

# Development of anti-virulence drugs by targeting the SaeRS two-component system of *Staphylococcus aureus*

W. Yeo<sup>1</sup>, R. Arya<sup>2</sup>, D Hu<sup>3</sup>, H. Jeong<sup>3</sup>, K Kim<sup>2</sup>, & T. Bae<sup>1,\*</sup>

<sup>1</sup> Department of Microbiology and Immunology, Indiana University School of Medicine-Northwest, Gary, USA

<sup>2</sup> Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon, Korea

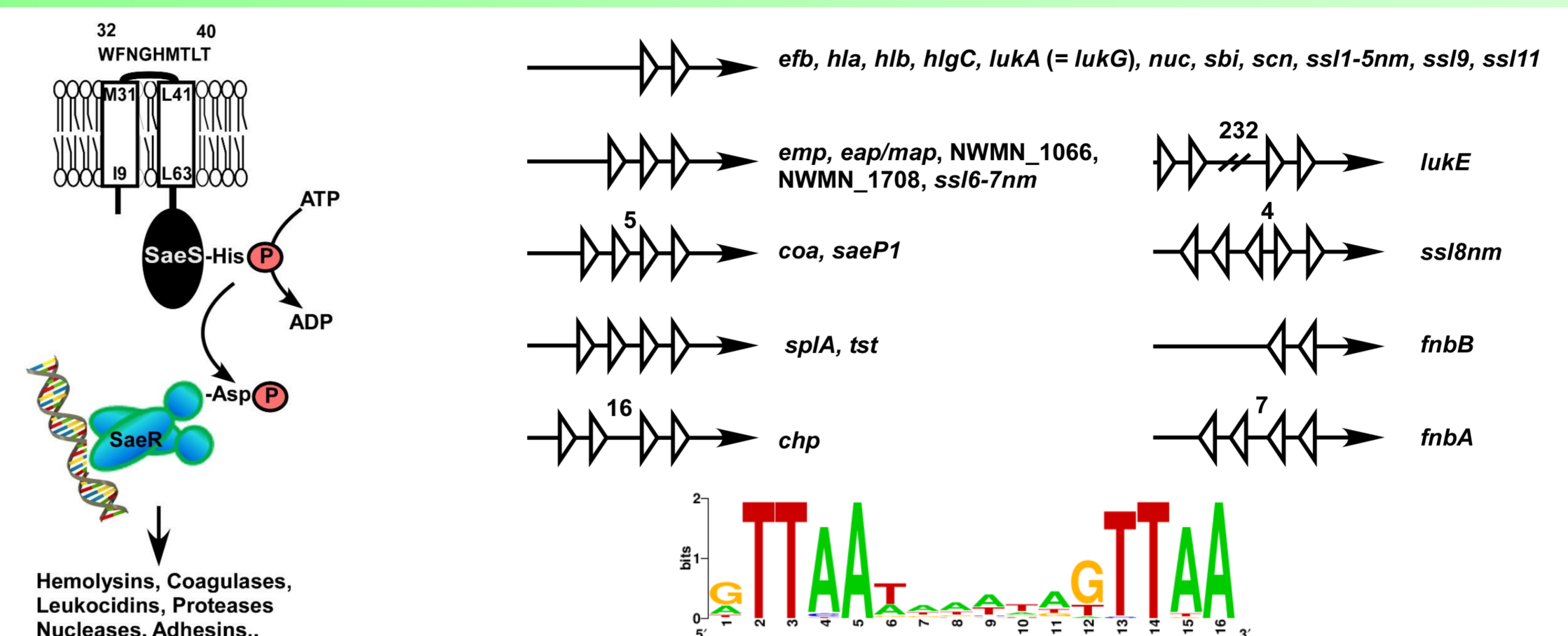
<sup>3</sup> College of Pharmacy, University of Illinois at Chicago, Chicago, USA

