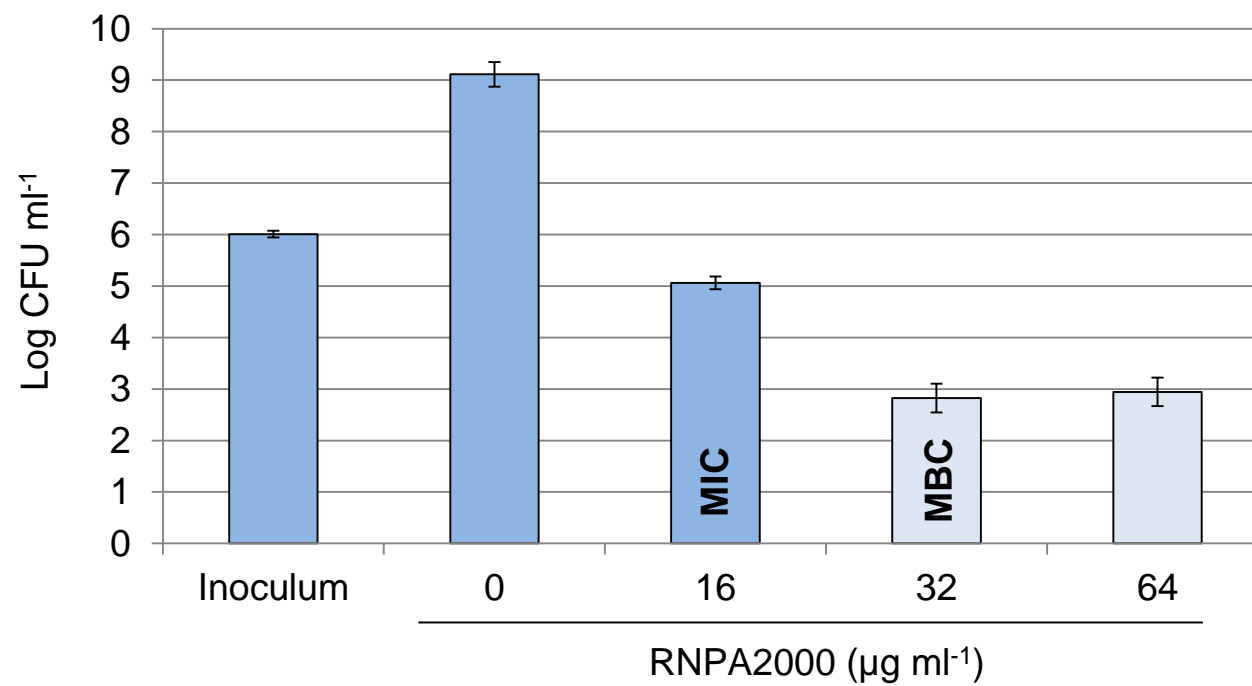
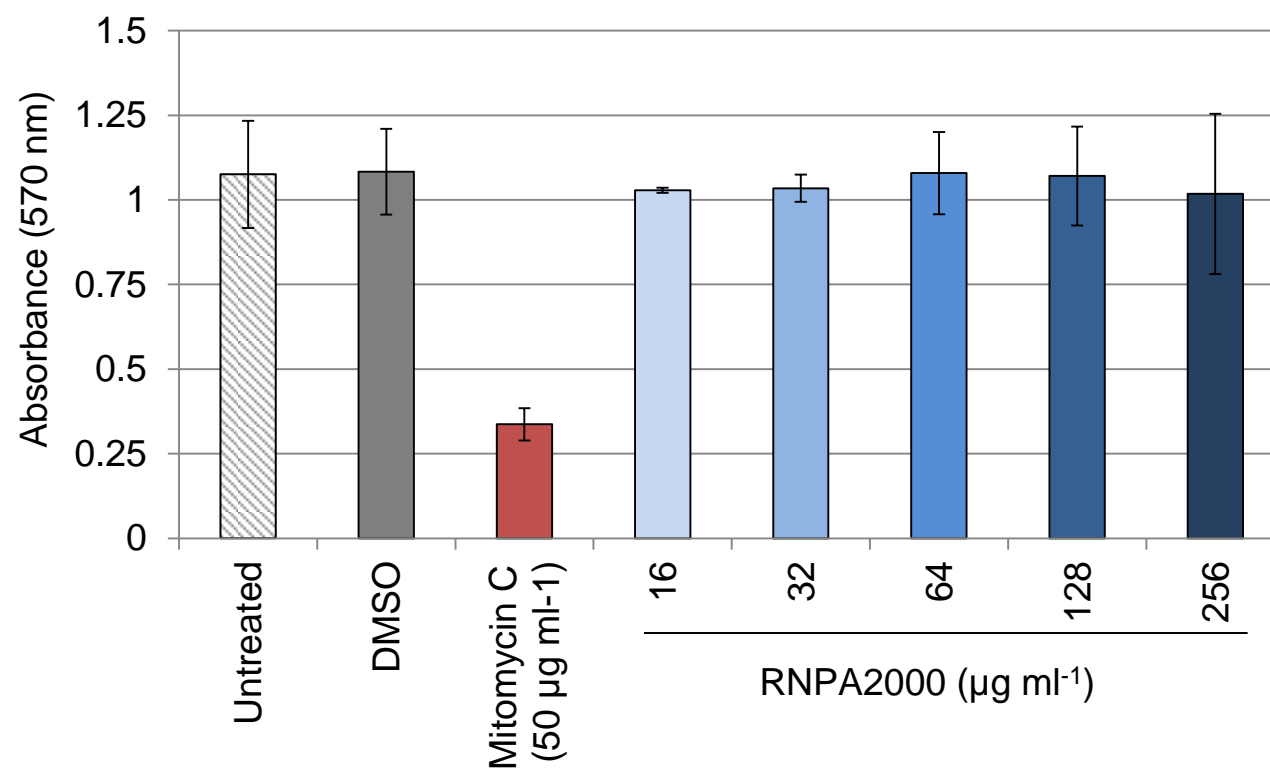
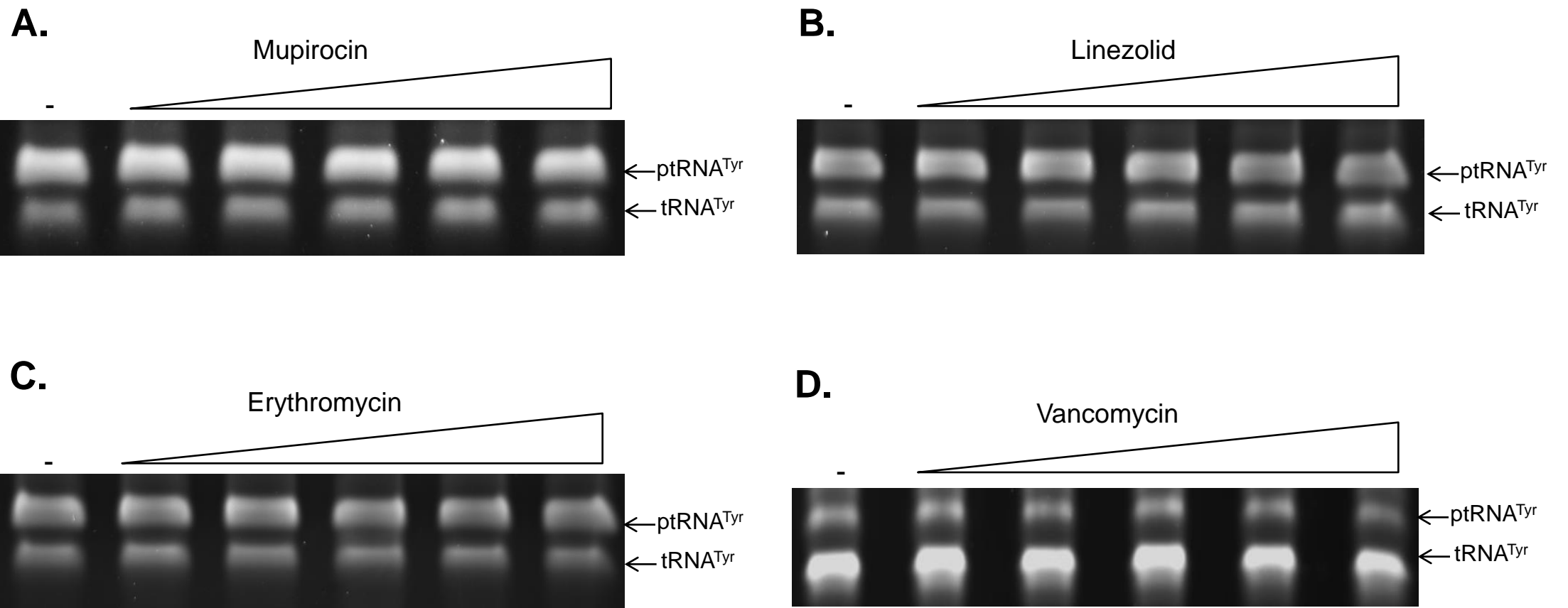
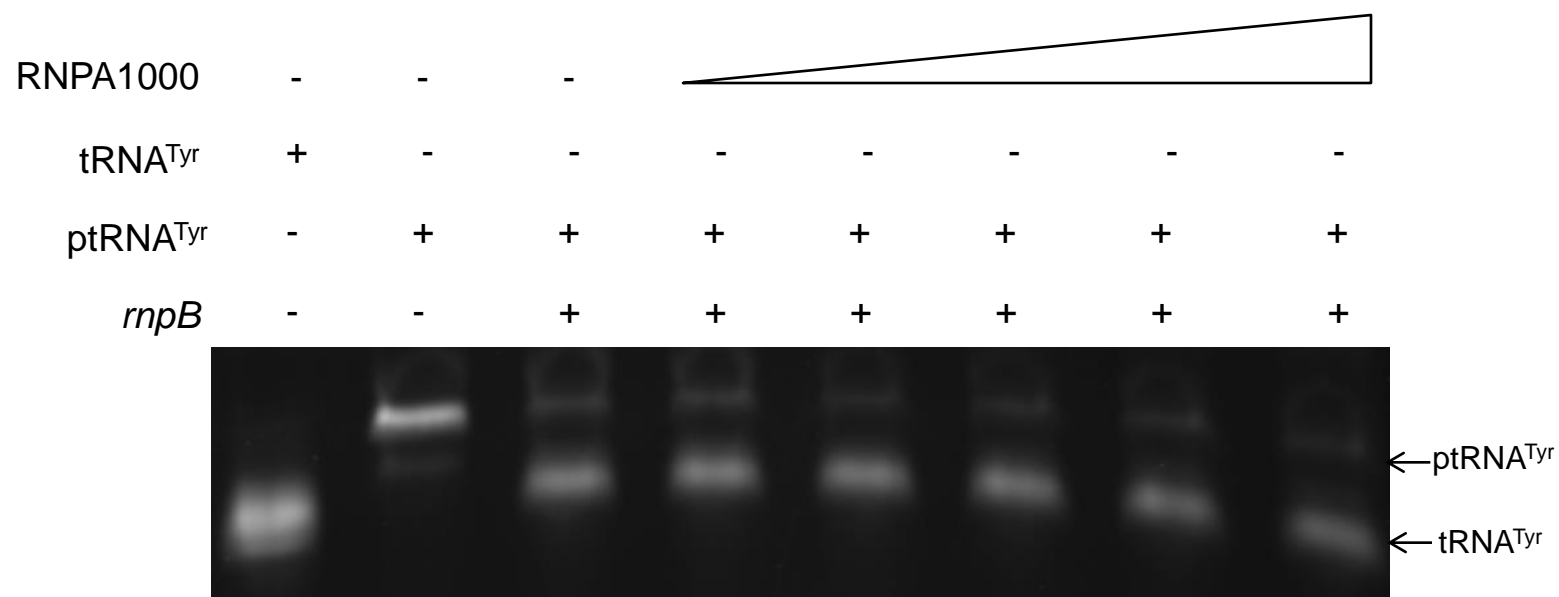
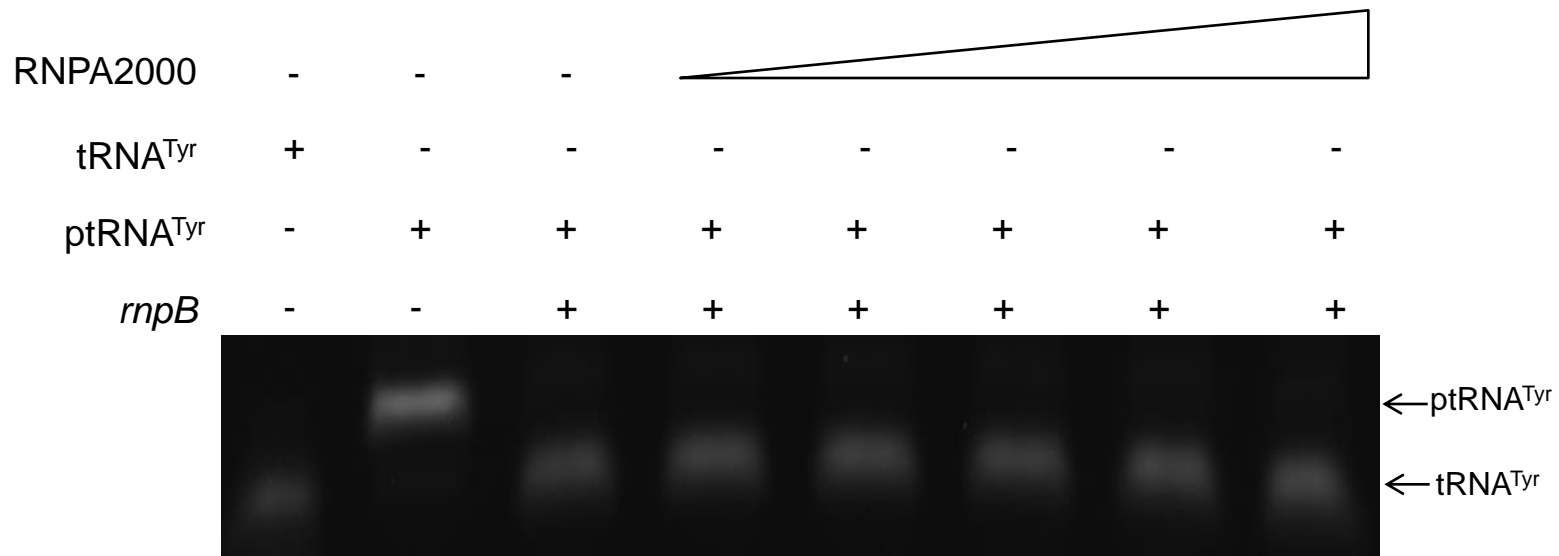


