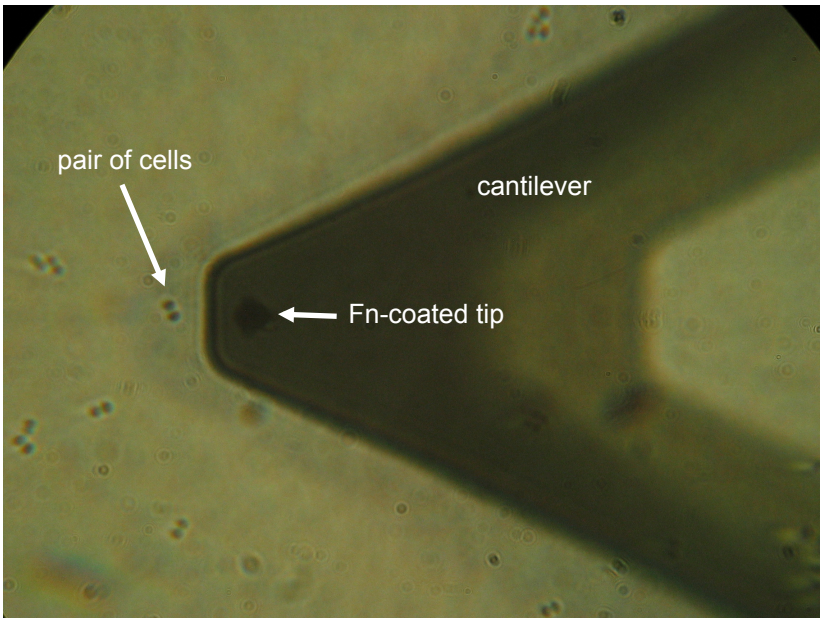
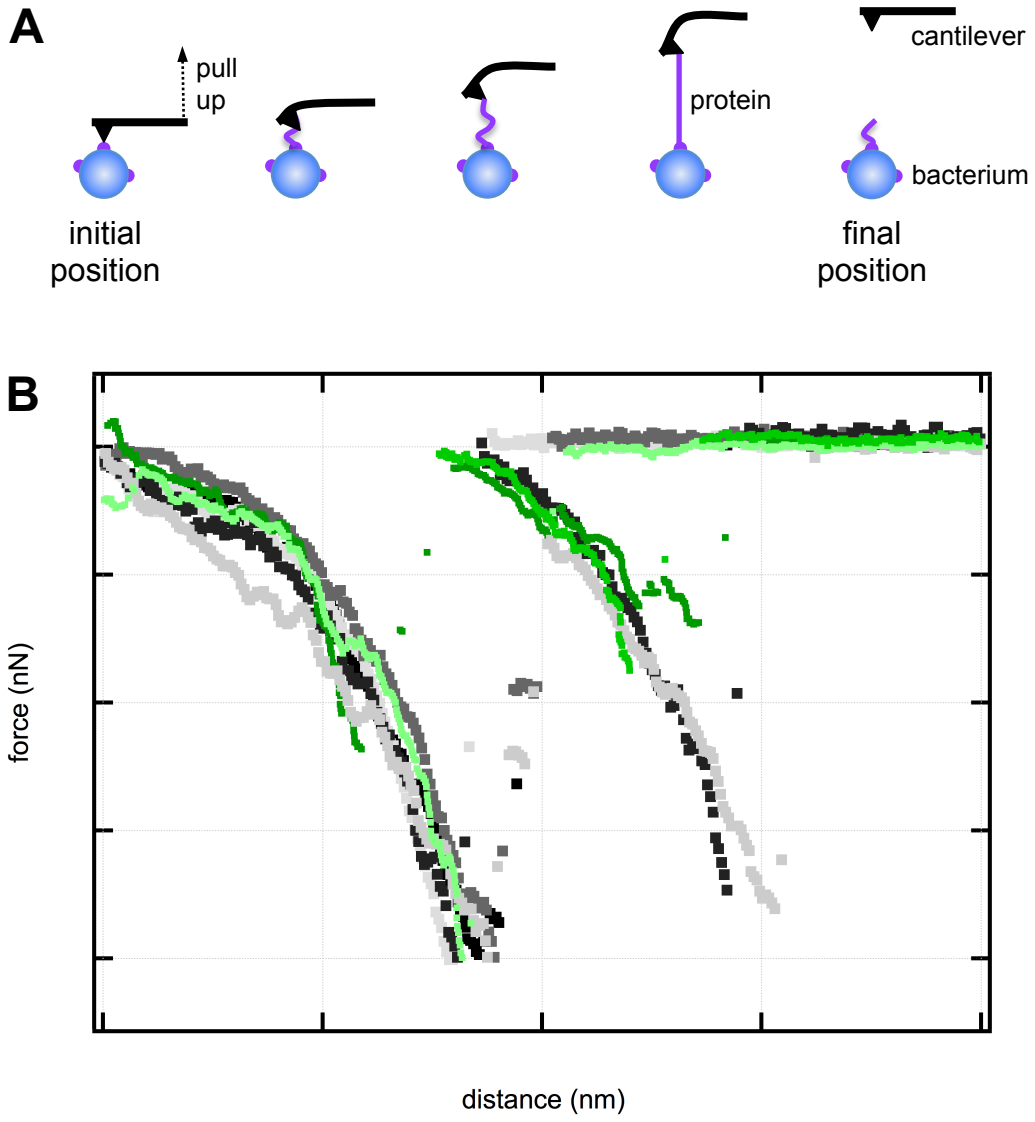
Supplemental Table 1. Sequences of template and sequencing primers for *fnbA*.

