

1 **Supporting Information**

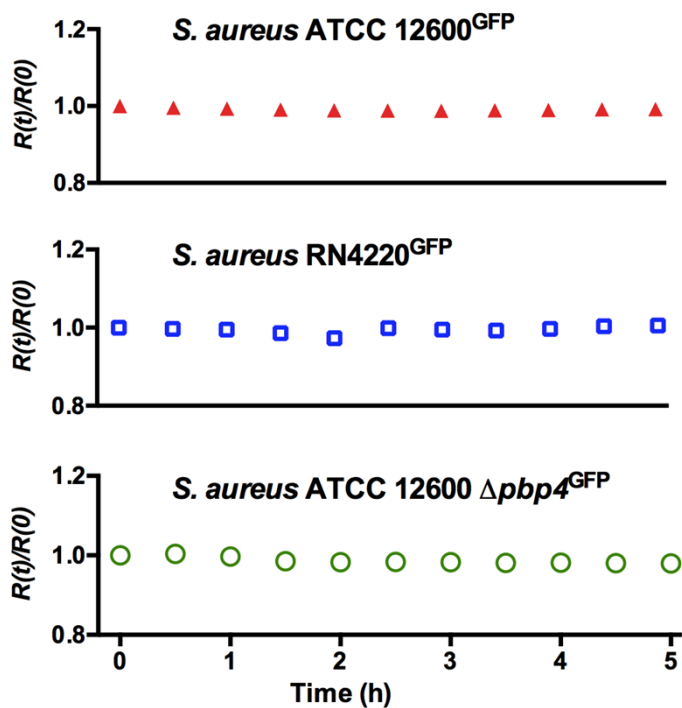
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3 **Residence-time dependent cell wall deformation of different**
4 ***Staphylococcus aureus* strains on gold measured using surface-**
5 **enhanced-fluorescence**

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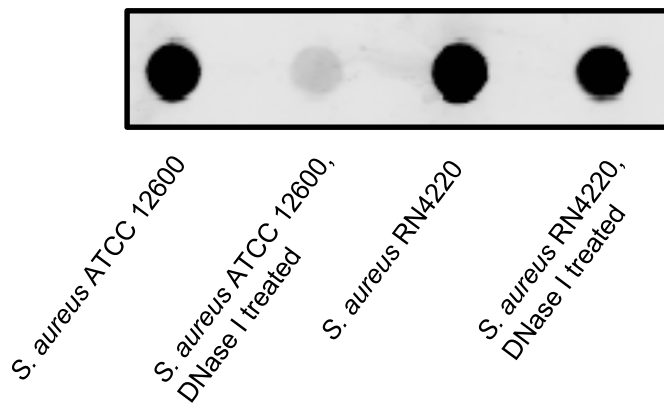
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12 **Fig. S1** Fluorescence radiance, $R(t)$, normalized against the radiance at $t=0$, $R(0)$, arising from green-fluorescence staphylococci
 13 adhering to a glass surface as a function of time for three different strains involved in this study. Fluorescence is constant over a
 14 time period of at least 5 h, demonstrating the absence of significant photo-bleaching upon repetitive excitation.

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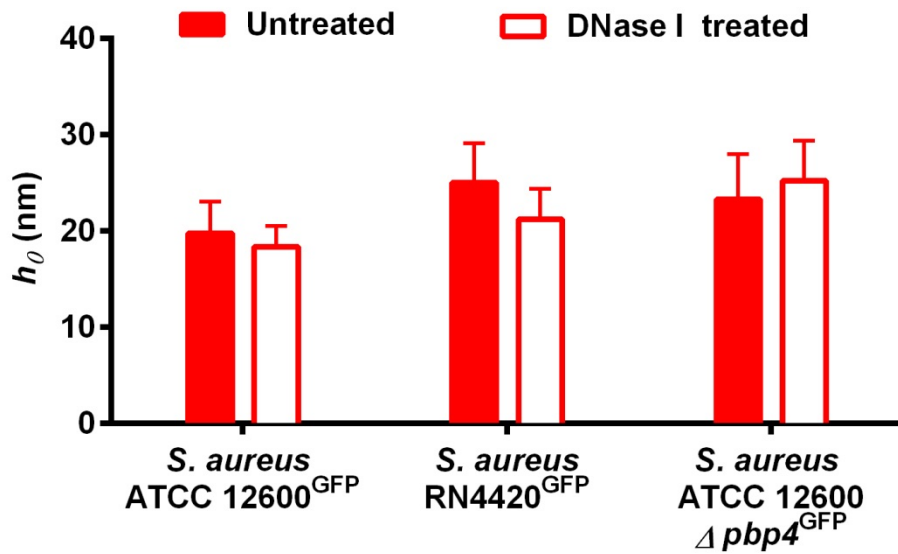


