



## BACKGROUND

- Secondary bacterial infection with *Staphylococcus aureus* is a common complication of influenza A virus (IAV) infection [1].
- Presence of *S. aureus* increases the production of viral particles in the host [2].
- The *sae* gene encodes bacterial surface proteins and has genetic regulatory functions [3].
- *S. aureus sae* knockdown mutants lead to decreased viral particle production compared to wild-type *S. aureus* (WT).
- FnbA is a *S. aureus* virulence factor that mediates bacterial adhesion and invasion [4].

## RESEARCH QUESTIONS

1. Is the *sae*-mediated pro-viral activity genetically encoded?
2. Is the pro-viral effect of *sae* due to the surface protein or its regulatory effects?
3. How does the FnbA protein affect bacterial adhesion and invasion in virus-infected host cells?

## METHODS

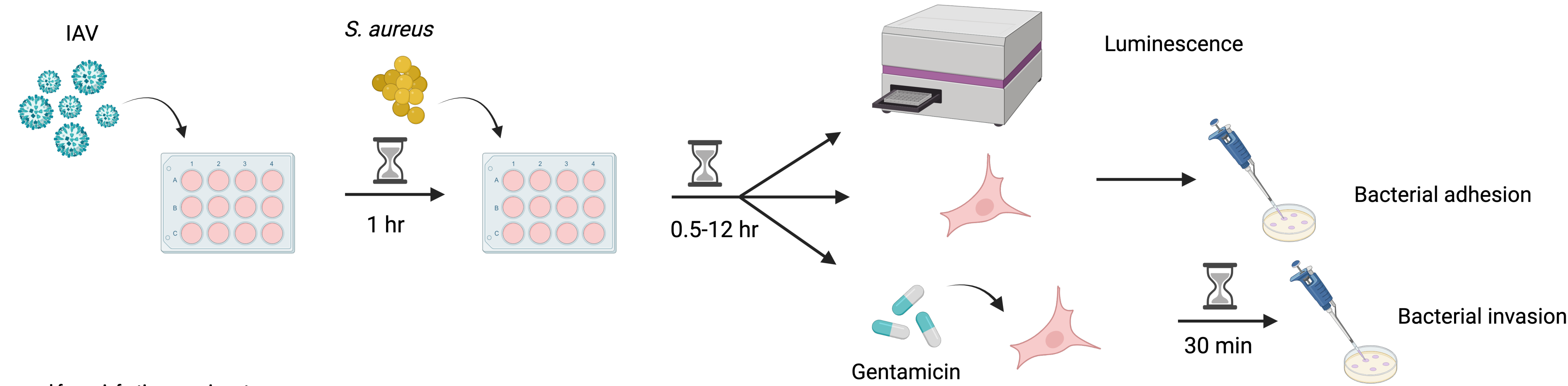


Figure 1. Schematic of the workflow used for co-infection experiments.

