Supporting information: Development and validation of a high-throughput whole cell assay to investigate *Staphylococcus aureus* adhesion to host ligands

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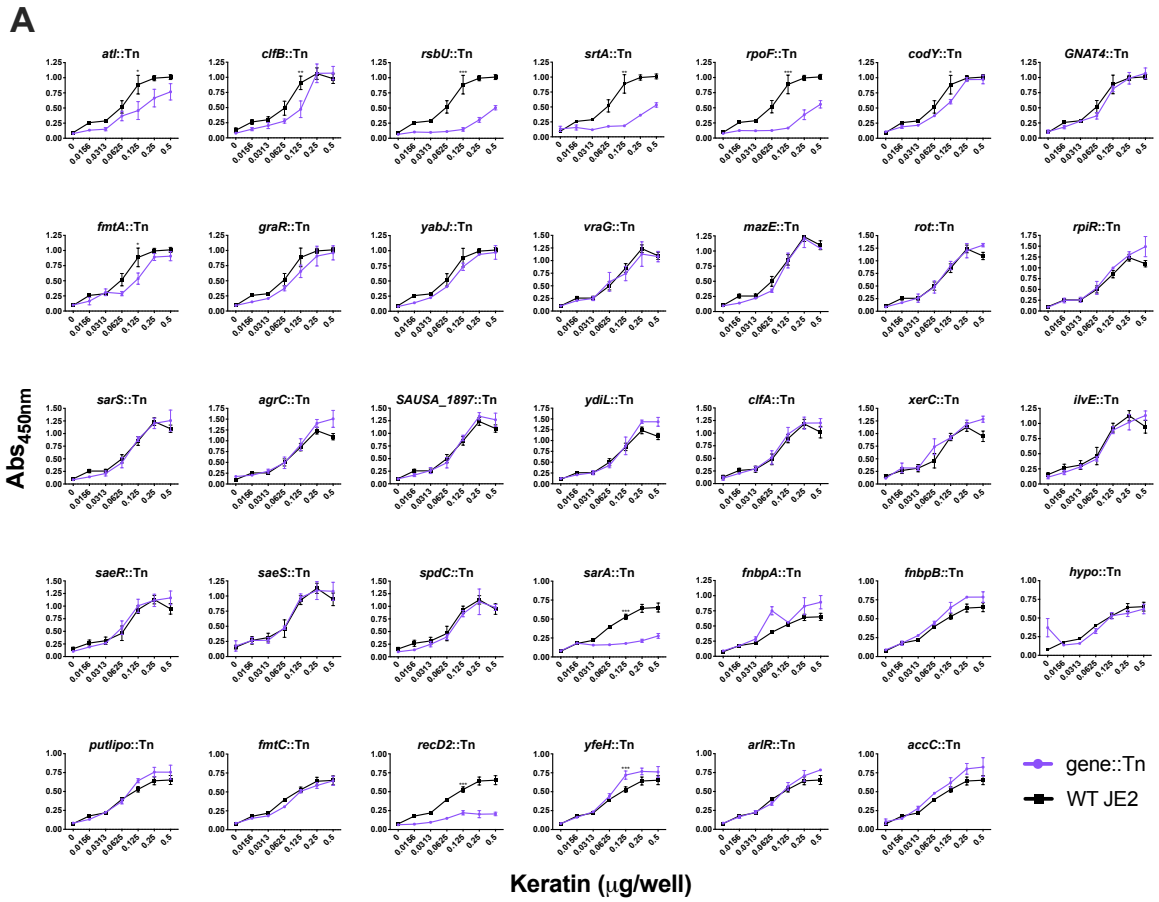
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accC::Tn
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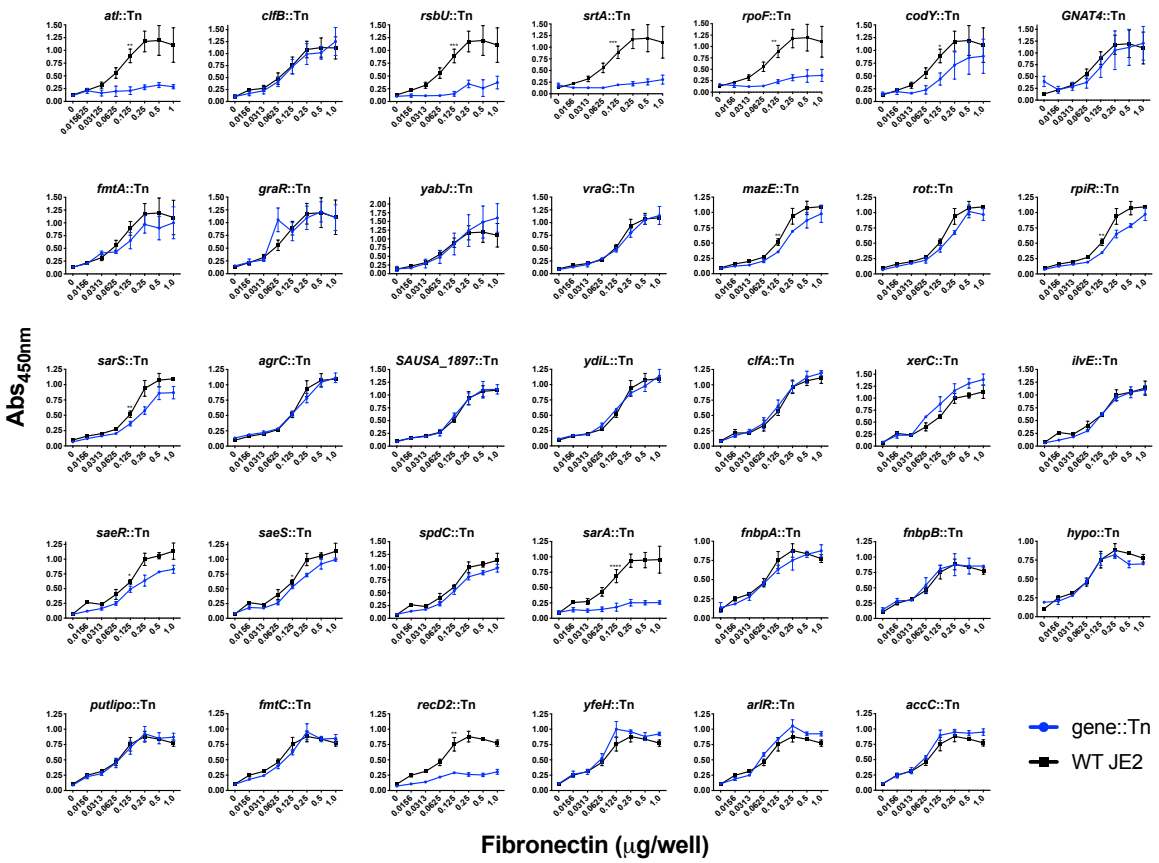
WT JE2
Abs450nm
Keratin (μg/well)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WT JE2
Abs450nm
Fibronectin (μg/well)

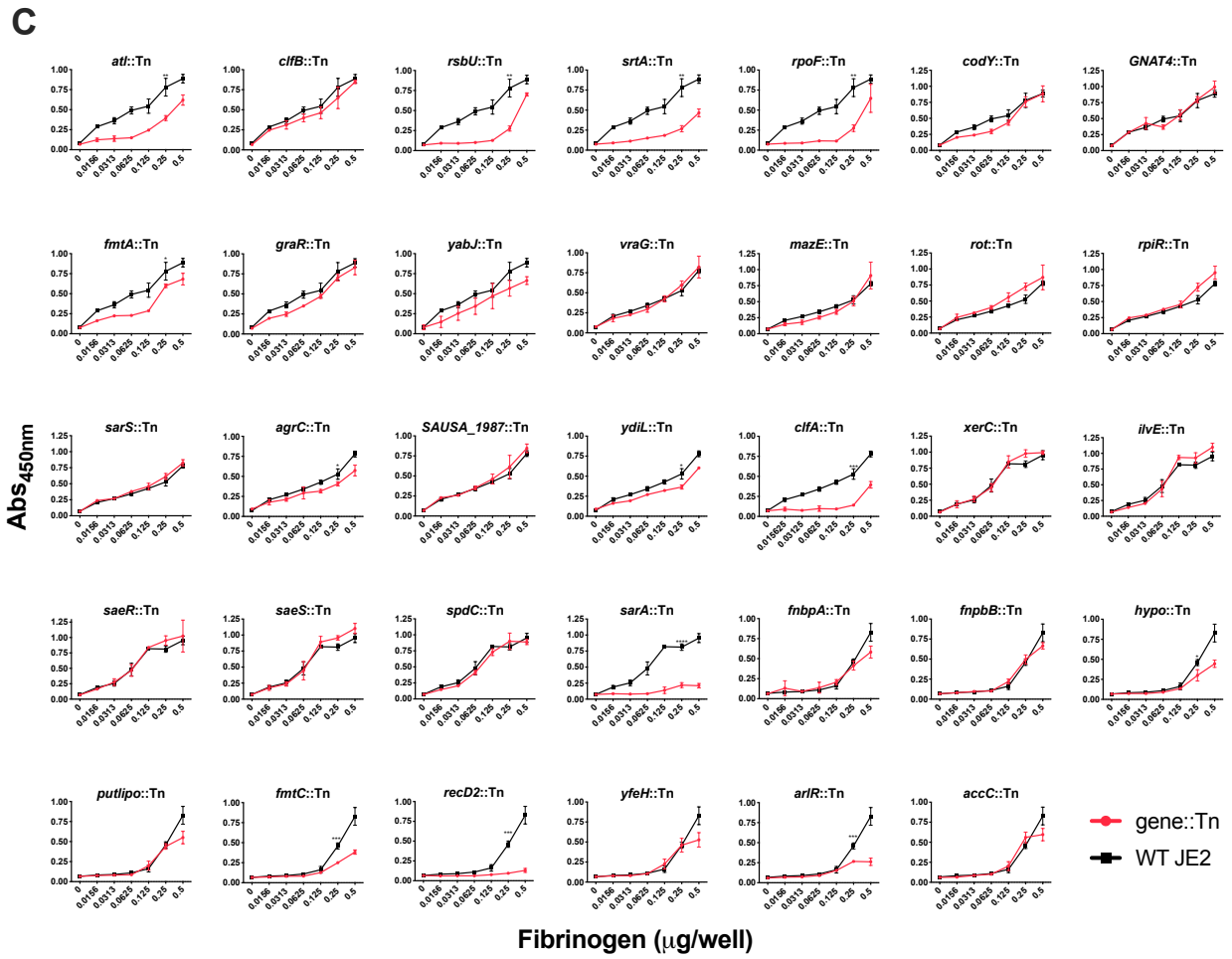

Figure S1. Construction of a *S. aureus* Genetic Adhesion Network: genes associated with *S. aureus* adhesion to a minimum of