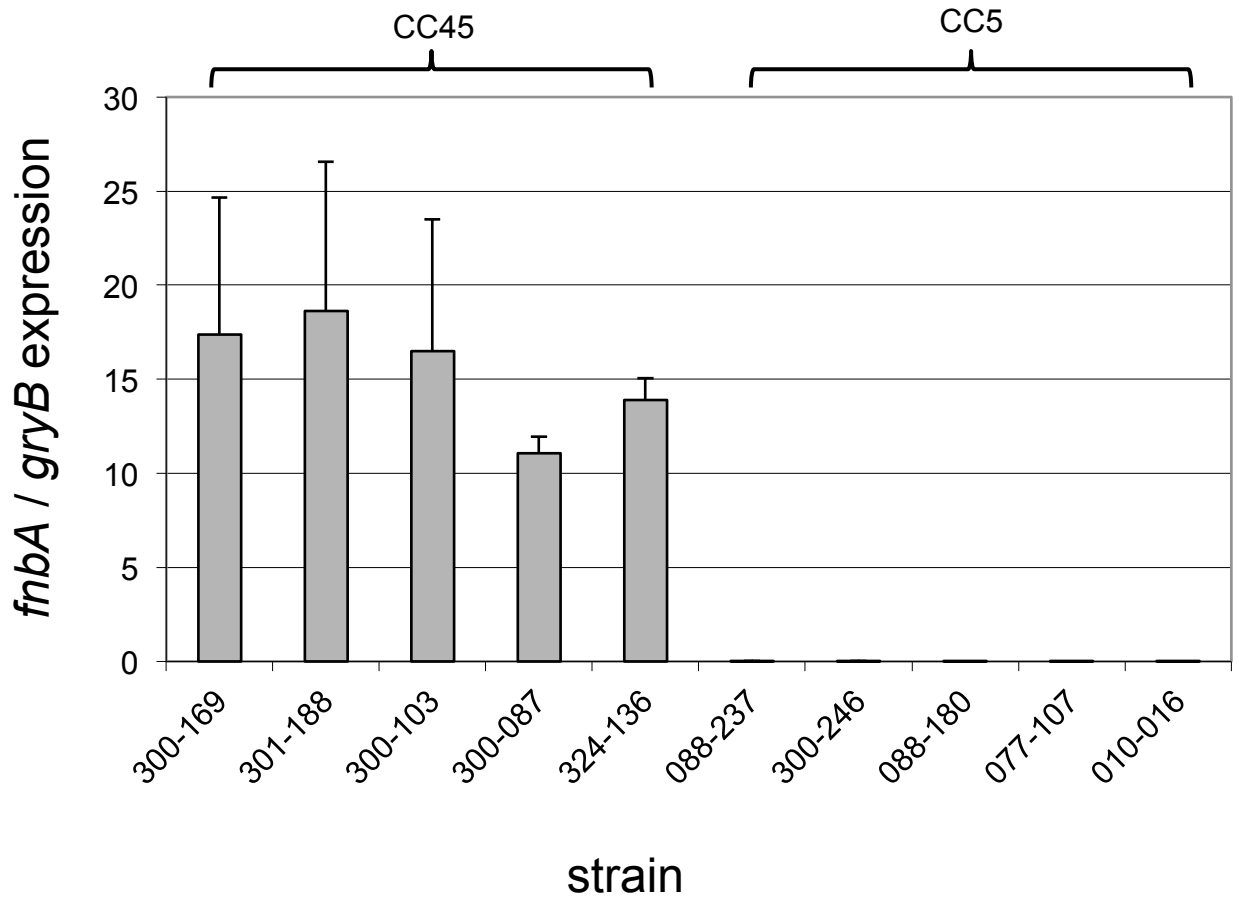
Primer direction and identifier	Sequence
Forward	
fnbA-F	5'-CCGAACAATATAGACTTGCATTTATTAAG-3'
fnbA-F1	5'-GAACACAAATGGTAGGACATCCAGAGCAAC-3'
fnbA-F2	5'-CAATAGAAGAAACGGATTCATCAGCTATTG-3'
fnbA-F3	5'-CTGGTTTAGGAACTGAAAATGGTCACG-3'
fnbA-F4	5'-CAGTCATTCGAGGAAGATACAGAAAAAGAC-3'
Reverse	
fnbA-R	5'-CATCAGACATAAACCAATGAAGCAATCAG-3'
fnbA-R1	5'-CTTCATATTCAACAACATCAGCGTGATG-3'
fnbA-R2	5'-CCCGTGACCATTTTCAGTTCCTAAACC-3'
fnbA-R3	5'-GTTGTATCTTCAATCGTTTGTTGACCTTC-3'
fnbA-R4	5'-CTTTACCTTGTTCCACTGGTTTAGAAGG-3'



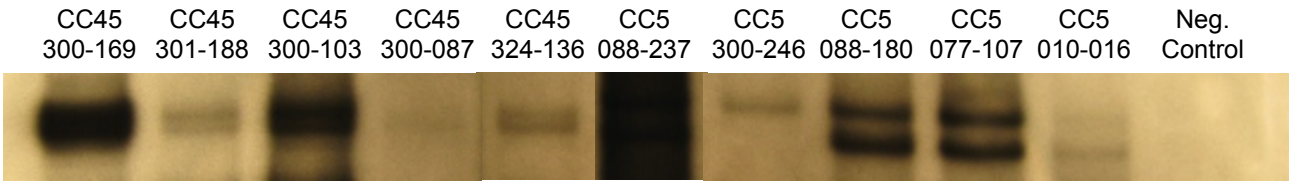
Supplemental Figure 1. Optical microscope image of AFM experiment. Binary fission pair of *S. aureus* cells about to be probed with a fibronectin-coated tip, which is integrated into the end of the force-sensing cantilever. The cells are supported on a glass slide. The entire system is immersed in buffer.



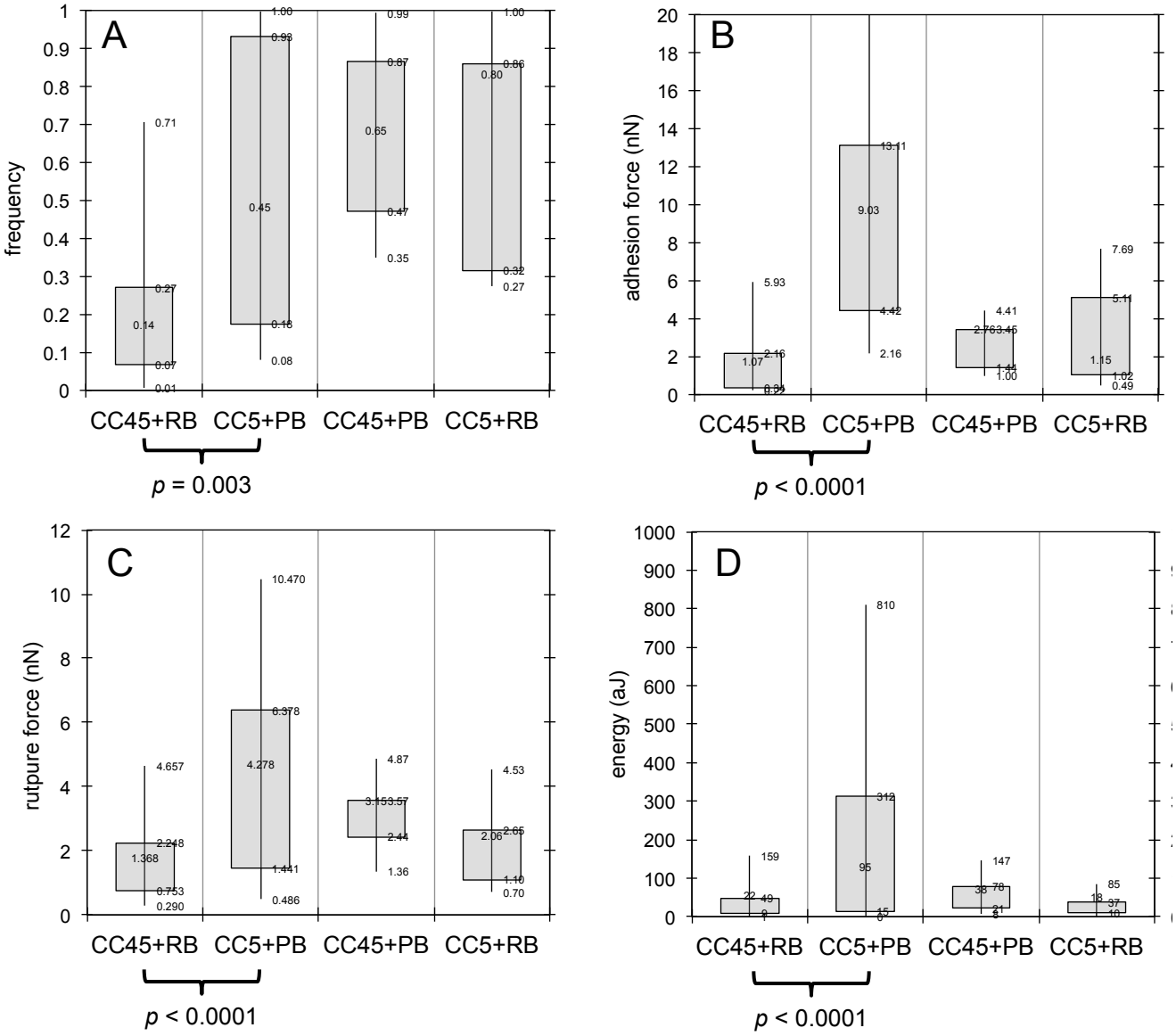
