



**Figure S1. A.** Biofilm formation measured at OD<sub>550</sub> for WT,  $\Delta pilY1$ ,  $\Delta pilA$ ,  $\Delta pilA \Delta pilY1$ , the vWA variants, and the Cys152S mutant in a static 96 well biofilm assay performed in M8 medium salts plus supplements incubated at 37 °C for 24 h. The  $\Delta pilA$  strain shows a less severe phenotype than the  $\Delta pilY1$  strain indicating that PilY1 has functions independent of its impact on T4P biogenesis, as previously described [3]. **B-C.** Partial functionality of vWA variants and phenotypic analysis of other cysteine vWA mutants. **B.** Plaquing assay with phage DMS3<sub>vir</sub> versus the WT and the indicated mutants as hosts. Zones of clearing shown for WT and the strain expressing the vWA-Cys152S mutant protein are similar, which indicates a similar degree of TFP function. The  $\Delta pilY1$  mutant serves as the negative control. **C.** Representative images of twitch zones stained with crystal violet shown for WT, the  $\Delta pilY1$  or strains expressing PilY1 variants with point mutations in the Cys residues in the vWA domain following incubation at 37 °C for 24 h plus one additional day at room temperature. Twitching serves as a measure of TFP function. **D.** Biofilm level measured at OD<sub>550</sub> for WT and the mutants shown in panel B using the 96 well static biofilm assay after 24 hrs at 37 °C, as described in the Materials and Methods.