A.**B.****Supplemental Figure 1. RNPA2000 bactericidal and human cytotoxicity measures.**

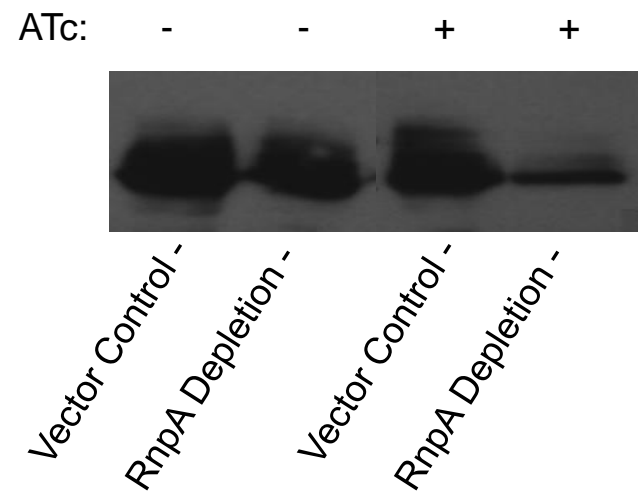
Panel A. Graphed are the colony forming units (CFUs) per milliliter of *S. aureus* strain UAMS-1 following 16 hr incubation with the indicated concentration of RNPA2000; starting inoculum and standard deviations (N=8) are shown. **Panel B.** Human HepG2 cell viability as measured by the MTT proliferation assay following 24 hr treatment with DMSO (negative control), mitomycin C (positive control), and the indicated concentration of RNPA2000.



