Supporting information

Covalent Sortase A Inhibitor ML346 Prevents *Staphylococcus aureus* Infection of *Galleria mellonella*

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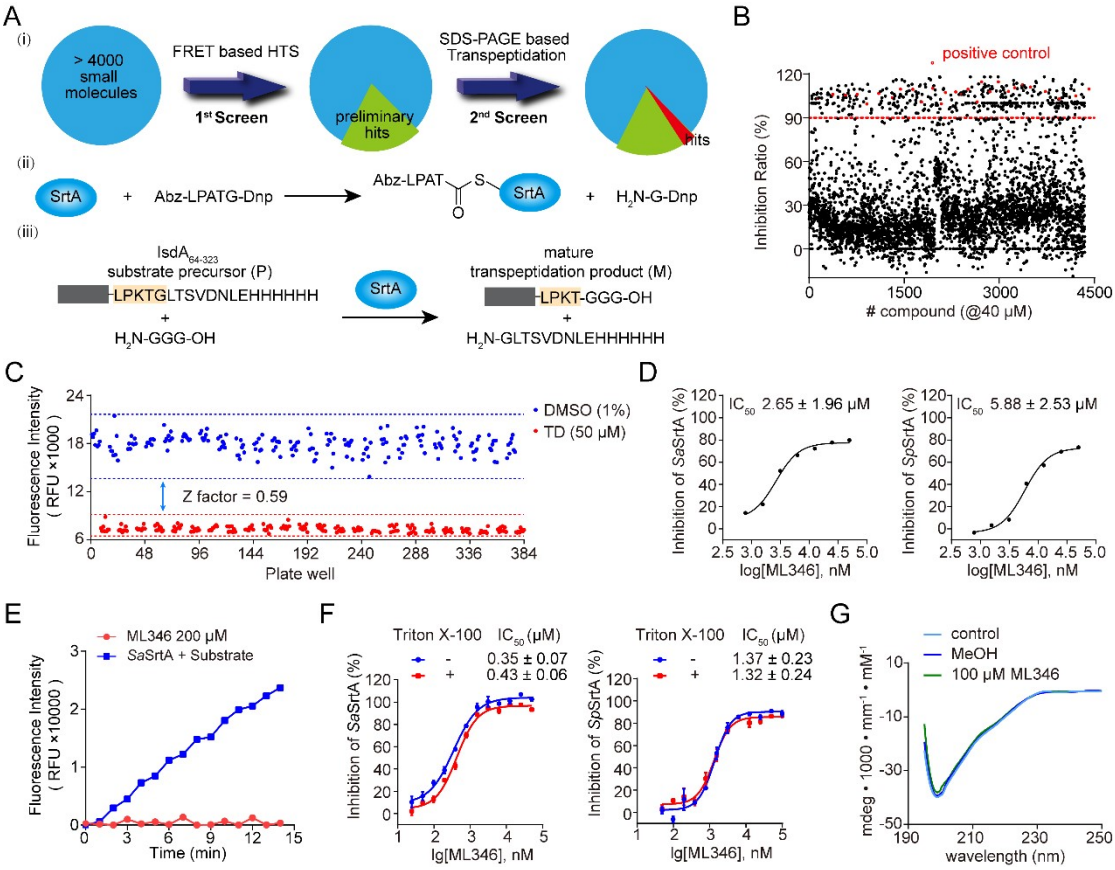

Figure S1. Identification of SrtA inhibitors. (A) Overview of SrtA inhibitors screening. (i) Schematic diagram of SrtA inhibitor screening procedure. (ii) The reaction scheme of FRET-based screening. (iii) The reaction scheme of PAGE-based screening. (B) High throughput screening was performed in FRET based assay and compounds with inhibition ratio > 90% at 40 μM were considered as preliminary hits. (C) Z factor plot of HTS of SrtA inhibitors using the FRET assay in the black 384-well plates. (D) IC_{50} curves of ML346 against *Sa*SrtA$_{\Delta N24}$ (left) and *Sp*SrtA$_{\Delta N81}$ (right) quantified by band intensify in Figure 1C. (E) Detection of fluorescence of compound ML346. (F) Effect of 0.1% detergent Triton X-100 on IC_{50} of ML346 against *Sa*SrtA$_{\Delta N24}$ (left) and *Sp*SrtA$_{\Delta N81}$ (right) in FRET based assay. (G) CD spectra of *Sa*SrtA$_{\Delta N24}$ with or without ML346 in methanol (MeOH).

