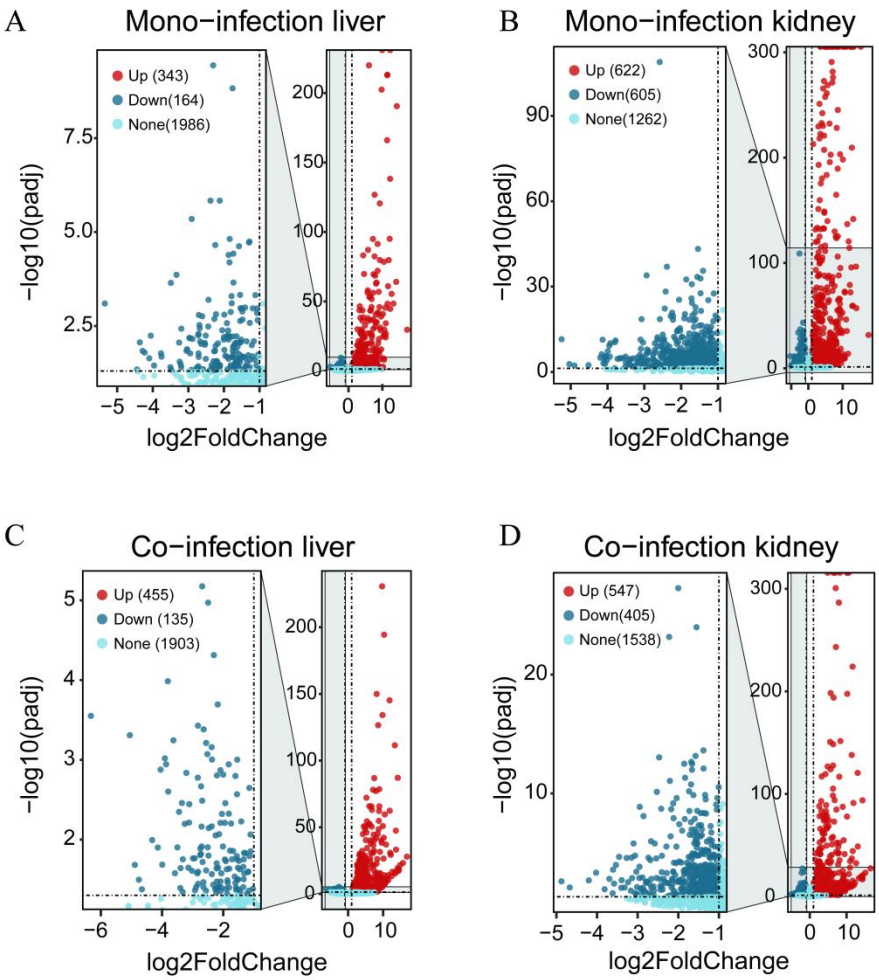

Figure S1. Tn-seq mutant abundance changes during mono-infection and co-infection.

(A-D) Volcano plots show changes in mutant abundance in the liver (A) and kidney (B) during mono-infection and in the liver (C) and kidney (D) during co-infection with *P. aeruginosa*. The x-axis indicates $\log_2$(fold change) relative to the input library, and the y-axis indicates −log10(adjusted *P*). Genes whose corresponding mutants showed increased abundance relative to the input were classified as Up, whereas genes whose corresponding mutants showed decreased abundance were classified as Down; all remaining genes were classified as None.