## Abstract

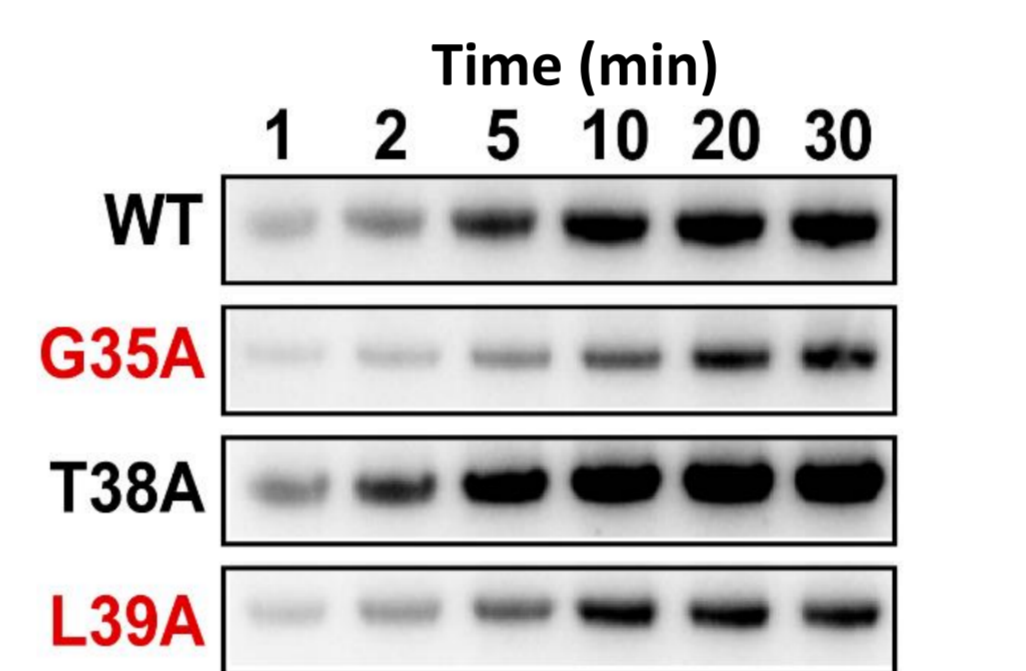
*Staphylococcus aureus* is a Gram-positive pathogen causing diseases in both humans and animals. In humans, the bacterium causes a wide range of diseases from skin and soft-tissue infections to various life-threatening diseases whereas, in animals such as cow, mastitis is a major staphylococcal disease. The success of *S. aureus* as a pathogen is due to the production of multiple virulence factors. The emergence of drug resistant strains including MRSA (methicillin resistant *S. aureus*) and VISA (vancomycin intermediate *S. aureus*) has made the treatment of the bacterial disease more difficult, calling for development of novel classes of drugs. One excellent target for novel drug development is the SaeRS two-component system, which controls the production of more than 20 staphylococcal virulence factors. The SaeRS TCS is composed of the sensor kinase SaeS and the response regulator SaeR. Animal experiments showed that the kinase activity of SaeS correlates with the bacterial virulence. To explore whether the SaeRS TCS is a viable target for anti-virulence drug development, we screened small molecule libraries for Sae-inhibitors by employing a promoter-GFP reporter system. By screening 10,000 compounds, we identified ~150 compounds that repress the Sae-regulated promoter. One (SKK1010) of those with low IC<sub>50</sub> (4 μM) was further characterized. At 8 μM, the compound blocked the hemolysis of human erythrocytes by *S. aureus*, and protected HeLa cells from *S. aureus*-mediated killing. The IC<sub>50</sub> cytotoxicity of SKK1010 was 62 μM, resulting in the therapeutic index of 15.5. In a microsomal stability assay, 80% of SKK1010 still remained intact at 30 min, showing an exceptional metabolic stability. Finally, in a murine infection model, SKK1010 showed a synergistic effect with vancomycin in protection of the mice from *S. aureus*-mediated killing, demonstrating the potential of the compound as an anti-virulence agent against *S. aureus* infection.

## Introduction

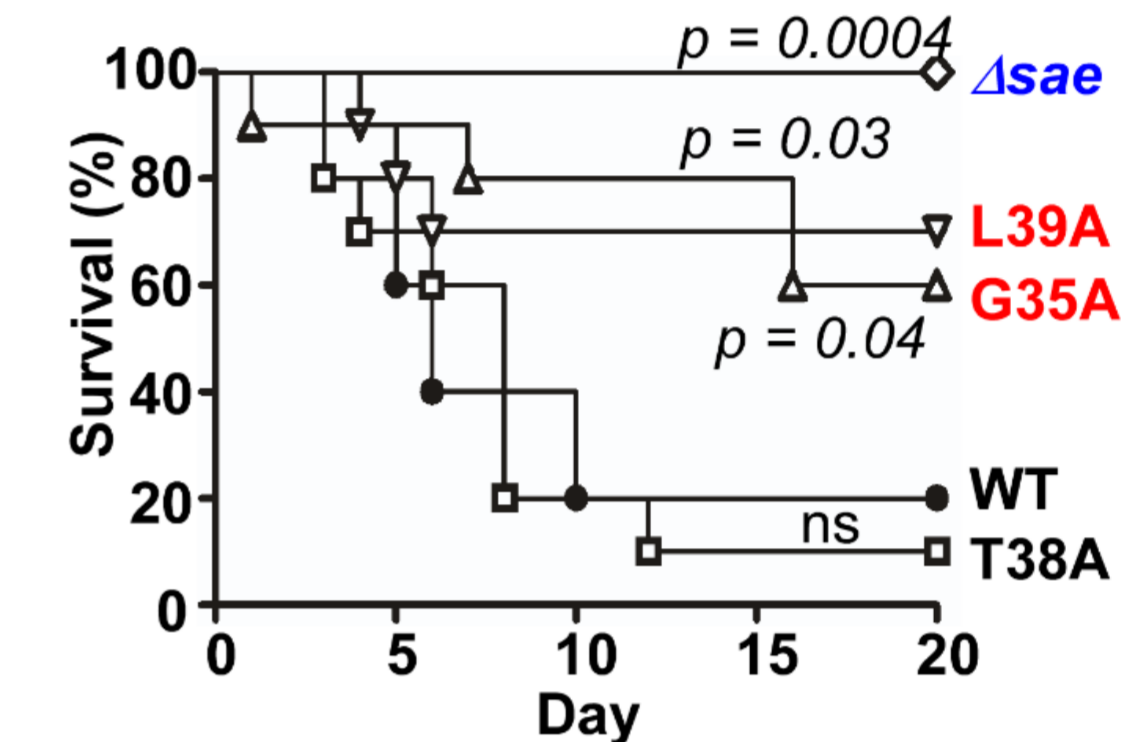
In *Staphylococcus aureus*, the SaeRS Two-component system controls the production of more than 20 virulence factors



