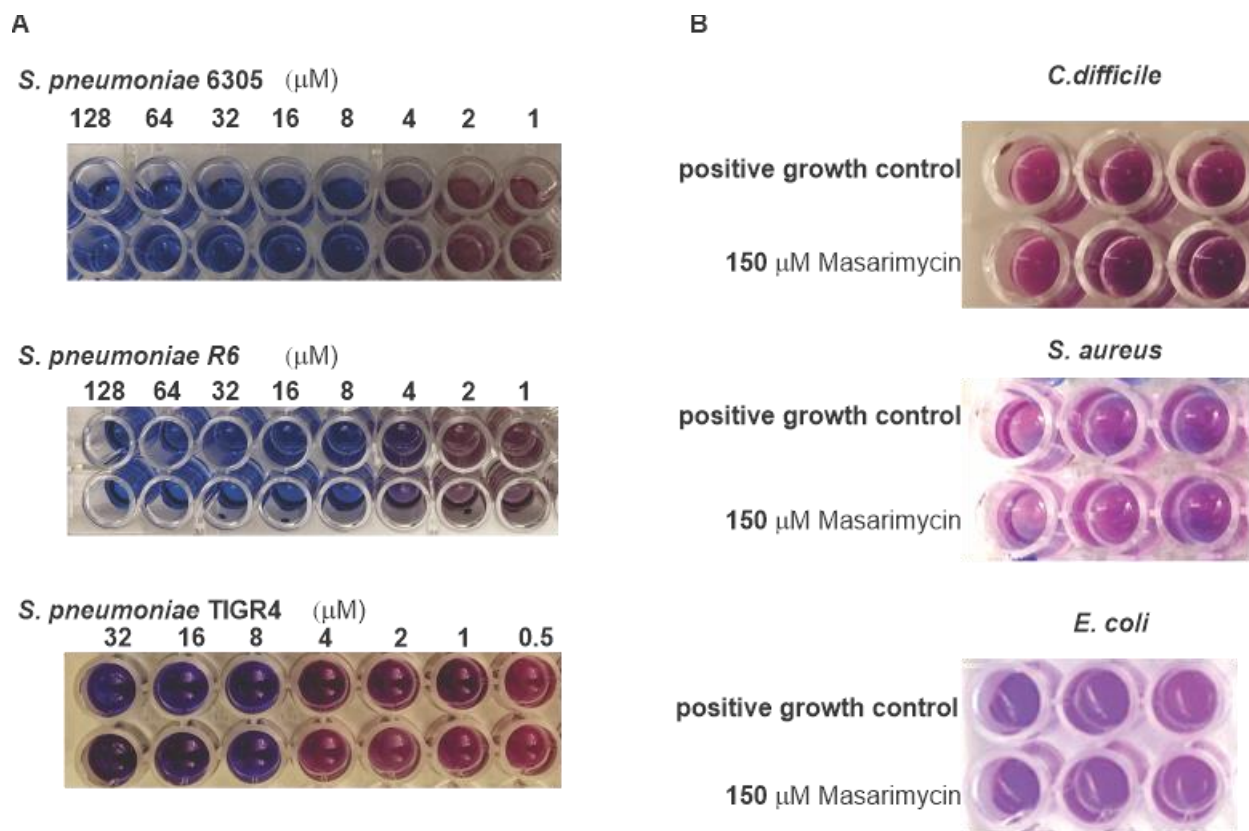


Figure S3. Minimum inhibitory concentration assays for *S. pneumoniae* strains 6305, R6, and TIGR4 (A), and screen against *C. difficile*, *S. aureus*, and *E. coli* at 150 μM masarimycin to assess compound spectrum.