one of three host ligands (A. Keratin, B. Fibronectin, and C. Fibrinogen). Adhesion attenuated mutants identified in Figure 4 were compiled into a sub-library and profiled for adhesion to all three host ligands. For mutants that showed confirmed adhesion-related phenotypes, the transposons were transduced using phage 80α into a fresh JE2 background, to eliminate the possibility of secondary-site mutations. These newly generated mutants (n=34 shown above) were profiled to confirm reduced adhesion. Each ligand was titrated (A-C), and the mutant strains (shown in each individual graph) were propagated in microtiter plates to the appropriate OD_{600nm}, with three biological replicates. The ELISA was performed to assess adhesion (Abs_{450nm}) and the values shown are the average of three biological replicates ± the standard deviation. A final concentration was selected for each ligand (keratin 0.125 ug/well; fibronectin 0.125 ug/well; fibrinogen 0.25 ug/well) and was used to calculate the average percentage of adhesion relative to wild-type JE2 propagated in the same microtiter plate (see Table 1). *P*-values were calculated using the two-tailed unpaired Student's *t*-test; statistically significant ($P \leq 0.05$*; $P \leq 0.01$**; $P \leq 0.001$***; $P \leq 0.0001$****) decreases in adhesion, compared to wild-type JE2, were used to create the Genetic Adhesion Network (Figure 5).