## RESULTS

1. The *sae*-mediated pro-viral activity is genetically encoded.

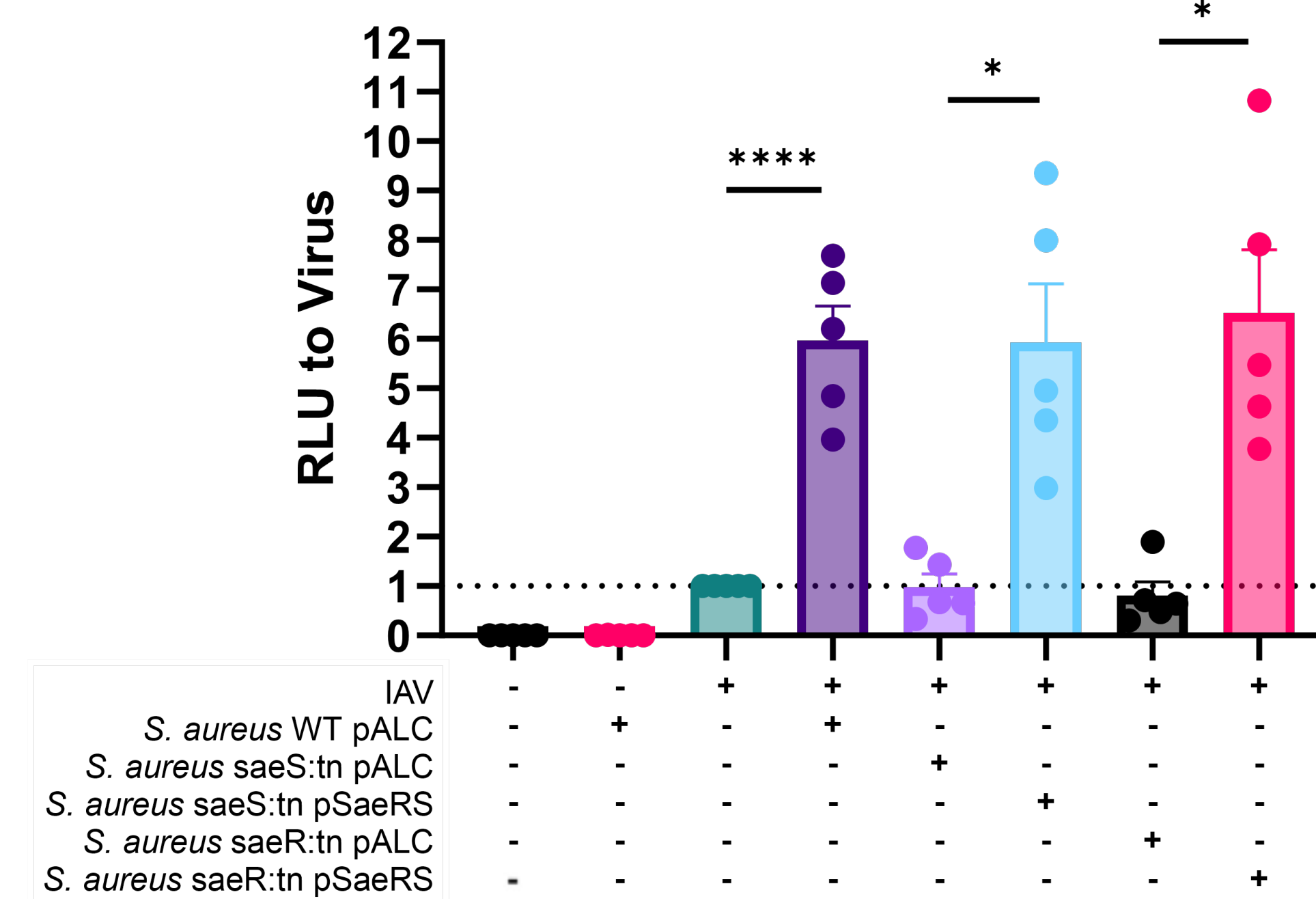


Figure 2. Complementation of *sae* mutants restores the pro-viral phenotype. A549 cells were infected with WSN-luciferase virus (MOI 2) for 1 hour. Post-inoculum removal,  $10^8$  CFU/ml *S. aureus sae* mutants containing pALC or pALC+*saeRS* plasmid were added. At 12 hpi, cell supernatant was combined with luciferase substrate and luminescence was read with a plate reader. Luminescence readings were normalized to virus sample (n = 5). Statistical analysis: Student's t-test, \* $p < 0.05$ .