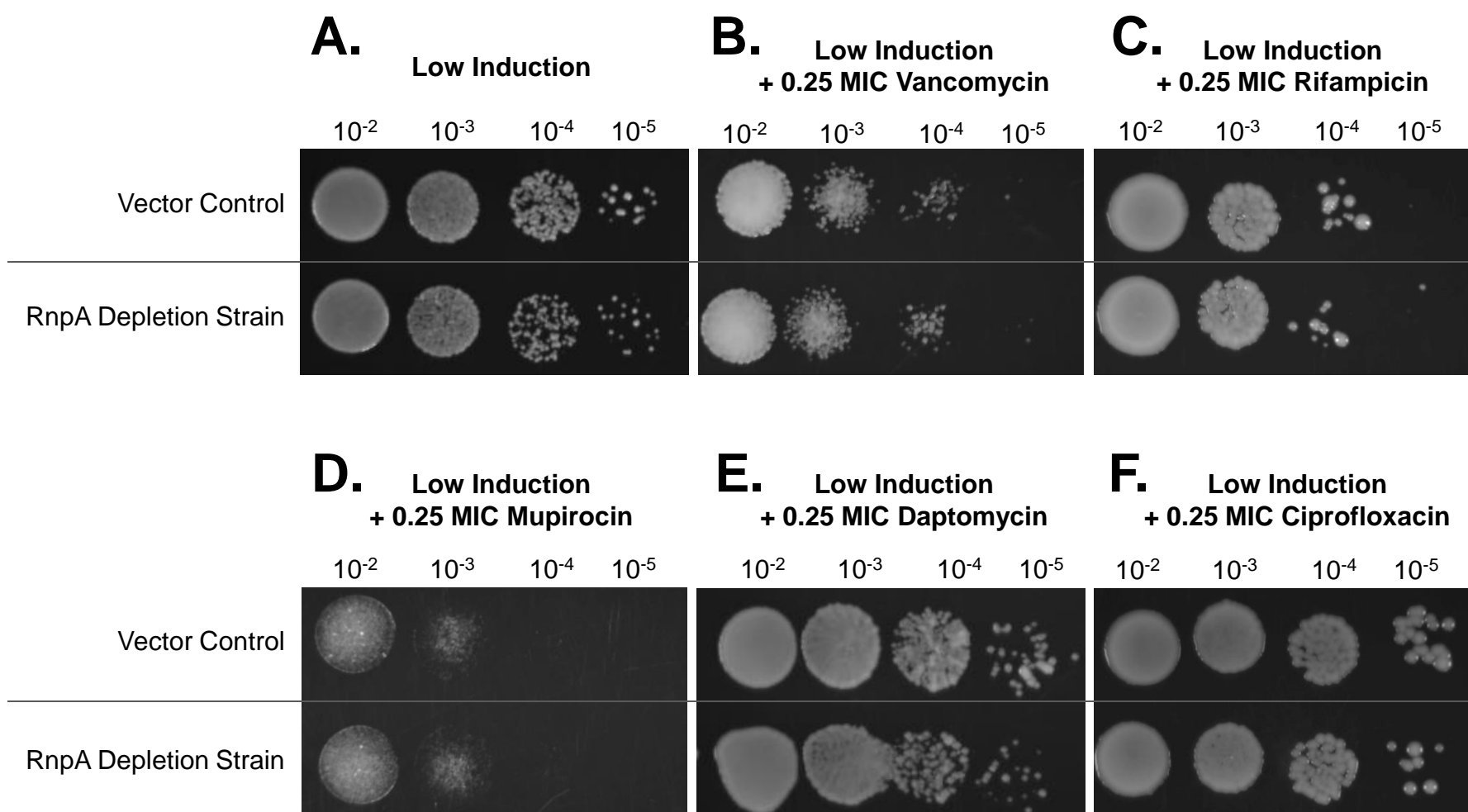
Supplemental Figure 2. *S. aureus* RNase P activity assays. Shown are RNase P $\text{ptRNA}^{\text{Tyr}}$ activity assay results for reactions performed in the absence (-) or presence of increasing concentrations (0, 31.25, 62.5, 125, 250, 500 μM) of mupirocin (**Panel A**), linezolid (**Panel B**), erythromycin (**Panel C**), and vancomycin (**Panel D**).

A.**B.**

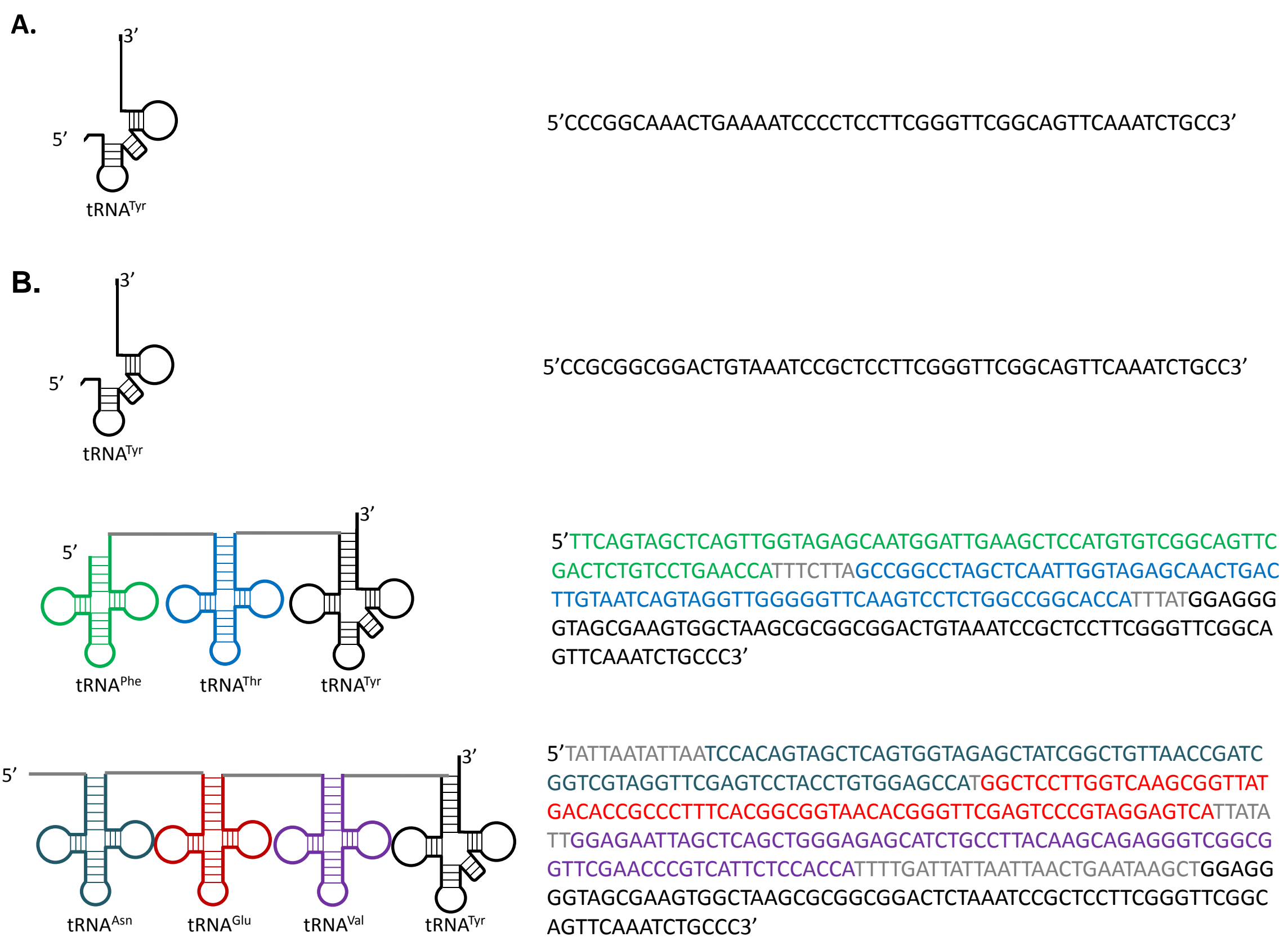
Supplemental Figure 3. *S. aureus* *rnpB* activity assays. Shown are *rnpB* ptRNA^{Tyr} activity assay results in high salt buffer conditions performed in the absence (-) or presence (+) of increasing concentrations (0, 31.25, 62.5, 125, 250, 500 μ M) of RNPA1000 (**Panel A**) or RNPA2000 (**Panel B**).