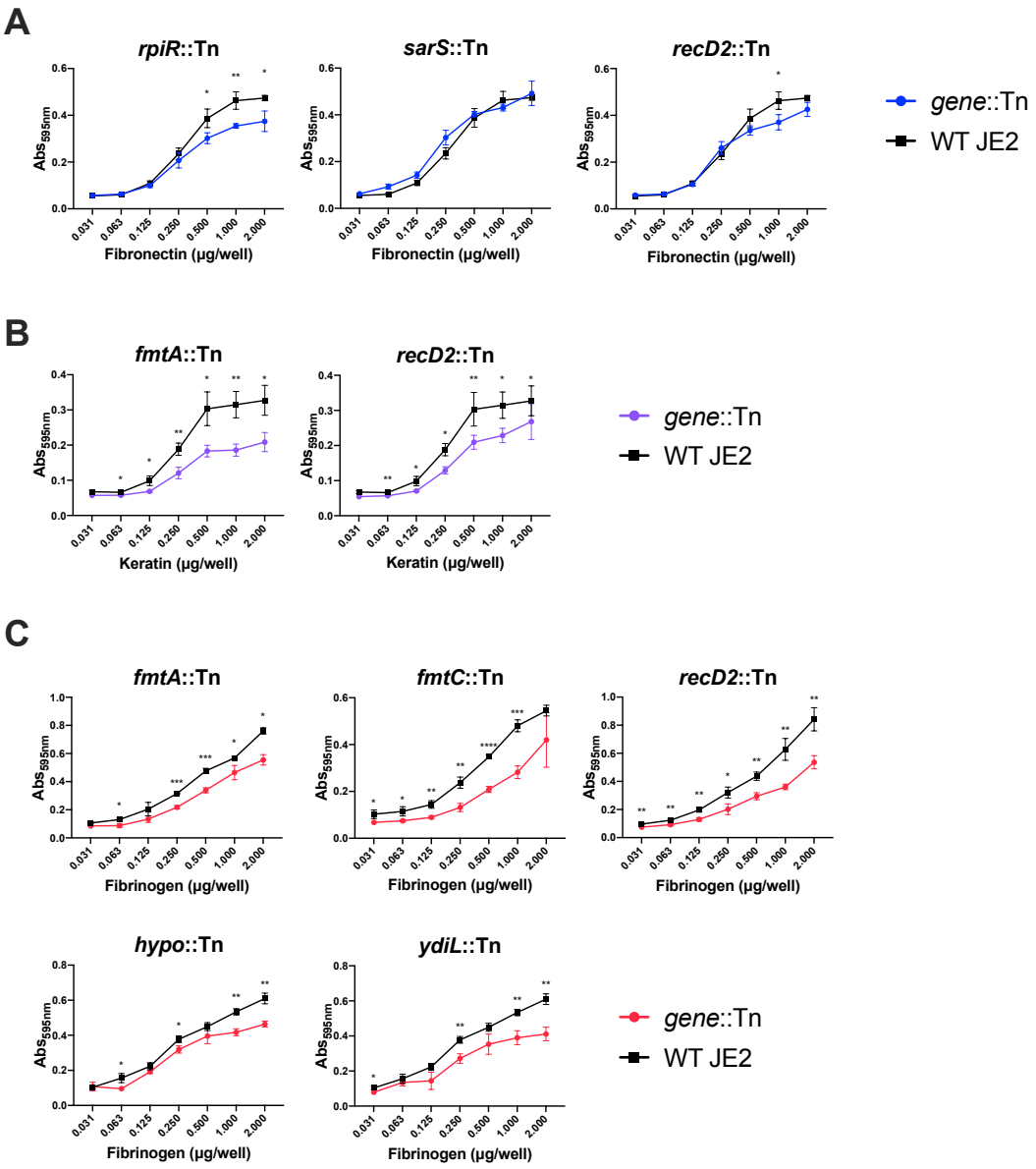

Figure S2. Crystal violet detection of poorly characterized adhesion-defective *S. aureus* mutants (A. Fibronectin, B. Keratin, and C. Fibrinogen). To confirm the adhesion defects were not due to decreased affinity of the ELISA antibodies, we profiled adhesion of the poorly characterized *S. aureus* mutants to their respective ligand(s) using crystal violet as an alternative detection method. Each ligand was titrated (A-C), the strains (shown in each individual graph) were propagated in 10 mL TSB to the appropriate OD_{600nm}, with three biological replicates. Crystal violet was used to assess adhesion (Abs_{595nm}) and the values shown are the average of three biological replicates ± the standard deviation. *P*-values were calculated using the two-tailed unpaired Student's *t*-test. Statistically significant decreases, compared to wild-type JE2, were denoted as $P \leq 0.05$*; $P \leq 0.01$**; $P \leq 0.001$***; $P \leq 0.0001$****.