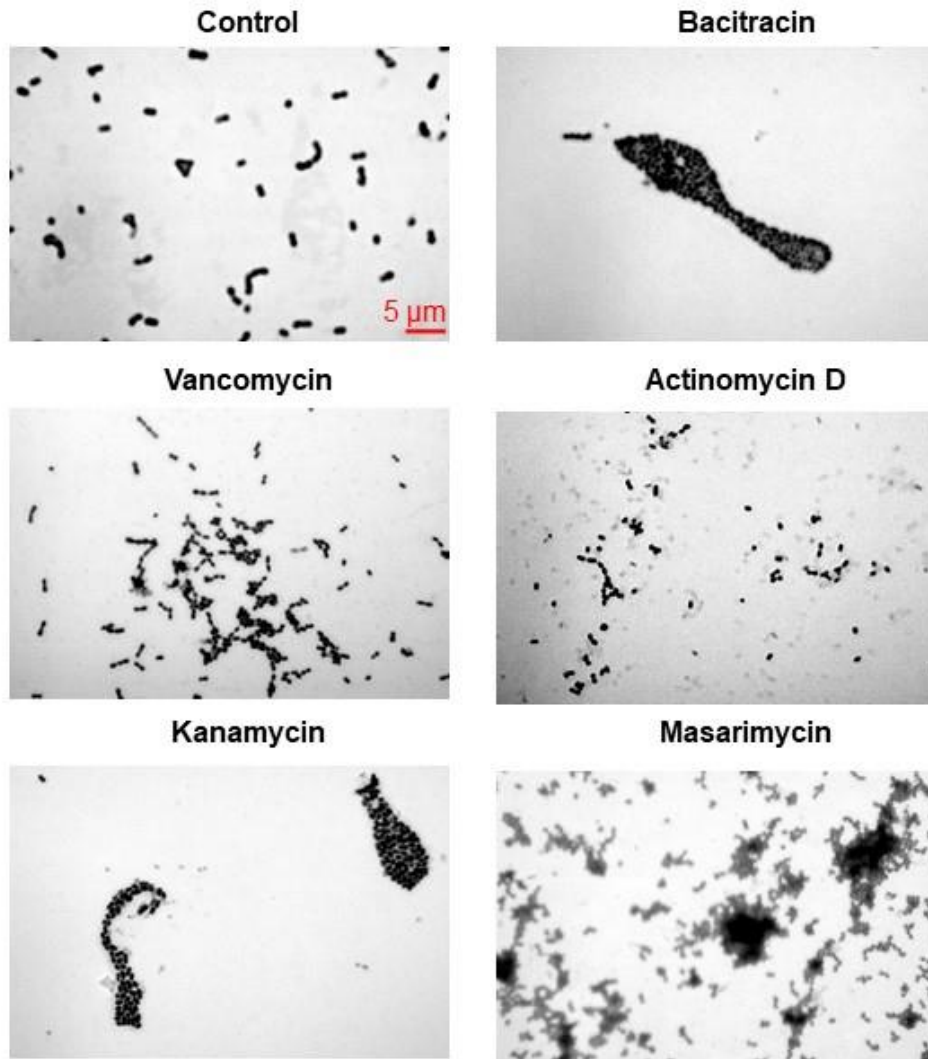


Figure S4. Phenotypic analysis of wild-type *S. pneumoniae* R6 in the presence of sub-MIC (0.7x) of antibiotics with well-defined modes of action or with masarimycin. Cells were treated for 90 min with antibiotic or vehicle control, fixed in 20 mM HEPES pH 7.0, 1% formaldehyde, and stained with methylene blue. Images were acquired at 1000x magnification.

