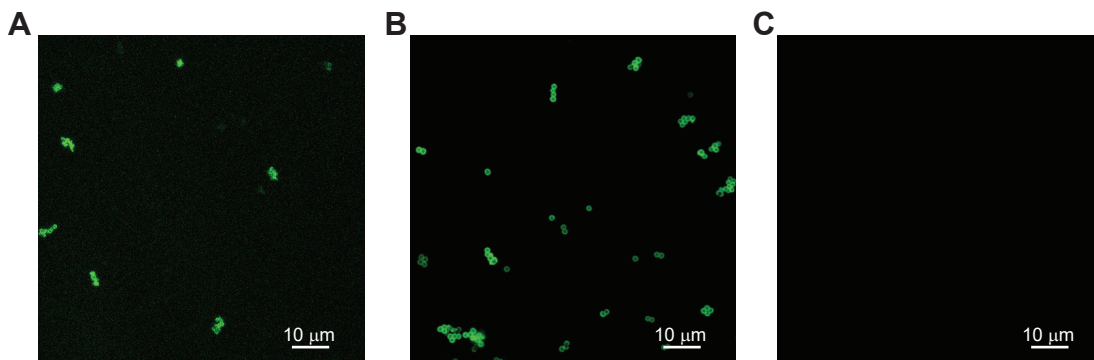


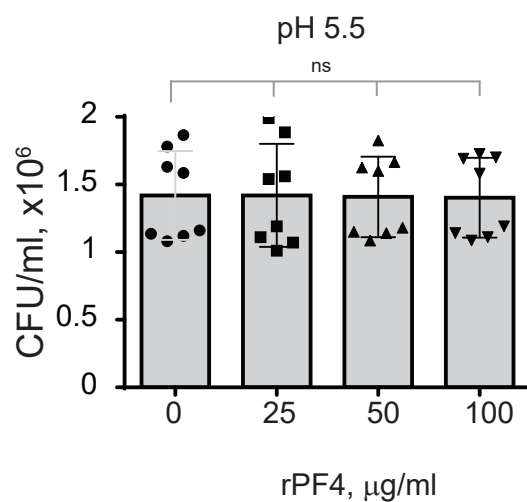
SUPPLEMENTARY MATERIAL

PLATELET FACTOR 4 (PF4) IMPROVES SURVIVAL IN A MURINE MODEL OF ANTIBIOTIC-SUSCEPTIBLE AND METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS PERITONITIS

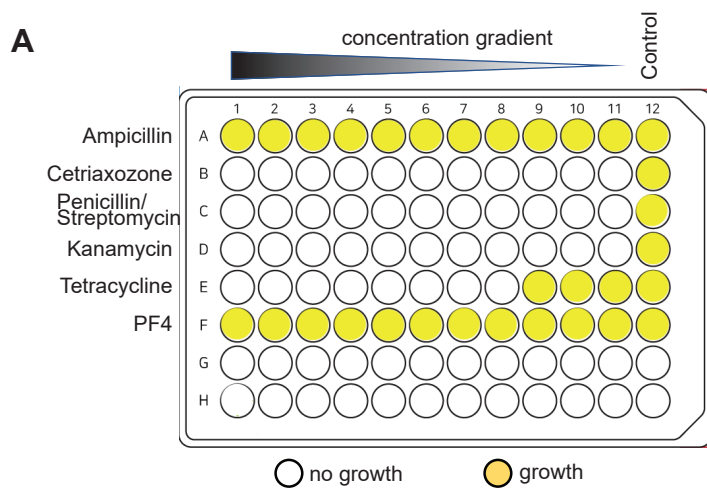
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Supplemental Figure 1. Confocal images showing specific binding of anti-PF4 antibody to rPF4- bound *S. aureus*. Nonencapsulated (A) and encapsulated (B) *S. aureus* was incubated with rPF4. After washing, bacteria were incubated with rabbit polyclonal anti-PF4 antibody (1:250) for 30 min at 22 °C, followed by Alexa Fluor 488-conjugated secondary antibody, fixed and observed using confocal system. (C) Control experiment showing the lack of the secondary antibody binding to the rPF4-treated noncapsulated bacteria in the absence of the primary antibody. The scale bars are 10 µm.