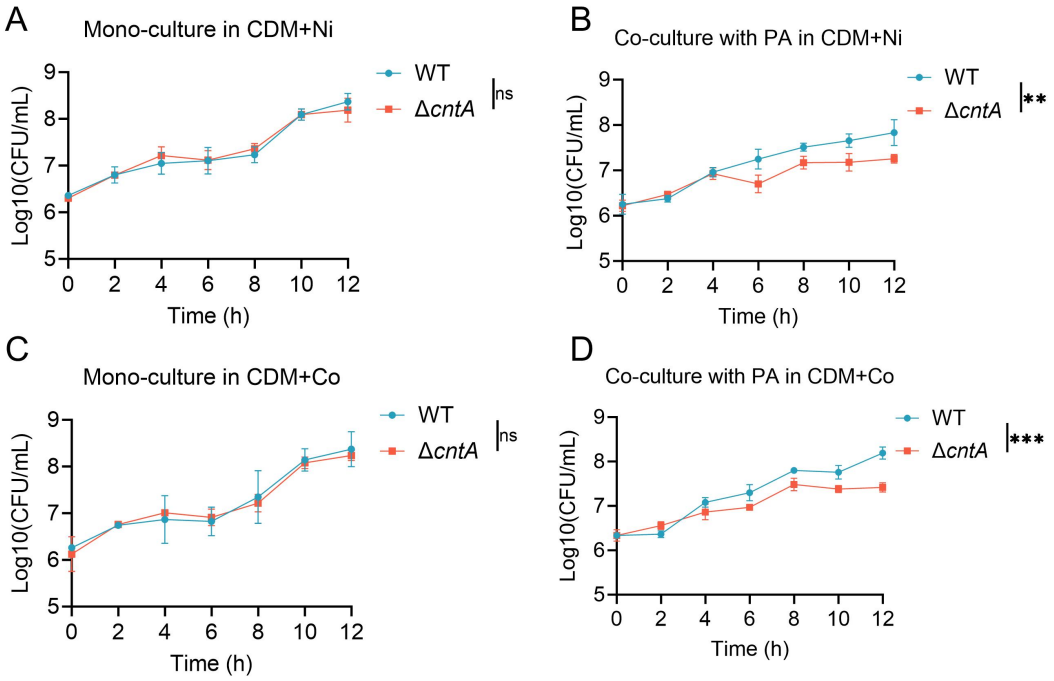

Figure S2. Growth of *S. aureus* WT and Δ*cntA* during mono- and co-culture with *P. aeruginosa*.

(A) Growth of *S. aureus* WT and Δ*cntA* under mono-culture conditions in CDM supplemented with 1 μM $NiCl_2$ (CDM+Ni). (B) Growth of *S. aureus* WT and Δ*cntA* during co-culture with *P. aeruginosa* in CDM+Ni. (C) Growth of *S. aureus* WT and Δ*cntA* under mono-culture conditions in CDM supplemented with 1 μM $CoCl_2$ (CDM+Co). (D) Growth of *S. aureus* WT and Δ*cntA* during co-culture with *P. aeruginosa* in CDM+Co. Bacterial abundance was determined by CFU enumeration. WT and Δ*cntA* showed comparable growth during mono-culture, whereas Δ*cntA* showed reduced growth relative to WT during co-culture with *P. aeruginosa*. Data are presented as mean ± SD from three independent experiments. Growth curves were analyzed using two-way ANOVA followed by Tukey's multiple-comparisons test. ns, not significant; $^{**}P < 0.01$, $^{***}P < 0.001$. PA, *P. aeruginosa* WT.

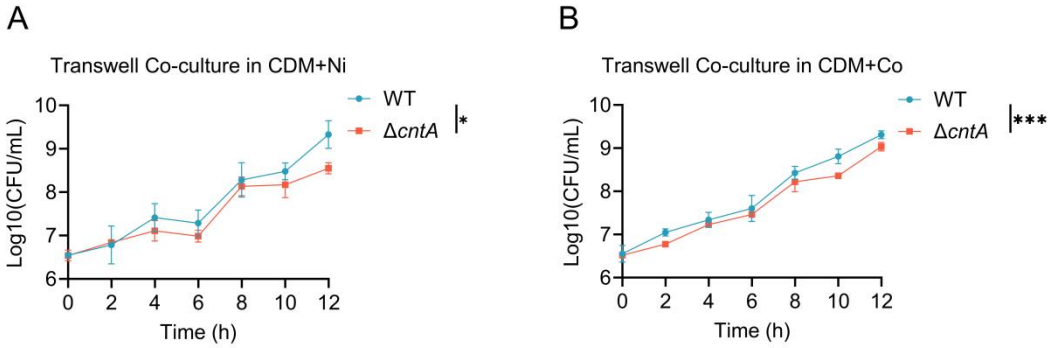

Figure S3. Growth of *S. aureus* WT and Δ*cntA* during Transwell co-culture with *P. aeruginosa*.