Supplemental Figure 2. Control experiment using AFM on *Lactococcus lactis*. (**A**) Schematic diagram (not to scale) showing a fibronectin-coated tip that was used to probe *L. lactis* strains engineered to express either *fnbA* or *fnbB* genes. Fibronectin on the tip was mechanically separated from FnBPs on a bacterium by pulling the cantilever away from the cell. Unraveling of a protein-protein complex resulted in a "sawtooth shaped" force spectrum. (**B**) Black- and gray-colored curves were obtained on *L. lactis* expressing FnBPA or FnBPB on their outer cell wall. Green-colored curves are some of the spectra obtained on the CC5 and CC45 clinical isolates of *S. aureus* as described in the manuscript. These spectra are not offset vertically (as is the case for Figure 1 in the manuscript) to highlight the overlapping similarities in the curves for *L. lactis* and *S. aureus*. Native *L. lactis* is an ideal control specimen because it is Gram-positive like *S. aureus*, but it does not naturally bind to fibronectin and lacks *fnbA* and *fnbB* genes.



Supplemental Figure 3. qRT-PCR of *fnbA* at 3 hour post-inculation. The effect of clonal variation on *fnbA* transcription by qRT-PCR, normalized by *gryB*, at 3 h post-inoculation.



Supplemental Figure 4. Western blots of fibronectin binding proteins. Western blot showing protein bands for FnBPs in the ten *S. aureus* isolates arranged from left to right in the same order as the RT-PCR data. A negative control was also performed with *S. aureus* DU5883, a double mutant that cannot express *fnbA* and *fnbB*.



Supplemental Figure 5. **AFM binding metrics for a fibronectin-coated probe on *S. aureus* grouped by both clonotype and persistence of infection**. Box and whisker plots of (**A**) frequency of binding, (**B**) adhesion force, (**C**) rupture force, and (**D**) energy (or work) of binding for a fibronectin-coated probe on *S. aureus* grouped as CC45+RB versus CC5+PB . CC corresponds to the clonal complex, RB indicates resolving bacteremia and PB is persistent bacteremia. Force is in nanoNewtons (10^{-9} N) and energy/work is in attoJoules (10^{-18} J). Box ends represent the first and third quartiles. Whisker ends represent the 9th and 91st percentile.