**Supplemental Material**

**Table S1**. Antibacterial specificity of PyS2-GN4 towards Gram-positive and Gram-negative bacteria.

| Gram-Positive | | | Gram-Negative | | |
|---|---|---|---|---|---|
| Species | Strain | MIC (µg/ml) | Species | Strain | MIC (µg/ml) |
| *B. cereus* | RSVF1 | > 256 | *A. baumaneii* | ATCC 17978 | > 256 |
| *E. faecium* | EFSK-2 | > 256 | *E. cloacae* | NR-50391 | > 256 |
| *S. aureus* | NR-45946 | > 256 | *E. coli* | ATCC 25922 | > 256 |
| *S. pyogenes* | D471 | > 256 | *K. pneumoniae* | NR-41916 | > 256 |

**Table S2.** List of bacterial strains used in this study.

| Bacteria | Source |
|---|---|
| *A. baumaneii* ATCC 17978 | ATCC |
| *B. cereus* RSVF1 | Vincent Fischetti, The Rockefeller University |
| *E. cloacae* NR-50391 | BEI Resources, NIAID, NIH |
| *E. coli* ATCC 25922 | ATCC |
| *E. faecium* EFSK-2 | Alexander Tomasz, The Rockefeller University |
| *K. pneumoniae* NR-41916 | BEI Resources, NIAID, NIH |
| *P. aeruginosa* ATCC 15692 | ATCC |
| *P. aeruginosa* 442 | Lars Westblade, Weill Cornell Medical College |
| *P. aeruginosa* 443 | Lars Westblade, Weill Cornell Medical College |
| *P. aeruginosa* 445 | Lars Westblade, Weill Cornell Medical College |
| *P. aeruginosa* 446 | Lars Westblade, Weill Cornell Medical College |
| *P. aeruginosa* 448 | Lars Westblade, Weill Cornell Medical College |
| *P. aeruginosa* 449 | Lars Westblade, Weill Cornell Medical College |
| *P. aeruginosa* 450 | Lars Westblade, Weill Cornell Medical College |
| *P. aeruginosa* 451 | Lars Westblade, Weill Cornell Medical College |
| *P. aeruginosa* 452 | Lars Westblade, Weill Cornell Medical College |
| *P. aeruginosa* 453 | Lars Westblade, Weill Cornell Medical College |
| *P. aeruginosa* MDR-M-3 | Daniel Green, Columbia University Medical Center |
| *S. aureus* NR-45946 | BEI Resources, NIAID, NIH |
| *S. pyogenes* D471 | The Rockefeller University Lancefield Collection |

ATCC, American Type Culture Collection; NIAID, National Institute of Allergy and Infectious Disease; NIH, National Institutes of Health

**Table S3**. List of primers used in this study.

| Oligonucleotide | Nucleotide Sequence |
|---|---|
| GN4_F | 5’-AACTTTAAGAAGGAGATATAATGCGCACCAGCCAGCGC-3’ |
| GN4_R | 5’-GTCGACGGAGCTCGAATTCGGATCCTTAGCTCAGCGGTTCCAGAAACAGTGC-3’ |
| PyS2_F | 5’-AACTTTAAGAAGGAGATATACCATGGCCGTGAACGATTATG-3’ |
| PyS2-GN4_R | 5’-TGGTGCGCATCGGGTCACGAAACATCAC-3’ |
| PyS2-GN4_F | 5’-TCGTGACCCGATGCGCACCAGCCAGCGC-3’ |
| PyS2-GN4ΔTBB_F | 5’-[Phos]GTTCAGGGTGGTGGTCGTGACATTATCCAG |
| PyS2-GN4ΔTBB_R | 5’-[Phos]GCTGCCCGGCTCATAATCGTTCACGGCCATG |
| PyS2-GN4_KO_1F | 5’-[Phos]CGCCTATCAGGCTAGCGTGGGTGTGTGGACC-3’ |
| PyS2-GN4_KO_1R | 5’-[Phos]CTCAGGCGCAGGCCCTCAAAGCTCTTAATC-3’ |
| PyS2-GN4_KO_2F | 5’-[Phos]GCGTGGGTGTGTGGGCCATTGGTTATGGTAC-3’ |
| PyS2-GN4_KO_2R | 5’-[Phos]TAGCCTGATAGGCGCTCAGGCGCAGGCCC-3’ |
| FpvAI_F | 5’-CGAAGGCCAGAACTACGAGA-3’ |
| FpvAI_R | 5’-TGTAGCTGGTGTAGAGGCTCAA-3’ |
| FpvAII_F | 5’-TACCTCGACGGCCTGCACAT-3’ |
| FpvAII_R | 5’-GAAGGTGAATGGCTTGCCGTA-3’ |
| FpvAIII_F | 5’-ACTGGGACAAGATCCAAGAGAC-3’ |
| FpvAIII_R | 5’-CTGGTAGGACGAAATGCGAG-3’ |

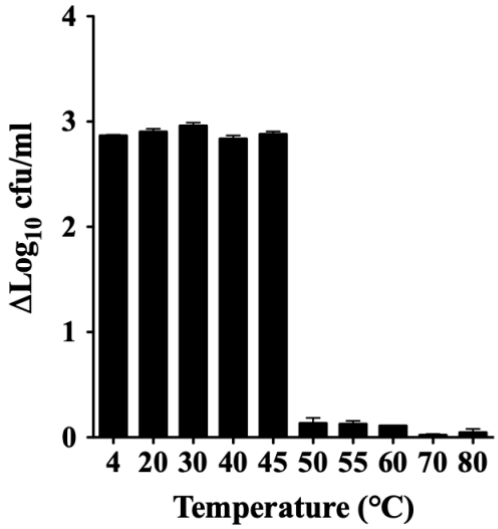


**Figure S1.** Lysocin thermal stability. PyS2-GN4 was incubated in PBS for 30 min at temperatures ranging from 4°C to 80°C. After cooling on ice, each sample at 50 μg/ml was incubated statically with *P. aeruginosa* strain 453 for a total of 4 h at 37°C. The average residual antipseudomonal activity of each sample was equated to the $\log_{10}$ decrease in viable bacterial cells when compared to the untreated control. All error bars correspond to ± SEM of triplicate experiments.

## SI Methods

### *Determining Thermal Stability*

Using an EchoTherm™ bench-top incubator (Torrey Pines Scientific), PyS2-GN4 was initially incubated at 1 mg/ml in PBS, pH 7.4, for 30 min at temperatures ranging from 4°C to 80°C. Following temperature treatment, the lysocin was immediately cooled on ice. Residual antipseudomonal activity was determined by incubating each lysocin sample at 50 μg/ml with *P. aeruginosa* strain 453 at $10^6$ cfu/ml in CAA medium with EDDHA for a total of 4 h at 37°C. The samples were plated on CAA agar in order to calculate viable cell counts. A control absent of lysocin was included.