Supplemental Figure 4. RnpA Western-blotting. *S. aureus* RnpA levels within cells harboring pML100 (vector; control) or pML100::AS-*rnpA* (*rnpA* mRNA directed antisense molecule; RnpA Depletion) following growth in the absence (-) or presence (+) of 5 ng ml⁻¹ anhydrotetracycline (ATc).



Supplemental Figure 5. Cellular RnpA levels do not influence *S. aureus* susceptibility to RnpA-independent classes of antibiotics. Serially diluted *S. aureus* vector (pML100) or isogenic RnpA Depletion (pML100::AS-*rnpA*; *rnpA* mRNA directed antisense molecule; RnpA Depletion strain) cells plated on TSA agar containing low induction conditions (5 ng ml⁻¹ ATc; **Panel A**) or medium supplemented with 0.25X MIC vancomycin (0.5 µg ml⁻¹; **Panel B**), rifampicin (1.95 ng ml⁻¹; **Panel C**), mupirocin (0.125 µg ml⁻¹; **Panel D**), daptomycin (0.5 µg ml⁻¹; **Panel E**), or ciprofloxacin (0.25 µg ml⁻¹; **Panel F**).



Supplemental Figure 6. 5' Rapid Amplification of cDNA Ends (RACE) sequencing results. Panel A. RNA isolated from mock treated *S. aureus* cells generated a single 5'RACE product representing a 5' truncated tRNA^{Tyr} species by sequencing. **Panel B.** Three predominant 5'RACE products were detected following 5'RACE of RNA isolated from *S. aureus* cultures treated with 2x MIC RNPA2000, corresponding to tRNA^{Tyr} species, and two polycistronic tRNA^{Tyr} species by sequencing (tRNA^{Asn,Glu,Val,Tyr} and tRNA^{Phe,Thr,Tyr}); secondary structures are approximate and were generated using tRNAscan.