The kinase activity of SaeS is critical for the virulence of *S. aureus*

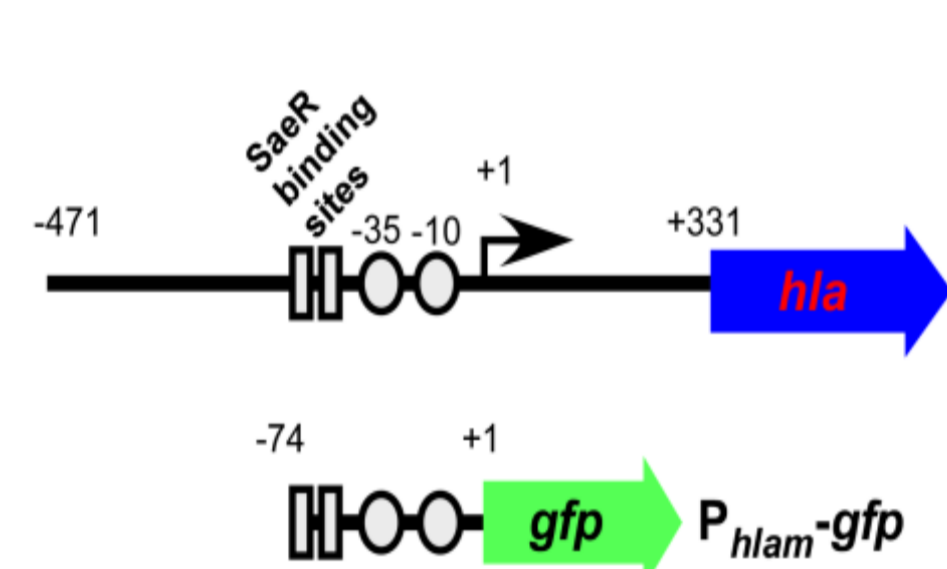


Autokinase assay for various SaeS mutants



Virulence of *S. aureus* mutants of SaeS assayed by a murine model of blood infection

