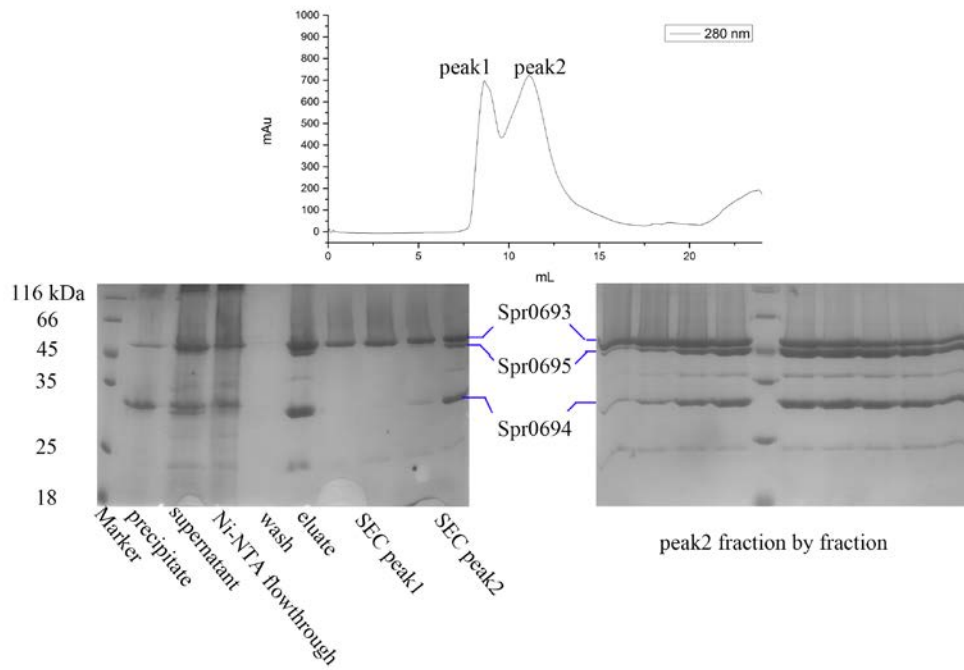


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2 **Supplementary Figure 1** The full SDS-PAGE image of Figure 3a. The area within the rectangle was

3 adopted for Figure 3a.



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18 **Supplementary Figure 3** Size-exclusion chromatography profile with co-purification of
 19 Spr0693-0694-0695.

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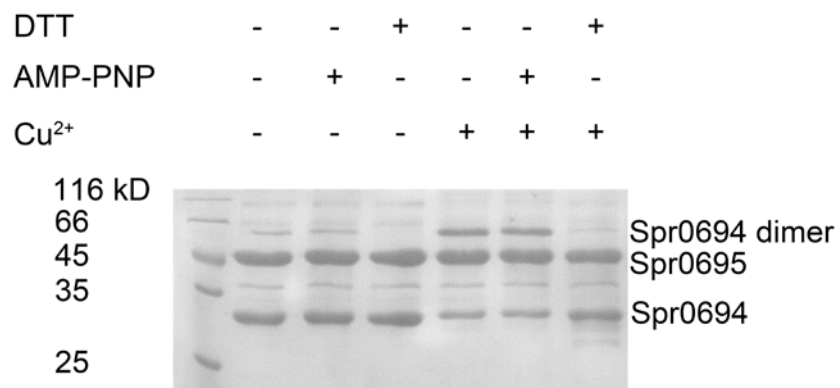
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40 **Supplementary Figure 4** The disulfide-crosslinking experiments of Spr0694_{G173C}-0695 mutant at
 41 different conditions. The reaction mix was separated by SDS-PAGE. Molecular masses are indicated
 42 on the left and the corresponding proteins are indicated on the right.

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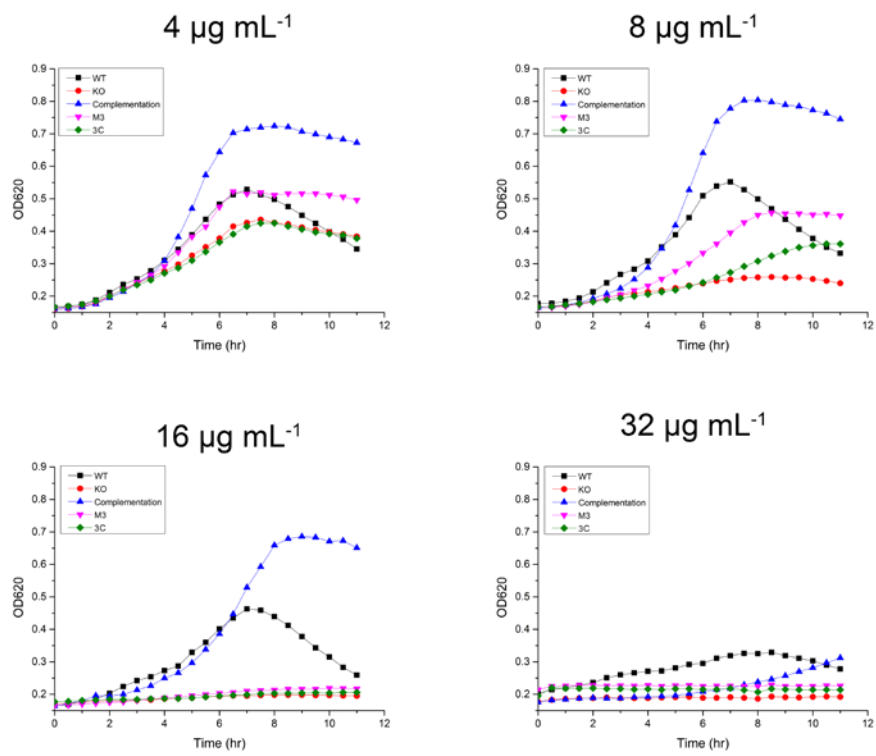
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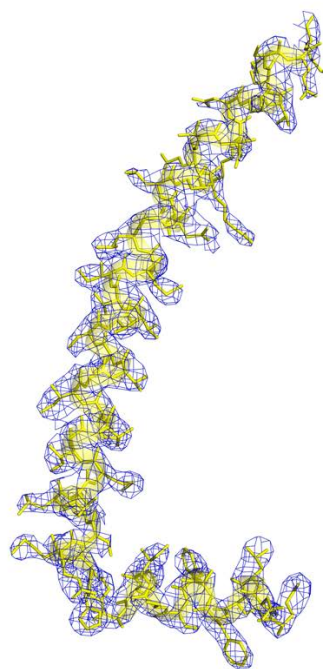
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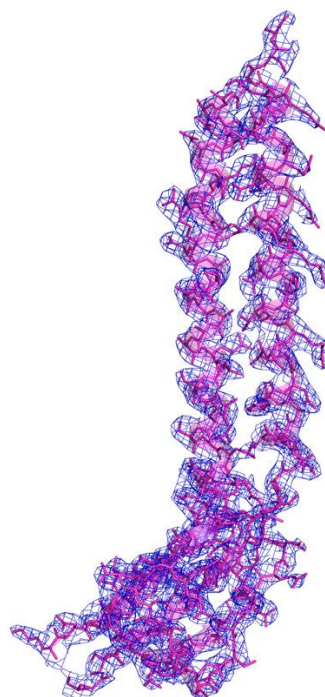
Supplementary Figure 5 Growth curves of *S. pneumoniae* R6 and mutants in the presence of LL-37 at different concentrations.

