## **Supporting Information for:**

## Bacterial self-resistance to the natural proteasome inhibitor salinosporamide A

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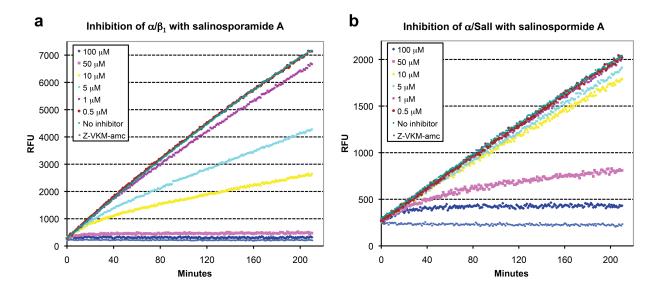
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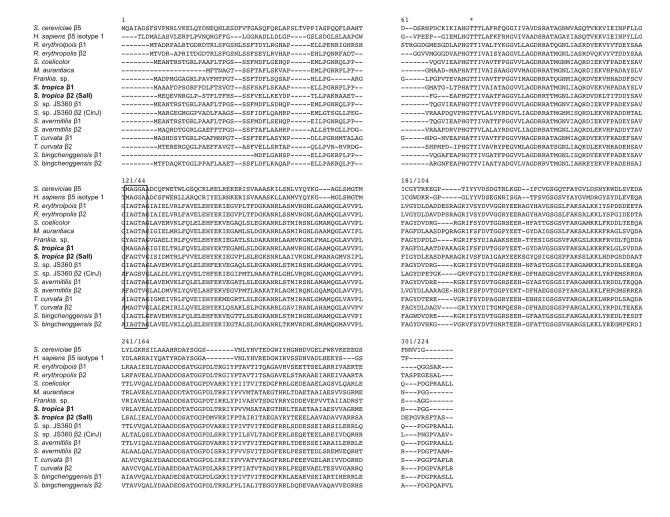
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## SUPPLEMENTAL FIGURES



**Supplementary Figure 1.** Time-dependence of salinosporamide A inhibition on the a)  $\alpha/\beta_1$  and b)  $\alpha/\text{SalI}$  complexes. Various concentrations of salinosporamide A were premixed with fluorogenic Z-VKM-amc substrate. Proteasome complex was then added at 20 µg ml<sup>-1</sup> and substrate hydrolysis was measured once per minute. RFU = Relative fluorescence units. Salinosporamide A is spontaneously hydrolyzed in aqueous buffer with an estimated half-life of 20–30 minutes at pH 8.0 (1).



**Supplementary Figure 2.** Alignment of actinobacterial 20S proteasome β-subunits, including prosequences, with the CT-L β5-subunits of *Saccharomyces cerevisiae* and *Homo sapiens*. Previously characterized β-subunit sequences from actinomycetes as well as organisms closely related to *S. tropica* (see Figure 3) are listed above those from *S. tropica*. The sequences listed below those from *S. tropica* are from organisms containing a secondary β-subunit (Table 4). Thr1 is denoted by "\*" above the alignment. Amino acid positions (from start of prosequence/from Thr1) are indicated at the start of each 60 residue block. Residues 45–49, found within the S1 binding pocket, are enclosed inside a box. Sequences obtained from the following organisms: *Saccharomyces cerevisiae*, *Homo sapiens*, *Rhodococcus erythropolis* PR4, *Streptomyces coelicolor* A3(2), *Micromonospora aurantiaca* ATCC 27029, *Frankia* sp. ACN14a, *Salinispora tropica* CNB-440, *Streptomyces* sp. JS360, *Streptomyces avermitilis* MA-4680, *Thermomonospora curvata* DSM 43183, *Streptomyces binchenggensis* BCW-1.

## SUPPLEMENTAL REFERENCES

1. Denora, N., Potts, B. C. M., and Stella, V. J. (2007) A mechanistic and kinetic study of the β-lactone hydrolysis of salinosporamide A (NPI-0052), a novel proteasome inhibitor, *J. Pharm. Sci. 96*, 2037–2047.