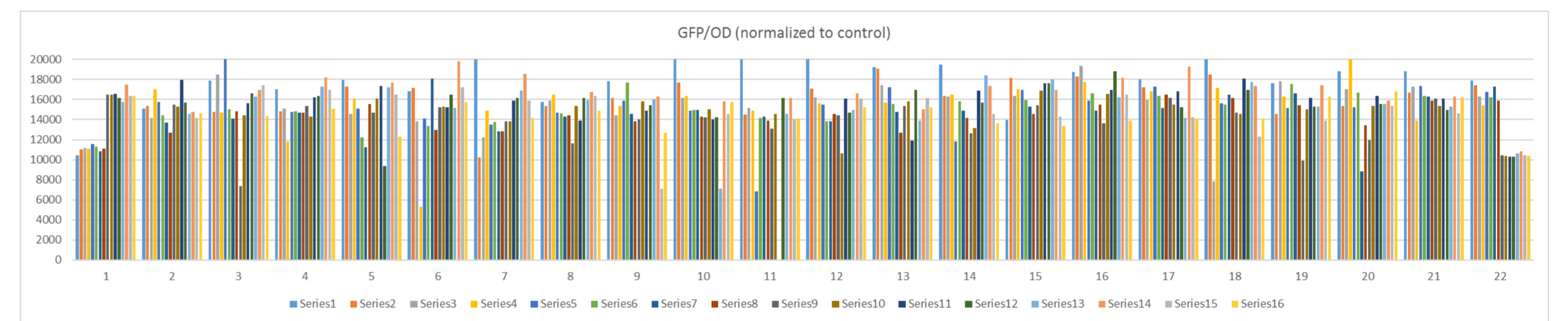
## Methods



A GFP-reporter strain



High-throughput screening of compound libraries



Identification of compounds inhibiting GFP expression

## Results

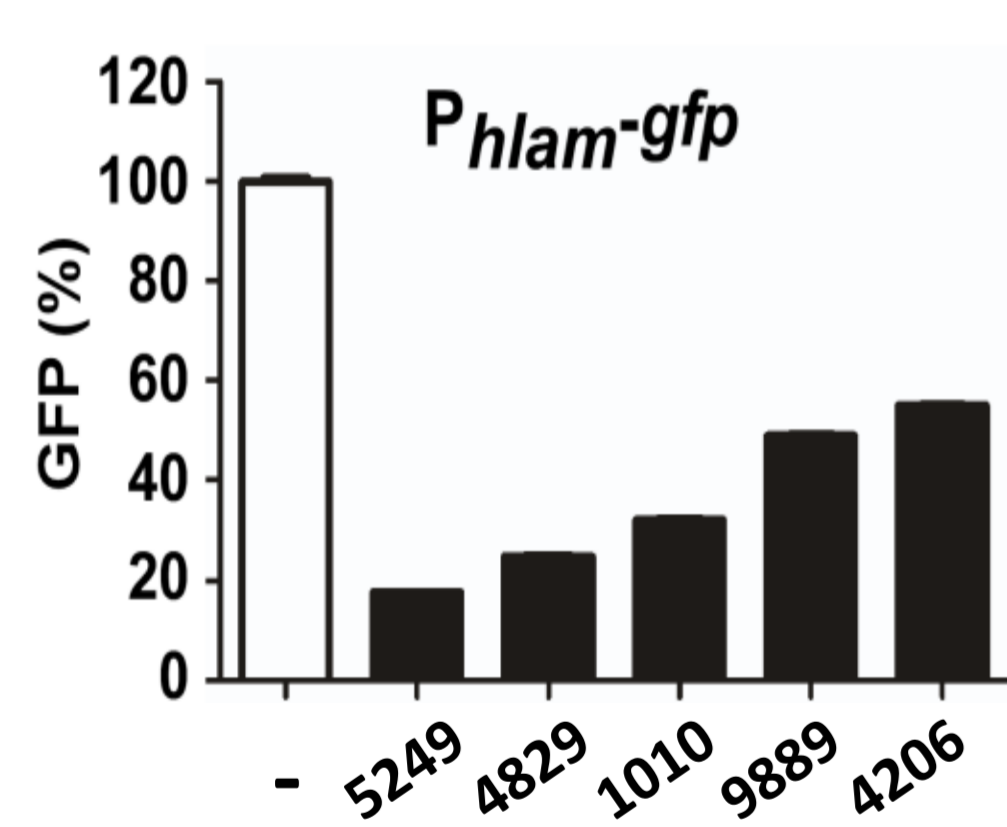


Fig. 1. Inhibition of Phlam-gfp by compounds selected by the library screening.