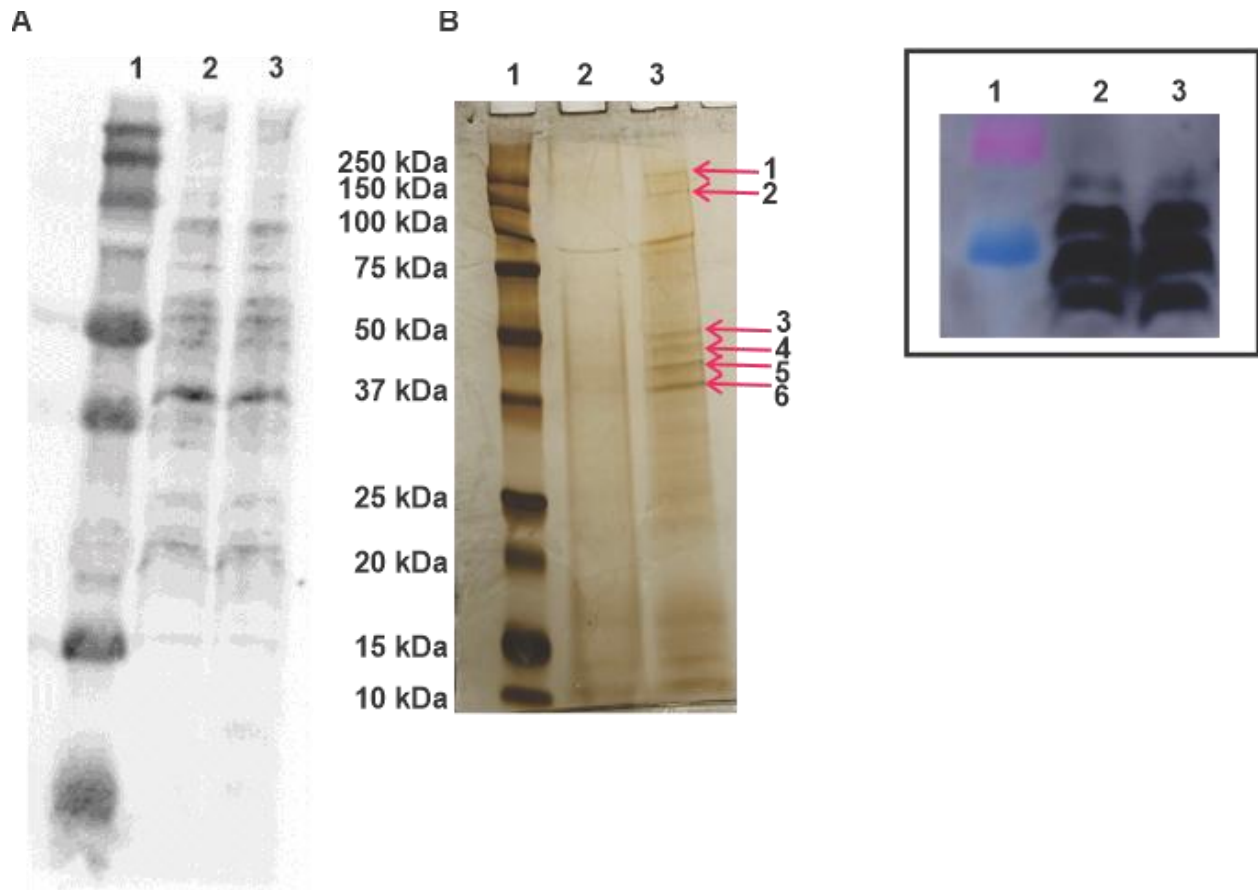


Figure S5. Changes in wall teichoic acid (WTA) and cell surface protein profiles in *S. pneumoniae* under sub-MIC (0.75x) treatment with masarimycin. (A) Wall teichoic acid profile, (B) profile of cell-wall associated proteins after high pH (pH 12.0), arrows with corresponding numbers indicate bands that were selected for protein identification by mass spectrometry (Table 1). Inset: changes to phosphocholine levels in WTA. Lane 1, MW marker; Lane 2, Control; Lane 3, masarimycin treatment.