(A) Growth of *S. aureus* WT and Δ*cntA* during Transwell co-culture with *P. aeruginosa* in CDM+Ni. (B) Growth of *S. aureus* WT and Δ*cntA* during Transwell co-culture with *P. aeruginosa* in CDM+Co. Bacterial abundance was determined by CFU enumeration. Reduced growth of Δ*cntA* relative to WT was observed during Transwell co-culture with *P. aeruginosa*. Data are presented as mean ± SD from three independent experiments. Growth curves were analyzed using two-way ANOVA followed by Tukey's multiple-comparisons test. *$P < 0.05$, ***$P < 0.001$.

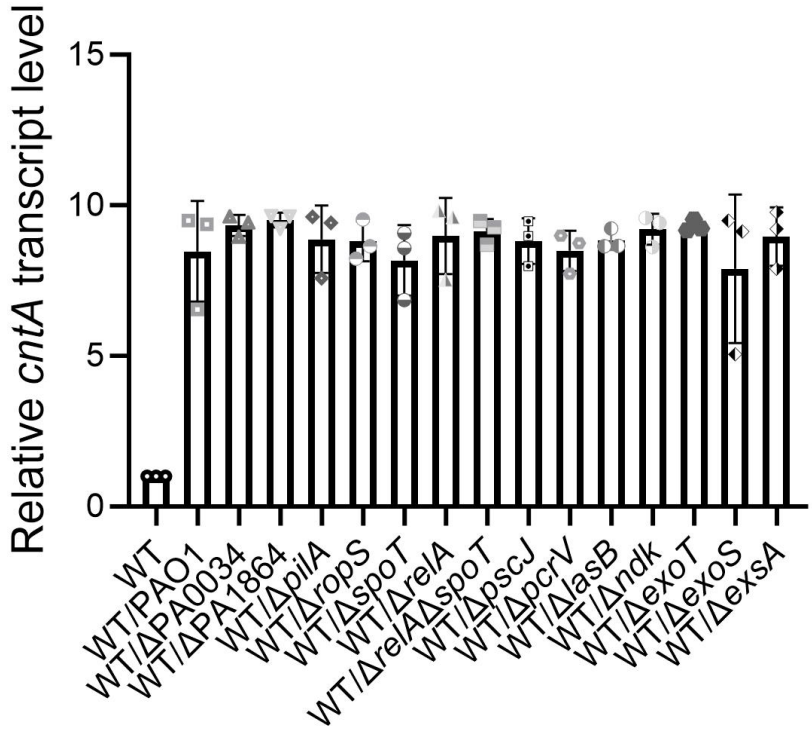

Figure S4. Screening of candidate P. aeruginosa mutants for effects on S. aureus cntA transcription.

Relative *cntA* transcript levels in *S. aureus* WT during mono-culture or co-culture with *P. aeruginosa* PAO1 or the indicated PAO1 mutants were measured by RT-qPCR. The figure shows the results for all mutant strains listed in Table S4, except Δ*cntM*. Relative transcript levels were normalized to *gyrA*. Compared with *S. aureus* mono-culture, co-culture with PAO1 or any tested PAO1 mutant strain significantly increased *cntA* transcription. No significant differences were observed between co-culture with PAO1 mutants and co-culture with PAO1, indicating that deletion of these candidate genes did not affect the *P. aeruginosa*-mediated increase in *cntA* transcription. Therefore, these mutants were excluded from further investigation. Detailed statistical analyses are provided in Table S7. Data are presented as mean ± SD from three independent experiments. Statistical significance was assessed using one-way ANOVA followed by Tukey's multiple-comparisons test. WT, *S. aureus* WT.