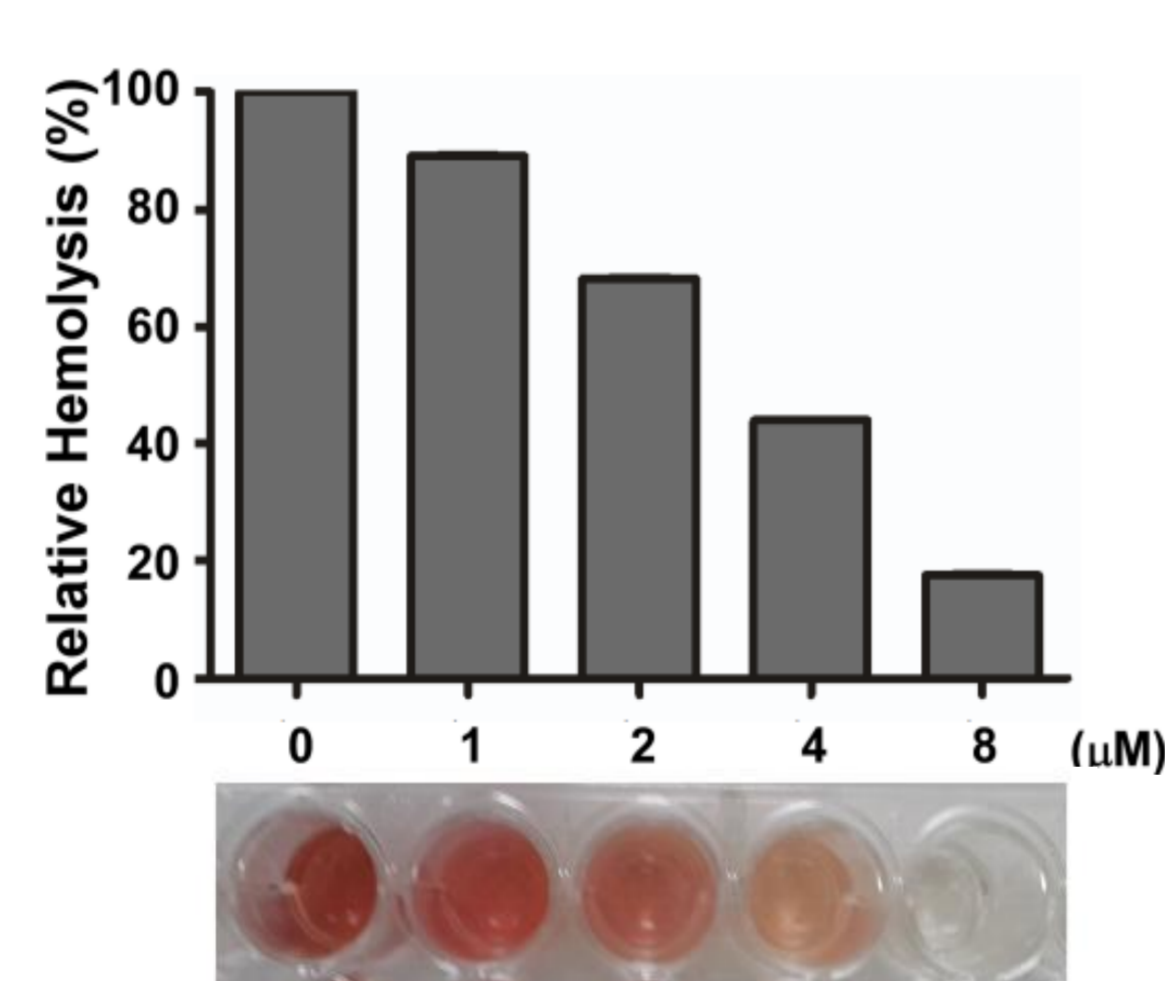


Fig. 2. Inhibition of the hemolysis activity of *S. aureus* by SKK1010. Human erythrocytes were mixed with *S. aureus* USA300 in the presence of various concentrations of SKK1010. At 3 h incubation.

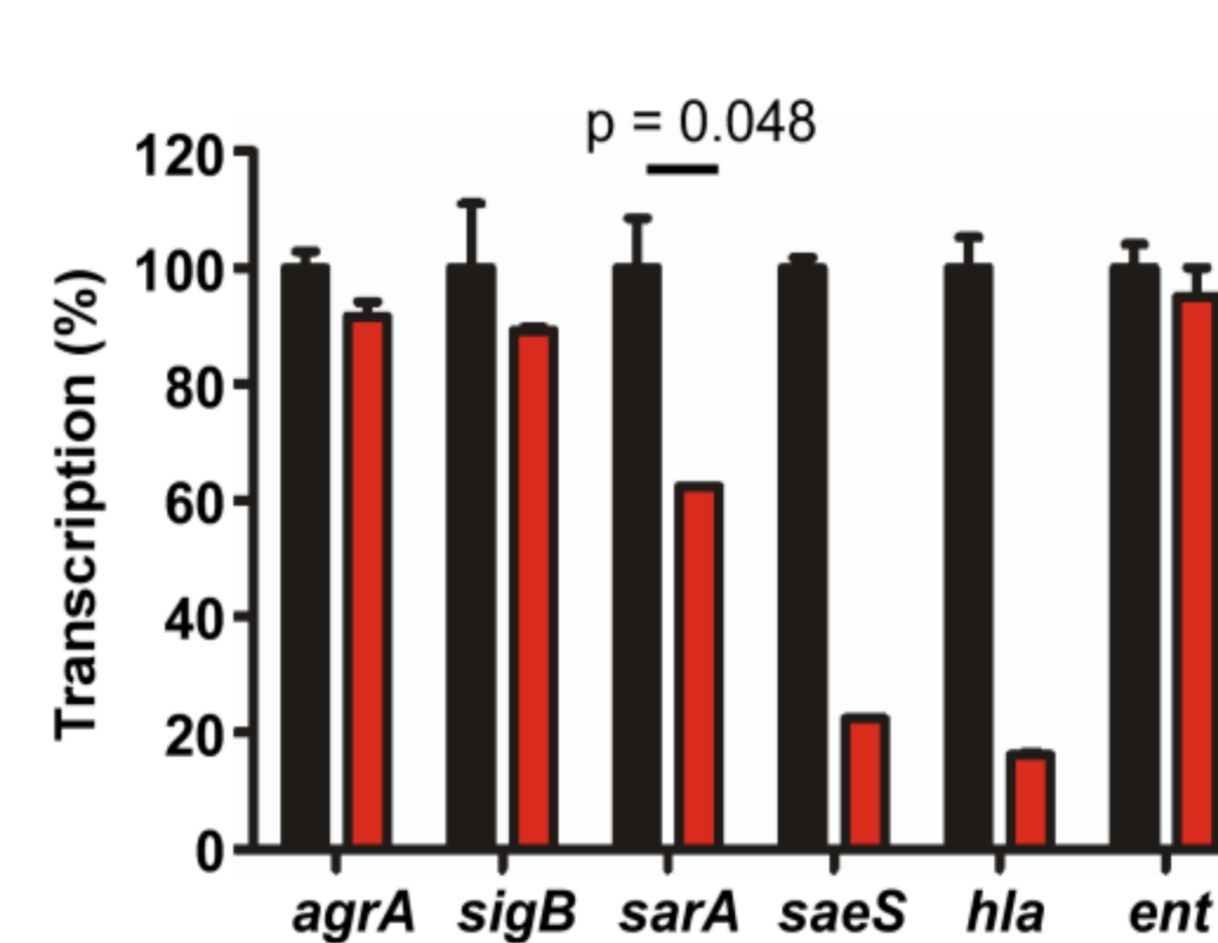


Fig. 3. Effect of SKK1010 on the transcription of select global regulator and toxin genes of *S. aureus*.