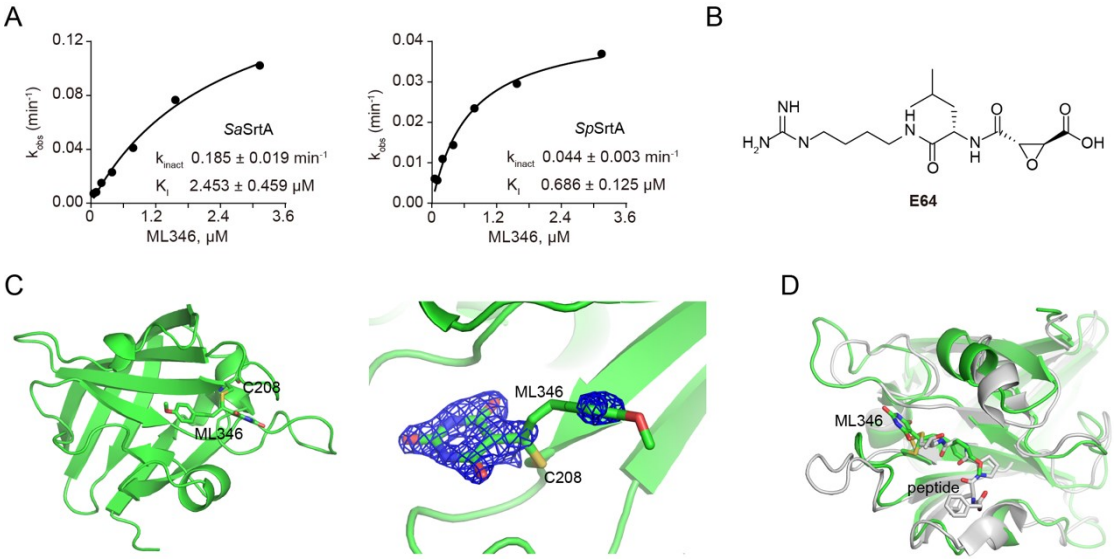

Figure S2. Characterization on mode of action of ML346. (A) Determination of inhibition constant (k_{inact} and K_I). (B) Chemical structure of E64. (C) X-ray crystal structure of *Sp*SrtA$_{\Delta N81}$/ML346 complex. Fo-Fc omit map is colored in blue. (D) Structural alignment of *Sa*SrtA$_{\Delta N59}$/peptide (gray, PDB ID: 2KID) and *Sp*SrtA$_{\Delta N81}$/ML346 (green, PDB ID: 7V6K). The structural superimposition was performed in PyMol.

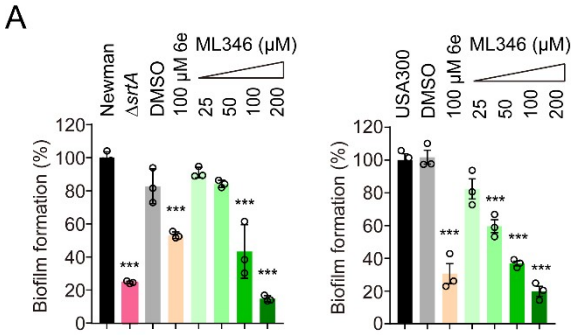

Figure S3. Effect of ML346 on biofilm formation of *S. aureus* Newman and USA300 strains. A. The reversible inhibitor 6e was assayed as a positive control. Statistical significance (*P < 0.05, **P < 0.01, ***P < 0.001) was determined using the unpaired, two-tailed Student's t test (n = 3). Data were presented as mean ± SEM.