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Spr0694-0695

b



Spr0693

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83 **Supplementary Figure 6** A portion of the *2Fo-Fc* electron density map of Spr0694-0695 and Spr0693,
84 countered at 1.5 σ . **(a)** Electron density map of TM1 of Spr0695. **(b)** Electron density map of the
85 α -helical hairpin and lipoyl domains of Spr0693 monomer.

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Strains, plasmids or primers	Description	Source
Strains		
WT	<i>S. pneumoniae</i> R6; <i>spr0693-0694-0695</i> ⁺ ; Kan ^s	¹
KO	<i>S. pneumoniae</i> R6 derivative; Δ <i>spr0693-0694-0695</i> ::Janus cassette; Kan ^r	This study
Complementation	KO strain transformed with pJWV25- <i>spr0693-0694-0695</i> ; Kan ^r , Tet ^r	This study
M3	KO strain transformed with pJWV25-M3; Kan ^r , Tet ^r	This study
3C	KO strain transformed with pJWV25-3C; Kan ^r , Tet ^r	This study
Plasmids		
pJWV25	amp ^r , tet ^r , bgaA, P _{czeD} ; integrate Zn ²⁺ -controlled target genes by double cross-over at the <i>S. pneumoniae</i> bgaA locus.	²
pJWV25- <i>spr0693-0695</i>	amp ^r , tet ^r , bgaA, P _{czeD} - <i>spr0693-0694-0695</i>	This study
pJWV25-M3	amp ^r , tet ^r , bgaA, P _{czeD} - <i>spr0693-0694-0695</i> _{K207A-R208A-K210A}	This study
pJWV25-3C	amp ^r , tet ^r , bgaA, P _{czeD} - <i>spr0693-0694-0695</i> _{D49C-R208C/K52C-E205C/E374C-K207C}	This study
Primers for MICs		
Nucleotide sequence (5' to 3')		
Up-F	GCGATATAACCAGCGCCTAGAATGGCAACTG	This study
Up-R	CAAACCTCTGTGCTCGAGCACTCGCAGCACCGATTGCTGC ATAC	This study
Down-F	CAGCATTATCCTCTAGAGAAGTTGGAGTATCAATCCCAGT TGCCC	This study
Down-R	GCGTAGCCAAGAGCTGTGCGCATAGTTAAGAAG	This study
JC-F	GGTGCTGCGAGTGCTCGAGCACAGAGTTTGTAGAAACGC AAAAAGG	This study
JC-R	GATTGATACTCCAACCTTCTCTAGAGGATAATGCTGAAAAC TCCTTGAAG	This study
pJWV25-F	TGATAAGCTGAGCGCCGGTCGCTACCA	This study
pJWV25-R	CTTTTTCTTCATCTTGTGCATCGTCATCCTTGTAGTCG	This study
pJWV25-Spr0693-F	GACCGGCGCTCAGCTTATCATTACATAACGAAGGGCTTC	This study
pJWV25-Spr0695-R	GACGATGACAAGATGAAGAAAAAGAATGGTAAAG	This study
Spr0695 _{K207A-R208A-K210A} -F	GGAGGCTGCAGCGTCAGCAATATATGGGTTTGGTG	This study
Spr0695 _{K207A-R208A-K210A} -R	CCCATATATTGCTGACGCTGCAGCCTCCGGACTAGTA	This study
Spr0695 _{D49C-K52C} -F	CAAGTCAATAAATGTATGACTTGCTCTCAGAAAAATATT AGCGTC	This study
Spr0695 _{D49C-K52C} -R	GCTAATATTTTTCTGAGAGCAAGTCATACATTTATTGACT TGACGAGATAGG	This study
Spr0695 _{E205C-K207C-R208C} -F	GTTTATACTAGTCCGTGTGCTTGTGCTCAAAAATATATG GGTTTGG	This study