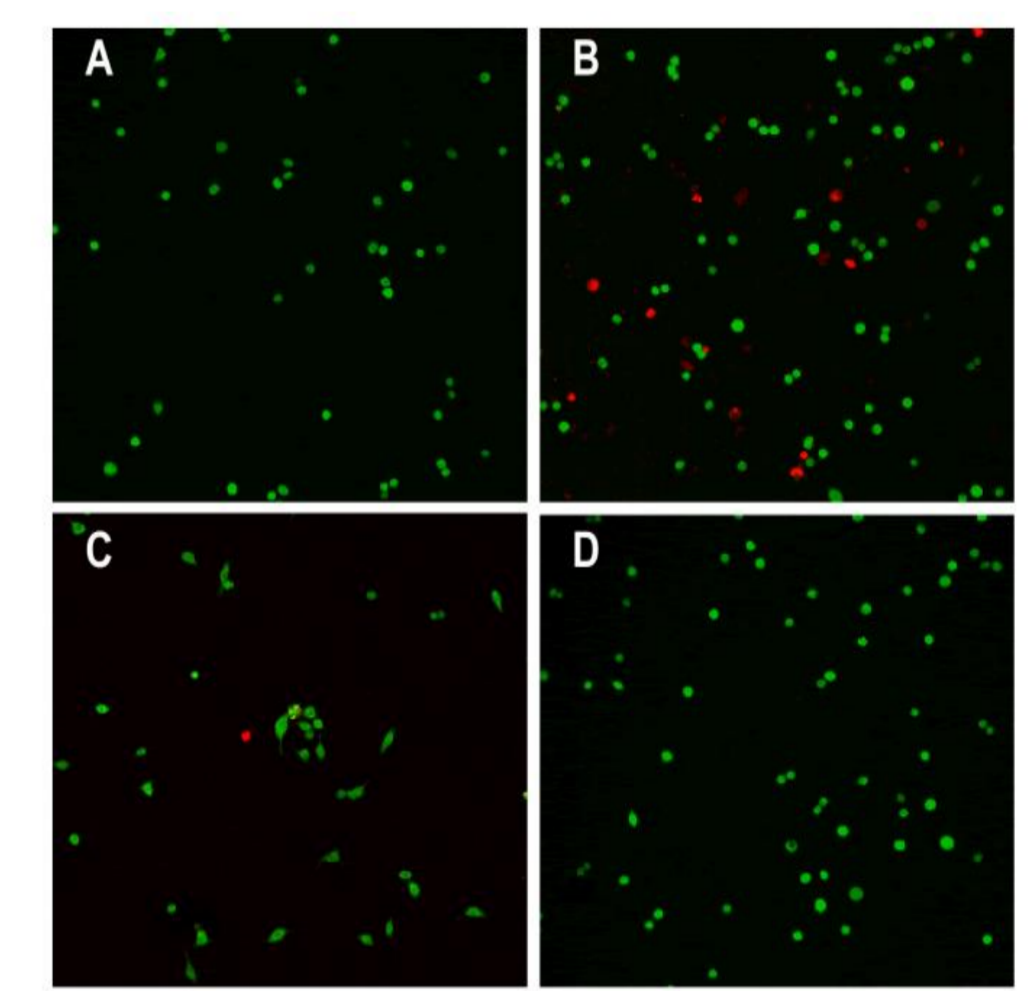


Fig. 4. SKK1010 protects HeLa cells from *S. aureus*-induced injury. A, no bacterium; B, 0 μM; C, 4 μM; D, 8 μM. Green, live cell; Red, dead cell.