2. The *fnbA* gene is required for pro-viral activity.

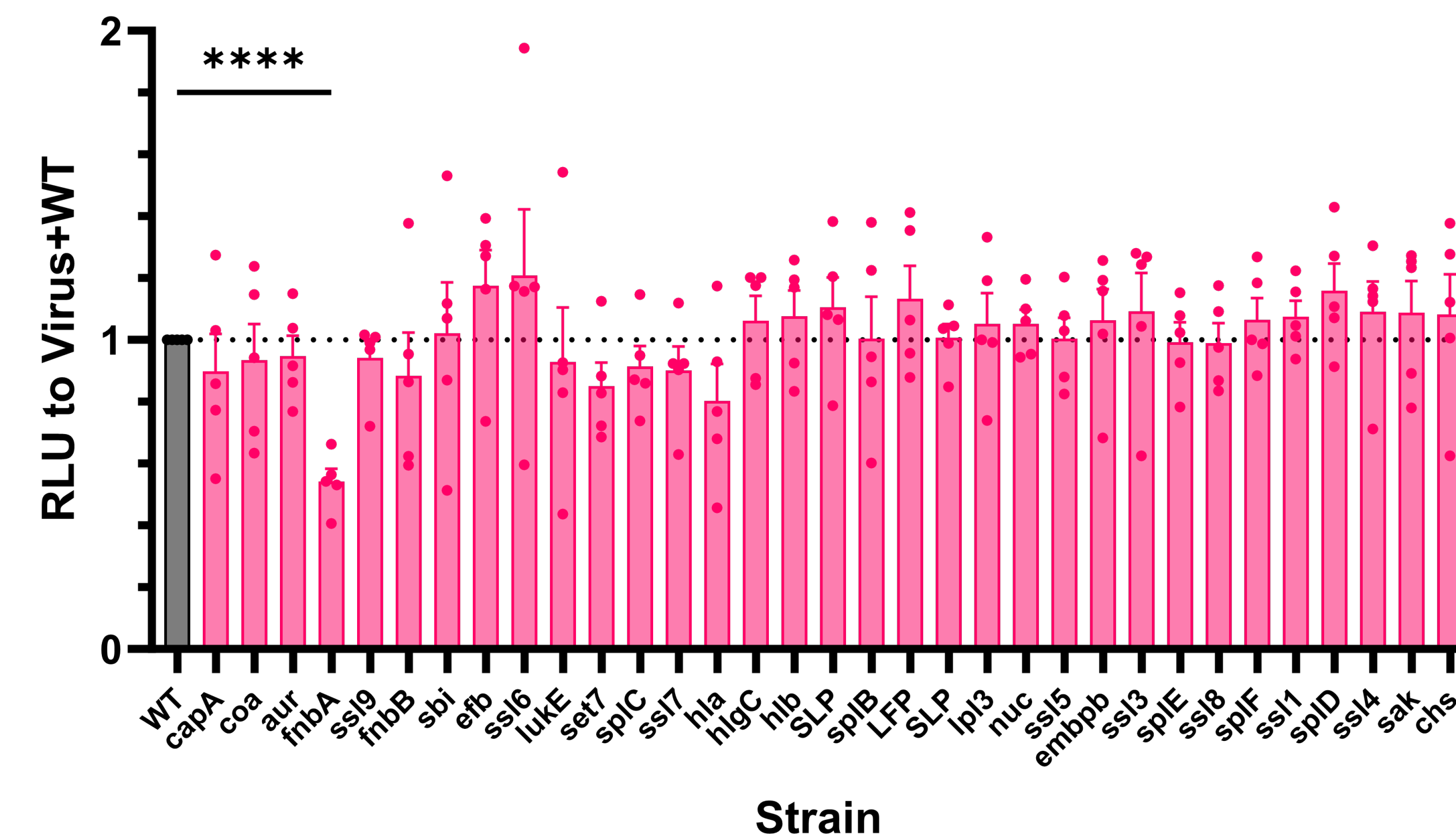


Figure 3. *S. aureus fnbA* mutants do not exhibit a pro-viral phenotype. A549 cells were infected with WSN-luciferase virus (MOI 2) for 1 hour. Post-inoculum removal,  $10^8$  CFU/ml *S. aureus* mutants of indicated genes were added. At 12 hpi, cell supernatant was combined with luciferase substrate and luminescence was read with a plate reader. Luminescence readings were normalized to virus sample (n = 5). Statistical analysis: Student's t-test, \* $p < 0.05$ .

3. *S. aureus* adhesion is FnbA-independent, invasion is FnbA-dependent at different points in the viral life cycle.

