

Supplementary Information

A highly active chimeric lysin with a calcium-enhanced bactericidal activity against *Staphylococcus aureus* in vitro and in vivo

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Table S1. Bacterial strains used in this study.

Strain	Description
BL21(DE3)	F ⁻ <i>ompT hsdS_B (r_B⁻ m_B⁻) gal dcm</i> λDE3 (harboring gene 1 of the RNA polymerase from the phage T7 under the <i>PlacUV5</i> promoter)
<i>E. coli</i> BL21/pET28a-ClyC	Constructed in this study
<i>E. coli</i> BL21/pET28a-Ply187CD	pET28a derivative carrying the catalytic domain of Ply187 gene
<i>E. coli</i> BL21/pET28a-SA97CBD	pET28a derivative containing the cell-wall binding domain of LysSA97 gene

Table S2: Primers used in the overlap PCR.

Code	Name	Sequence
A	Ply187CD-F	ATATCCATGGGCATGGCACTGCCTAAAACGGG
B	Ply187CD-R	AGTTCCAGGTGATCTTGTCCGCTGGTGGTG-TAGGTTTCGGTTC
C	SA97CBD-F	GAACCGAAACCTACACCACCAGCGGACAAGATACCTG-GAACT
D	SA97CBD-R	TATACTCGAGCGCCCATTCGATGGTGCCCCAG

Table S3. MIC of ClyC against different *S. aureus* strains.

<i>S. aureus</i> strains	Drug resistance	MIC of ClyC (μg/mL)
WHS11016	Methicillin-resistant	18
WHS11095	Methicillin-resistant	18
WHS11048	Methicillin-resistant	18
WHS11101	Methicillin-resistant	18
WHS11044	Methicillin-resistant	18
WHS11051	Methicillin-resistant	18
WHS11036	Methicillin-resistant	9
WHS11098	Methicillin-resistant	18
WHS11034	Methicillin-resistant	18
WHS11009	Methicillin-resistant	9
WHS11040	Methicillin-resistant	9
AB91118	Methicillin-sensitive	9

N315	Methicillin-resistant	18
WHS11024	Methicillin- sensitive	4.5
WHS11041	Methicillin-resistant	18
WHS11011	Methicillin-resistant	9
WHS11018	Methicillin-resistant	18
WHS11032	Methicillin-resistant	18
WHS11005	Methicillin-resistant	18
WHS11099	Methicillin-resistant	9
WHS11046	Methicillin-resistant	36
WHS11100	Methicillin-resistant	18
WHS11026	Methicillin-resistant	18
WHS11037	Methicillin-resistant	9
WHS11033	Methicillin-resistant	18
WHS11025	Methicillin-resistant	18
ATCC29213	Methicillin- sensitive	18
WHS11102	Methicillin-resistant	36
WHS11017	Methicillin-resistant	18

Figure S1. Lytic spectrum of ClyC.

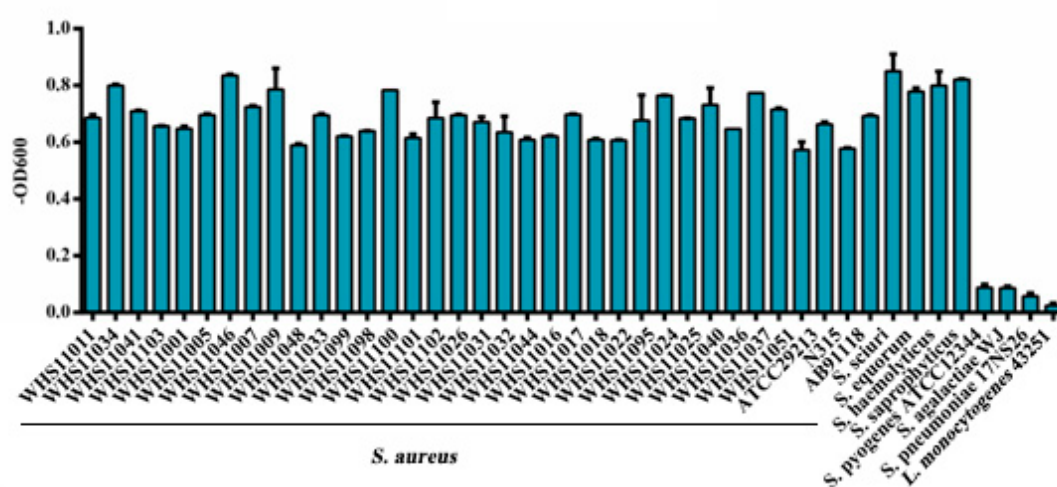


Fig S1. Susceptibility of ClyC to staphylococcus and single strains of *S. pyogenes*, *S. agalactiae*, *S. pneumoniae*, and *Listeria monocytogenes*. Strains were washed once with PBS and resuspended to a final OD₆₀₀ of 0.8~0.9. After treatment with 25 µg/mL of ClyC at 37 °C for 10 min, the final OD₆₀₀ value of treated wells were subtracted from the PBS-treated well to yield the net change in OD₆₀₀, and the bactericidal efficiency determined by the value change of OD₆₀₀. The experiment was repeated three times. Error bars represent the standard deviations.

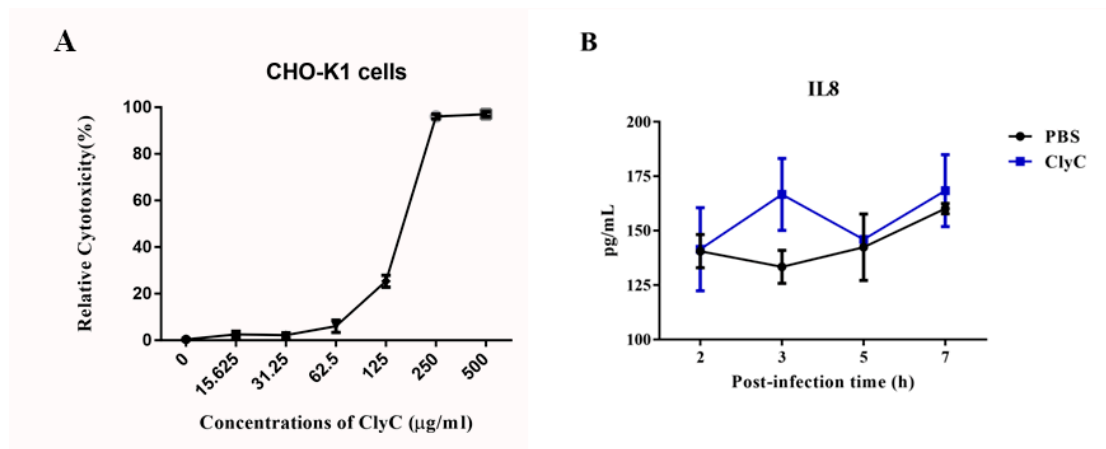
Figure S2. Cytotoxicity of ClyC.

Fig S2. Cytotoxicity of ClyC. (A) Cytotoxicity of ClyC to CHO-K1 cells. Cells were co-cultured with different concentrations of ClyC (0, 15.625, 31.25, 62.5, 125, 250, and 500 $\mu\text{g/mL}$) for 24 h and the relative viability was calculated by the change of OD₅₇₀ by MTT assay. (B) Concentration of IL-8 in mice serum at different times post ClyC treatment.