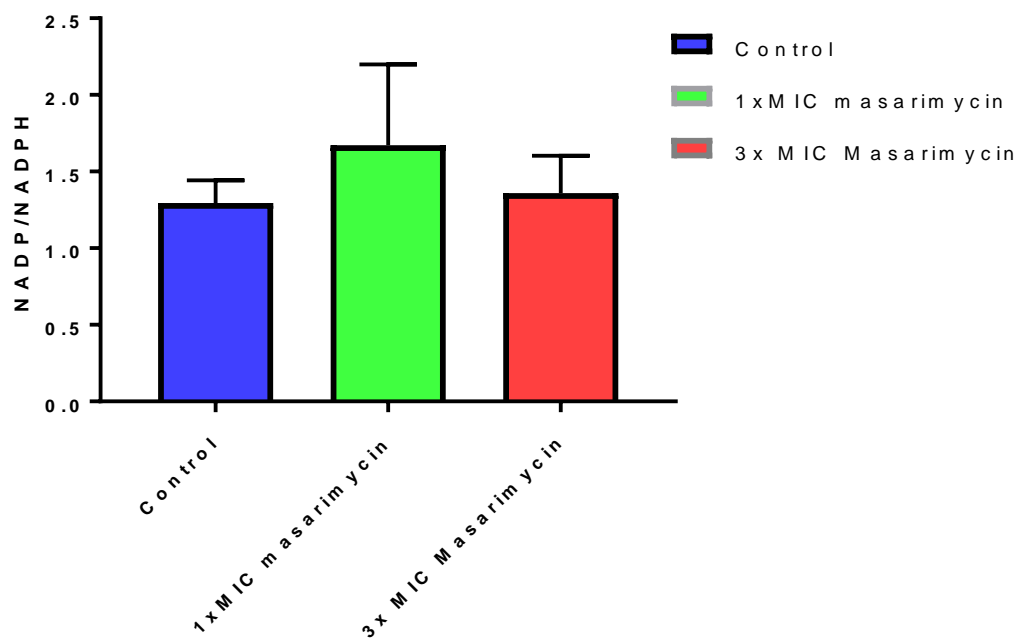
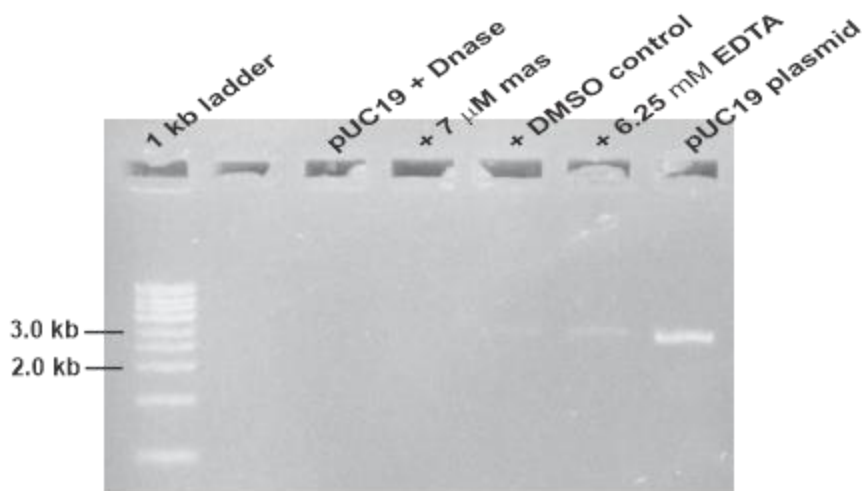


Figure S6. Ratios of NADP⁺/NADPH in *S. pneumoniae* in the presence of masarimycin

A



B

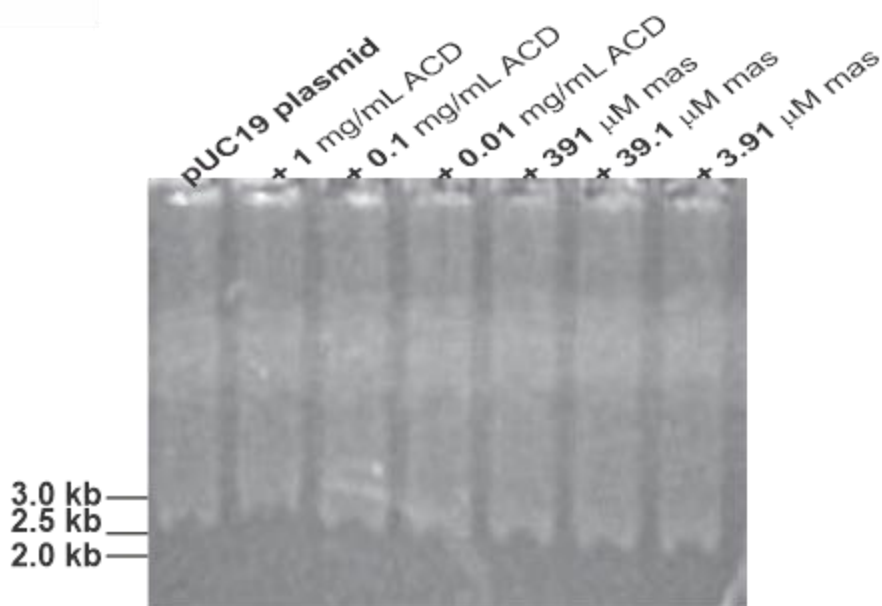


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