Table S1

Strains and Plasmids		
Strain Designation	Description	Reference or Source
1457	S. epidermidis clinical isolate	P. Fey, (1)
7291	S. epidermidis clinical isolate	P. Fey
RP62A	S. epidermidis clinical isolate	ATCC 35984
1457-FL	1457 (pHL007); fluorescent reporter strain	This study
1457 ∆ <i>phnD</i>	1457 phnD::dhfr, phnD deletion strain	This study
RN4220	S. aureus; restriction-deficient cloning intermediate	P. Fey, (2)
Lowenstein	S. aureus clinical isolate; capsular type 5	ATCC 49521
MRSA252	S. aureus clinical isolate; methicillin resistant	ATCC BAA-1720
SM131	S. haemolyticus clinical isolate	ATCC 29970
R22	S. hominis clinical isolate	ATCC 700236
E. coli TOP10	cloning strain	Invitrogen
<i>E. coli</i> BL21(DE3)	protein expression strain; lambda lysogen encoding T7 RNA polymerase under control of the lacUV5 promoter	Invitrogen
Plasmid	Description	Reference or Source
pUC19	general cloning vector; AmpR	New England Biolabs
pE194	staphylococcal plasmid conferring ErmR	P. Fey, (3)
pROJ6448	pE194 with pC221 nick site for mobilization, temperature-sensitive replicon; ErmR	P. Fey, (4)
pHL007	PftsA-RFP (codon-optimized red fluorescent protein behind the <i>ftsA</i> promoter) ligated into multiple cloning site of pUC19, then combined with pE194 via <i>Pst</i> l	This study
pHL008	pROJ6448-derivative; allele exchange vector with <i>phnD::dhfr</i>	This study



Fig. S1

FIGURE LEGENDS FOR SUPPLEMENTAL MATERIAL

Figure S1. PhnD antibodies inhibit *S. epidermidis* biofilm formation in the standard microtiter plate assay. Biofilms of *S. epidermidis* strains 1457 and RP62A were seeded at an OD_{600nm} of 0.05 and grown in TSB at 37°C. Cultures were incubated in triplicate with or without antibodies at 100 µg/mL. After 8 h of static growth, biofilms were washed and stained with crystal violet. (A, B) Pictures of stained wells for each treatment. (C, D) Quantification of staining in panels A and B by absorbance spectroscopy, normalized to the 'no antibody' control as 1.0.

Movie 1. PhnD antibodies inhibit *S. epidermidis* 1457 biofilm formation. Time lapse video microscopy of biofilms grown at 37° C in the presence of control (rabbit anti-rat IgG, left) or PhnD (right) antibodies at 200 µg/mL. Images were acquired at 20 min intervals for 10 h. Scale bar: 30 µm.

Movie 2. PhnD antibodies inhibit *S. epidermidis* 1457-FL biofilm formation when administered in prophylactic mode. Time lapse video microscopy of biofilms grown at 30°C in the presence of control (rabbit anti-rat IgG, left) or PhnD (right) antibodies at 100 µg/mL. Images were acquired at 20 min intervals for 18 h. Scale bar: 30 µm.

Movie 3. PhnD antibodies inhibit *S. epidermidis* 1457-FL biofilm formation when administered in early therapeutic mode. Time lapse video microscopy of biofilms grown at 30^oC in the presence control (rabbit anti-rat IgG, left) or PhnD (right) antibodies at 100 μg/mL. Images were acquired at 20 min intervals for 17 h. Scale bar: 30 μm.

Movie 4. Deletion of *phnD* results in defective biofilm formation. Time lapse video microscopy of *S. epidermidis* 1457 (left) and 1457 $\Delta phnD$ (right) strains grown at 30°C. Images were acquired at 20 min intervals for 18 h. Scale bar: 30 µm.

Movie 5. PhnD antibodies enhance the opsonophagocytosis of *S. epidermidis* biofilms. Neutrophils exhibit increased binding and active engulfment of biofilms in the presence of 5 µg/mL PhnD (right) versus control antibody (rabbit anti-rat IgG, left). Images were acquired at 2 min intervals for 4.25 h. Scale bar: 15 µm.

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Movie 6. Single neutrophil tracking of biofilm opsonophagocytosis. A single neutrophil is capable of moving toward and engulfing large numbers of biofilm bacteria. Images were acquired at 2 min intervals for 108 min. Scale bar: 5 µm.

Movie 7. PhnD antibodies inhibit *S. epidermidis* 1457-FL biofilms grown in human

plasma. Longitudinal development of biofilms grown at 37°C in the presence of rabbit

anti-rat IgG control (left) or PhnD antibodies (right) at 200 µg/mL. Antibodies were

administered in prophylactic mode at 200 µg/mL. Images were acquired at 20 min

intervals for 12 h. Scale bar: 30 µm

REFERENCES FOR SUPPLEMENTAL MATERIAL

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