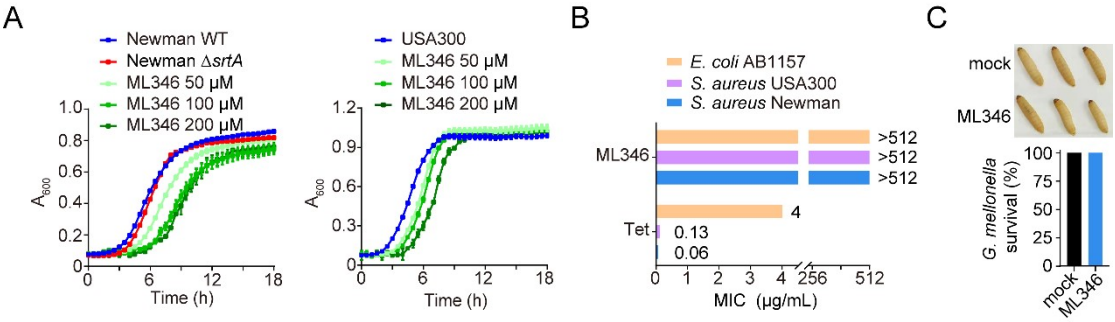

Figure S4. Toxicity study of ML346 on bacteria and *G. mellonella*. (A) Effect of ML346 on bacterial growth of *S. aureus* Newman (left) and USA300 (right) strains. (B) Determination of MIC values of ML346 for inhibition on the growth of *S. aureus* Newman, *S. aureus* USA300, and *E. coli* AB1157 strains. Tet, tetracycline was assayed as a control. (C) The appearance (top) and survival rate (bottom) of *G. mellonella* (n = 15) after a 120 h treatment with 60 mg/kg ML346.

Table S1. Inhibition of proteases by ML346.

Compound	Remaining activity (%)	
	cathepsin B	cathepsin L
E64 (6.25 nM)	33% ± 0.8%	18% ± 1.7%
ML346 (20 μM)	86% ± 0.7%	91% ± 2.3%

Table S2. Data collection and refinement statistics[a].

	*Sp*SrtA/ML346 (7V6K)
Data collection	
Space group	P 31 2 1
Cell dimensions	
a, *b*, *c* (Å)	34.33, 34.33, 396.23
α, β, γ (°)	90, 90, 120
Resolution (Å)	29.73-1.57 (1.61-1.57)[b]
No. of observations	658629
No. unique	40185 (2808)
R_{merge}[c]	0.067 (0.843)
$I/\sigma(I)$	20.7 (2.3)
Completeness (%)	99.8 (99.1)
Redundancy	16.4 (9.2)
Data refinement	
Resolution (Å)	29.0-1.57
No. reflections	40001
Completeness (%)	99.76
R_{work}/R_{free}	0.19/0.22
No. atoms	
protein	2579
water	120
Mean B value (Å^2)	23.86
Rmsd[d] in	
Bond lengths (Å)	0.008
Bond angles (º)	1.047
Ramachandran Plot	
Favored (%)	98.71
Allowed (%)	1.29

[a] The structure was solved using one crystal.

[b] Highest resolution shell is shown in parenthesis.

[c] $R_{merge} = \Sigma|(I - \langle I \rangle)|/\Sigma(I)$, where I is the observed intensity.

[d] Root mean squared deviation.

Table S3. MIC values of ML346.

Isolate		*S. aureus*	*S. aureus*	*E. coli*
Strain		Newman	USA300	AB1157
MIC (μg/mL)	Vancomycin	2	2	>64
	Kanamycin	5	>160	20
	Tetracycline	0.0625	0.125	4
	ML346	>512	>512	>512