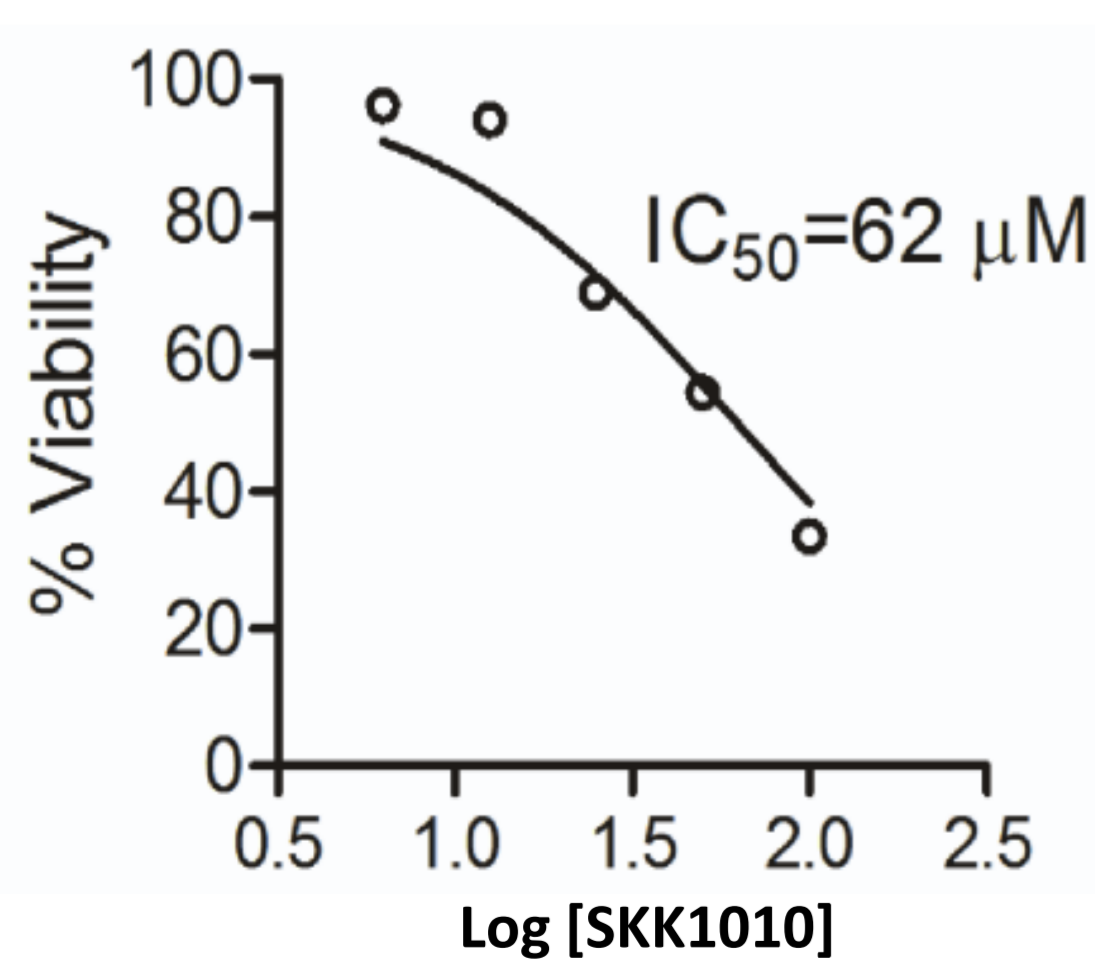


Fig. 5. Cytotoxicity of SKK1010 on HepG2 cells.

