Supporting Information

Dual Mode of Action for Plusbacin A_3 in *Staphylococcus aureus*

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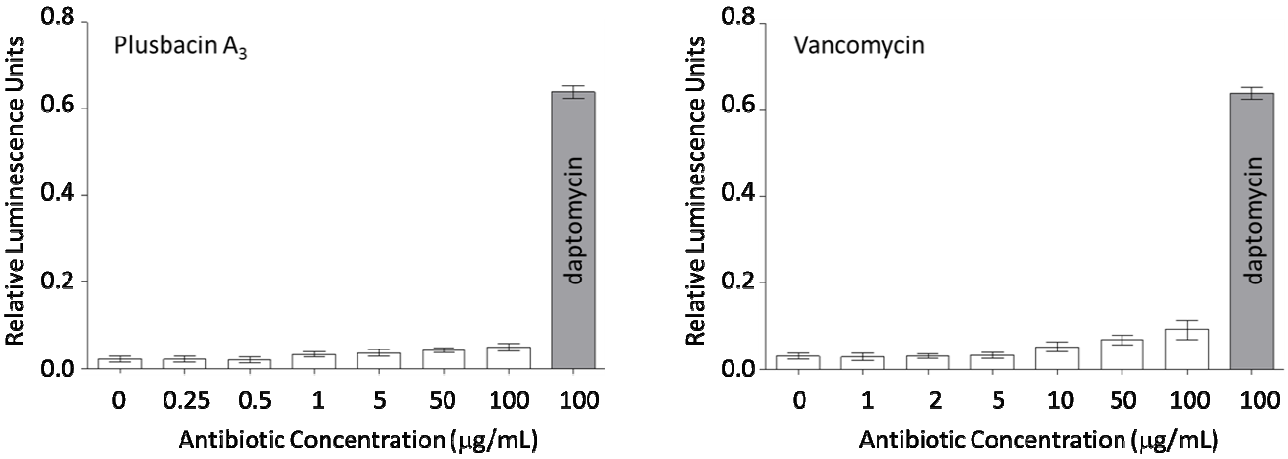
ATP-leakage assay

An ATP-leakage assay was performed on overnight culture of *S. aureus* (ATCC 6538P) grown in TSB harvested at OD_{600nm} 1.5. *S. aureus* was pelleted and then resuspended in phosphate buffered saline (PBS) supplemented with 20 mM Ca^{2+}. Plusbacin A_3 and vancomycin were respectively added to the suspension to final drug concentrations of 0, 0.25, 0.5, 1, 5, 50, and 100 μg/ml (plusbacin A_3), or 0, 1, 2, 5, 10, 50, and 100 μg/ml (vancomycin). As a control, daptomycin was added to the suspension at a final concentration of 100 μg/ml. The antibiotic-treated cells were incubated for 20 minutes at 37 °C and the supernatant containing ATP was collected following a brief centrifugation. The ATP that was leaked to the supernatant was quantified by adding 100 μL of CellTiter-Glo® 2.0 reagents (Promega, Madison WI) to an equal

volume of supernatant. After 10 min equilibration at room temperature, luminescence was measured using a Fluoroskan Ascent FL Luminometer (Thermo Scientific) with an integration time of 200 ms.



Supplementary Figure S1. ATP leakages from *S. aureus* treated with plusbacin A_3 and vancomycin. ATP leakage was induced to *S. aureus* harvested at OD_{660nm} 1.5 by the addition of plusbacin A_3 (left) to final concentrations of 0, 0.25, 0.5, 1, 5, 50, and 100 μg/ml, and vancomycin (right) to final concentrations of 0, 1, 2, 5, 10, 50, and 100 μg/ml. ATP leakage from *S. aureus* treated with daptomycin at 100 μg/ml is shown as a positive control. *S. aureus* treated with plusbacin A_3 or vancomycin did not induce significant ATP leakage which suggests that neither drug targets the bacterial membrane.