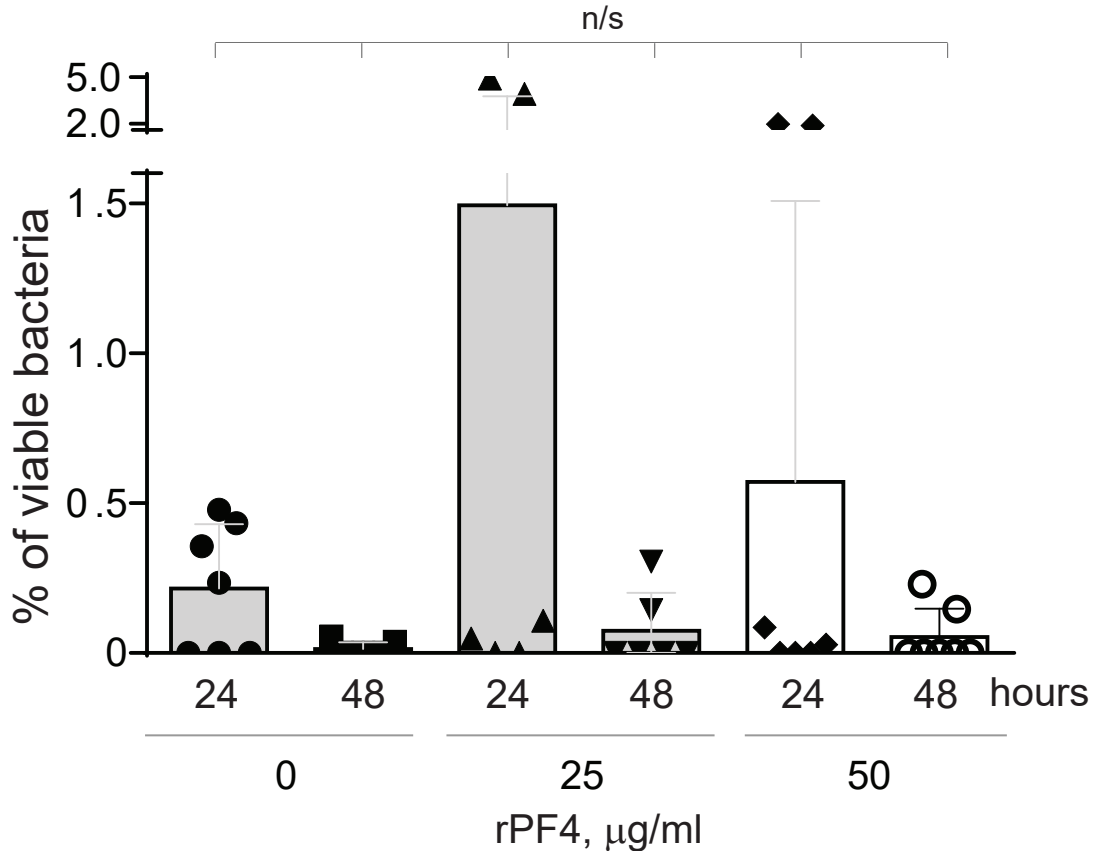
Supplemental Figure 2. Effect of rPF4 on bacterial growth. For the solution-phase microbicidal assays, *S. aureus* at 10⁶ CFU/ml were inoculated into MES buffer (pH 5.5) and incubated with different concentrations of rPF4 for 1 h at 37°C. Aliquots (100 μl) of diluted suspensions (1:500) were cultured on LB agar plates. Colonies were enumerated after incubation for 24 h at 37 °C. Data are expressed as CFU/ml and are means ± S.D. from three individual experiments. ns, no significant difference.



B

Antibiotic or PF4	Range of antibiotics, $\mu\text{g/ml}$	MIC, $\mu\text{g/ml}$
Ampicillin	5-5000	>5000
Ceftriaxone	0.02-20	< 0.02
Penicillin/Streptomycin	2-2500	< 2
Kanamycin	5-5000	< 5
Tetracycline	6-6000	23
PF4	0.5-500	>500

Supplemental Figure 3. Minimal Inhibitory concentration (MIC) of rPF4. (A) Aliquots of *S. aureus* (10^3 CFU) in LB were added to the wells containing serial dilutions (1:2) of rPF4 (0.4-500 $\mu\text{g/ml}$) and selected antibiotics. The visible growth of bacteria was evaluated after overnight incubation. Control, wells with no added inhibitors. **(B)** The concentrations of antibiotics or PF4 used in the MIC assay.



Supplemental Figure 4. Effect of rPF4 on intracellular bacterial killing. Adherent IC-21 macrophages (5×10^5) were incubated with 10^6 CFU of *S. aureus* without or with two concentrations of rPF4 (25 and 50 $\mu\text{g/ml}$) for 1 h at 37 °C. Cells were washed, and extracellular bacteria were inactivated by treatment with gentamicin for 1 h, after which macrophages were cultured in DMEM for 24 and 48 hours and then lysed. Aliquots of cell lysates were plated on LB agar plates for 16 h at 37 °C, and CFUs determined. The number of viable bacteria was expressed as a percent of viable bacteria present in macrophages 1 h after gentamicin treatment. Data are means \pm S.D. from six experiments.