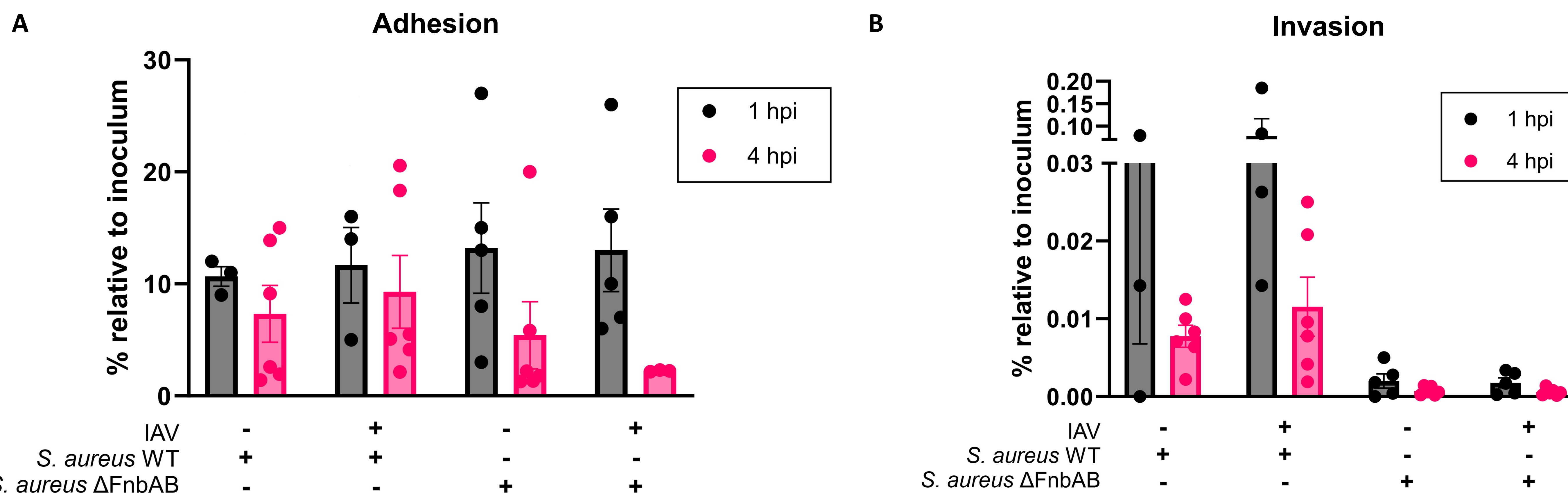


Figure 4. Bacterial adhesion, but not invasion, occurs without FnbA at different points throughout the viral life cycle. A) Cells were infected with PR8 BEI virus (MOI 3) for 1 hour. Post-inoculum removal, at 1 hpi or 4 hpi  $10^6$ - $10^7$  CFU *S. aureus* mutants were added. 30 min post bacterial addition, cells were lysed with Triton-X100, plated on TSA, and incubated overnight at 37°C before enumeration (n=5). B) Cells were treated as in A) but 30 mins post bacterial addition, 150  $\mu$ g/ml gentamicin were added for 30 min. Following gentamicin removal, cells were lysed with Triton-X100 and plated on TSA, followed by overnight incubation at 37°C and enumeration (n=5). Data shown are mean  $\pm$  SEM.

## CONCLUSIONS

- The *sae*-mediated pro-viral phenotype is genetically encoded.
- Knockdown of *sae* leads to decreased viral particle production because the *fnb* gene is no longer expressed.
- FnbA is not required for bacterial adhesion but is required for bacterial invasion.
- Bacterial adhesion and invasion are not affected by the presence of virus.

## FUTURE DIRECTIONS

- Is the *fnb*-mediated pro-viral effect genetically encoded?
- How does FnbA affect adhesion and invasion in virus-infected cells at different points in the viral life cycle?

## REFERENCES & ACKNOWLEDGEMENTS

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We acknowledge with respect the Lekwungen-speaking peoples on whose traditional territory the university stands and the Songhees, Esquimalt and WSÁNEĆ peoples whose historical relationships with the land continue to this day.

Images created using Biorender.com. Figures created using GraphPad Prism 10.0.3.