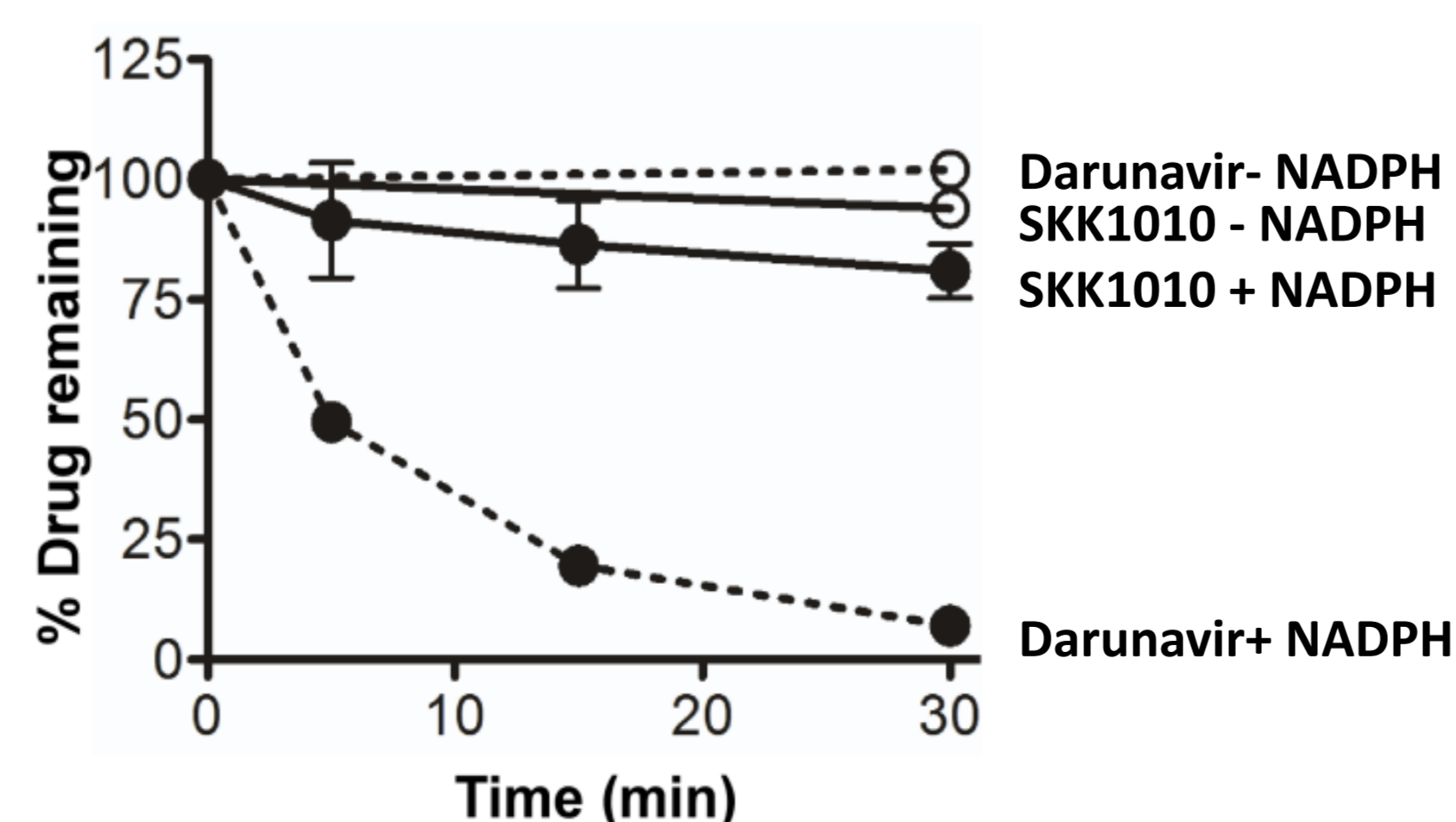


Fig. 6. SKK1010 is metabolically very stable. NADPH was used as a cofactor for drug-metabolizing enzymes in the murine liver lysate.

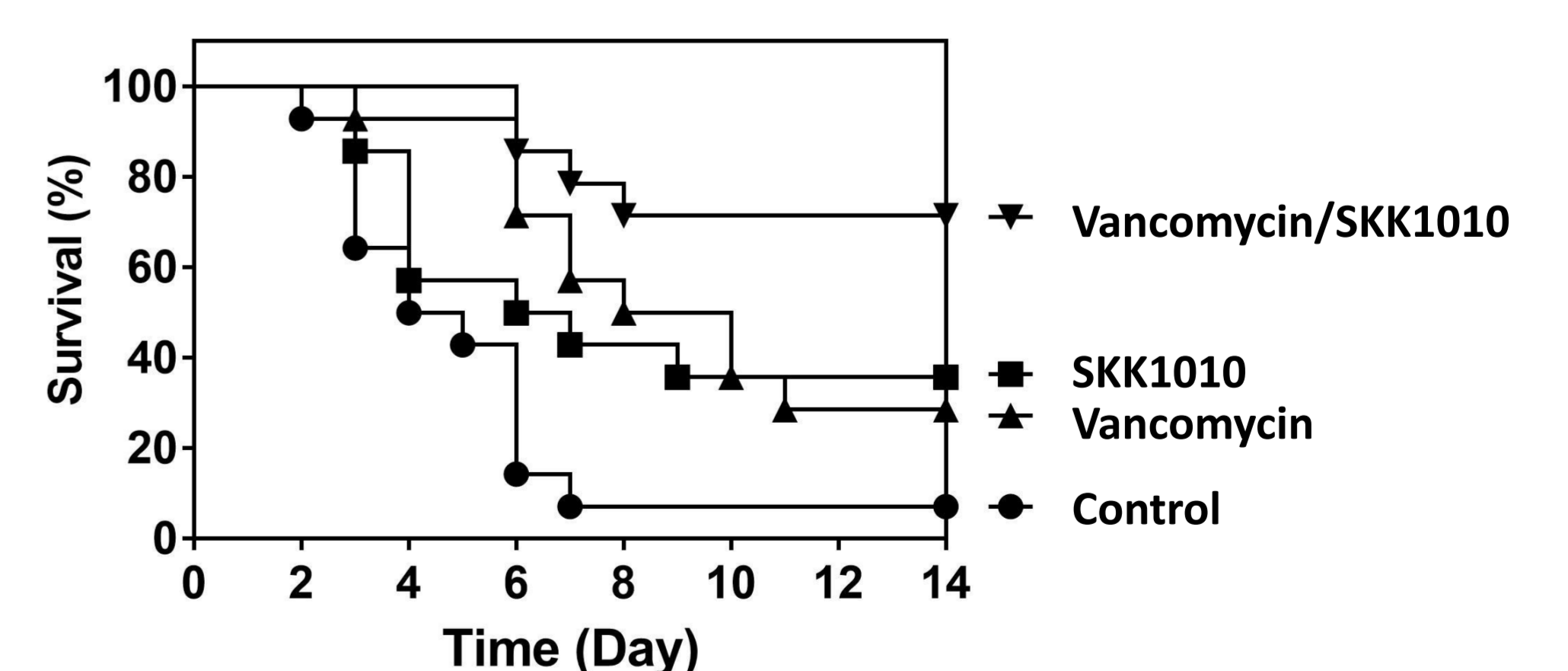


Fig. 7. SKK1010 is synergistic with vancomycin in protection of mice from *S. aureus*-mediated killing. Mice were injected with *S. aureus* USA300 ( $2 \times 10^7$  CFU); then 2 h post infection drugs were IP injected into mice. Drugs were injected everyday.

## Conclusions

- 10,000 compounds were successfully screened by *S. aureus* USA300 carrying a GFP-reporter plasmid.
- Initial screening identified ~ 150 compounds that inhibit the reporter gene expression.
- One of the identified compound, SKK1010, showed promising characteristics as an anti-virulence drug candidate for *S. aureus* infection.