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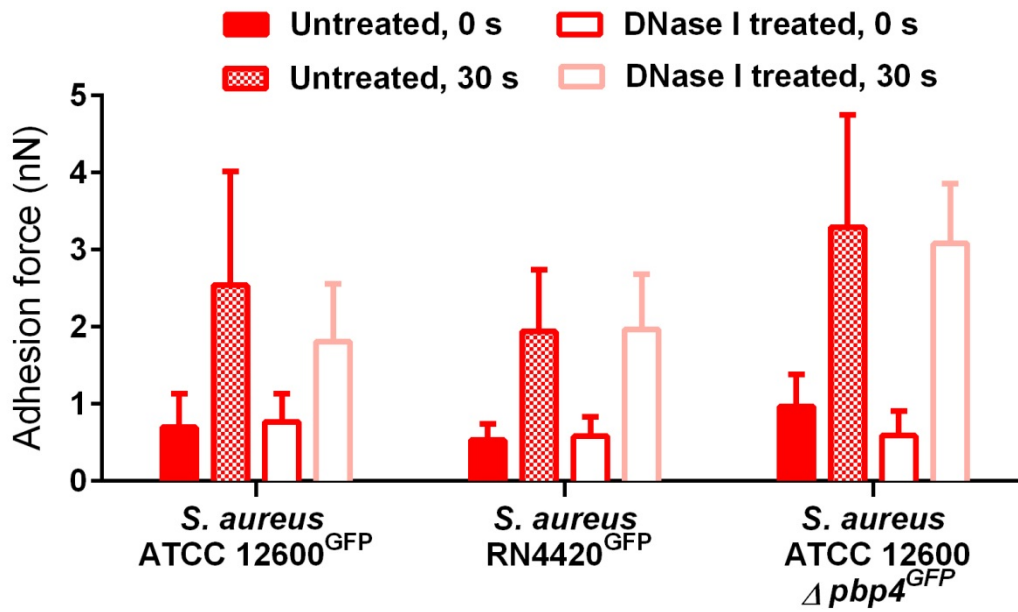
21 **Fig. S2** Stainable EPS expression in planktonic cultures of *S. aureus* ATCC12600 and *S. aureus* RN4220. Staphylococci, grown
22 and harvested as described in the main text, were suspended in 10 ml PBS to an optical density at 578 nm of 1. Subsequently, 1.5
23 mL suspension was pelleted at 5000 g for 5 min at 10°C, after which EPS was extracted by re-suspending the pellet in 50 µL of
24 0.5 M EDTA (pH 8.0) for 5 min at 100°C. Concentrated EPS was incubated at 37°C with 10 µl of 20 µg/ml proteinase K for 30
25 min and diluted 1:100 in water and 40 µL was blotted on a nitrocellulose membrane. The membrane was then blocked using 1%
26 bovine serum albumin-Tris buffered saline (20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 0.05% Tween20) for 1 h under mild shaking
27 at room temperature. The membrane was subsequently incubated with a 1: 10,000 dilution of Wheat Germ Agglutinin (Sigma-
28 Aldrich, St. Louis, USA) for 1.5 h under mild shaking at room temperature. Wheat Germ Agglutinin is a biotin labelled antibody
29 specific for poly-n-acetylglucosamine, a major constituent of staphylococcal EPS. Finally, Streptavidin IRDye (LI-COR
30 Biosciences, Lincoln, USA) was added in 1: 10,000 dilution for 30 min under similar conditions and the membrane was washed 3
31 times, for 10 min each, with Tween20-Tris buffered saline. The membrane was imaged using an Odyssey Infrared Imaging System
32 (LI-COR Biosciences, Lincoln, USA), yielding dark spots on the blot indicative of the amount of PNAG.

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35 **Fig. S3** Height of the contact cylinder of untreated and DNase I treated *S. aureus* adhering on gold-coated quartz surfaces, as
 36 derived from AFM adhesion force measurements under different loading forces. Staphylococci were grown, harvested and DNase
 37 I treated as described in the main text. Bacterial probes were prepared by immobilizing a bacterium to an α -poly-L-lysine coated
 38 tipless cantilever (Bruker, Camarillo, CA). Deformation was measured at different loading forces as exerted by the AFM
 39 cantilever up to 1 nN and applied in a recently published¹⁵ elastic deformation model, that self-defines the dimensions of an
 40 assumed cylindrical, contact volume between adhering bacteria and substratum surfaces based on the relation between deformation
 41 and the loading force applied. Error bars represent standard deviations of 90 force-distance measurements on 30 randomly chosen
 42 spots, equally divided over the surfaces of three different bacteria.



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44 **Fig. S4** Residence-time dependent adhesion forces for untreated and DNase I-treated *S. aureus* adhering on gold-coated quartz
 45 surfaces from AFM force measurements. Staphylococci were grown, harvested and DNase I treated as described in the main text
 46 and immobilized to an α -poly-L-lysine coated tipless cantilever (Bruker, Camarillo, CA) for residence-time dependent AFM
 47 adhesion force measurements in 10 mM potassium phosphate buffer (pH 7.0) at room temperature using a BioScope Catalyst
 48 AFM (Bruker) under a loading force of 1 nN. For each bacterial probe, force-distance curves were measured upon initial contact (0
 49 s) and after 30 s bond-maturation. Error bars represent standard deviations of 90 force-distance measurements on 30 randomly
 50 chosen spots, equally divided over the surfaces of three different bacteria.

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