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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.





Supplementary Figure 2 Mass spectrometry identification of proteins in the pull-down assays. The
bands on the SDS-PAGE gel were retrieved, lysed by trypsin and then analyzed by Mass spectrometry.
The representative mass profile of one peptide from each protein is shown, with the corresponding
sequence labeled on the top.



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



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



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69 Supplementary Figure 5 Growth curves of *S. pneumoniae* R6 and mutants in the presence of LL-37 at different concentrations.
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Spr0693

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

Strains, plasmids	Description	Source
or primers		
Strains		
WT	S. pneumoniae R6; spr0693-0694-0695 ⁺ ; Kan ^s	1
КО	S. pneumoniae R6 derivative; $\Delta spr0693-0694-0695::Janus$	This study
	cassette; Kan ^r	,
Complementation	KO strain transformed with pJWV25-spr0693-0694-0695; Kanr,	This study
-	Tet ^r	
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	ampr, tetr, bgaA, PczcD; integrate Zn2+-controlled target genes by	2
	double cross-over at the S. pneumoniae bgaA locus.	
pJWV25- <i>spr0693-0</i>	amp ^r , tet ^r , bgaA, P _{czcD} - <i>spr0693-0694-0695</i>	This study
695		
pJWV25-M3	amp ^r , tet ^r , bgaA, P _{czcD} - <i>spr0693-0694-0695</i> _{K207A-R208A-K210A}	This study
pJWV25-3C	amp ^r , tet ^r , bgaA,	This study
	P _{czcD} -spr0693-0694-0695 _{D49C-R208C/K52C-E205C/E374C-K207C}	
Primers for MICs	Nucleotide sequence (5' to 3')	
Up-F	GCGATATAACCAGCGCCTAGAATGGCAACTG	This study
Up-R	CAAACTCTGTGCTCGAGCACTCGCAGCACCGATTGCTGC	This study
	ATAC	
Down-F	CAGCATTATCCTCTAGAGAAGTTGGAGTATCAATCCCAGT	This study
	TGCCC	
Down-R	GCGTAGCCAAGAGCTGTCGCATAGTTAAGAAG	This study
JC-F	GGTGCTGCGAGTGCTCGAGCACAGAGTTTGTAGAAACGC	This study
	AAAAAGG	
JC-R	GATTGATACTCCAACTTCTCTAGAGGATAATGCTGAAAAC	This study
	TCCTTGAAG	
pJWV25-F	TGATAAGCTGAGCGCCGGTCGCTACCA	This study
pJWV25-R	CTTTTTCTTCATCTTGTCATCGTCATCCTTGTAGTCG	This study
pJWV25-Spr0693-F	GACCGGCGCTCAGCTTATCATTCATAACGAAGGGCTTC	This study
pJWV25-Spr0695-R	GACGATGACAAGATGAAGAAAAAGAATGGTAAAG	This study
Spr0695 _{K207A-R208A-K} _{210A} -F	GGAGGCTGCAGCGTCAGCAATATATGGGTTTGGTG	This study
Spr0695 _{K207A-R208A-K} _{210A} -R	CCCATATATTGCTGACGCTGCAGCCTCCGGACTAGTA	This study
Spr0695 _{D49C-K52C} -F	CAAGTCAATAAATGTATGACTTGCTCTCAGAAAAATATT	This study
	AGCGTC	
Spr0695 _{D49C-K52C} -R	GCTAATATTTTTCTGAGAGCAAGTCATACATTTATTGACT	This study
	TGACGAGATAGG	
Spr0695 _{E205C-K207C-R2}	GTTTATACTAGTCCGTGTGCTTGTTGCTCAAAAATATATG	This study
_{08C} -F	GGTTTGG	

105 Supplementary Table 1 Bacterial strains, plasmids and primers used in this study

Spr0695 _{E205C-K207C-R2}	CCATATATTTTTGAGCAACAAGCACACGGACTAGTATAA	This study
08C-K		
Spr0695 _{E374C} -F	C	This study
Spr0695 _{E374C} -R	CCAACTTCTATACCGCATATTAAACCTTGCAGTAACAAAC	This study
Primers for protein	Nucleotide sequence (5' to 3')	
preparation		
pBAD-Spr0694-069	CATCATGGTACCATGAAGCAACTAATTAGTCTAAAAAATA	This study
5-F1	TCTTCAG	
pBAD-Spr0694-069	CTAATTAGTTGCTTCATGGTACCATGATGATGATGATGATGATG	This study
5-R1	AGAACC	
pBAD-Spr0694-069 5-F2	CGTTATGAATGATGAAGCTTGGGCCCGAACAAAAACTC	This study
pBAD-Spr0694-069	GGCCCAAGCTTCATCATCATAACGAAGGGCTTCAATTGG	This study
5-R2	ATC	
Spr0694 _{E170Q} -F	GCGGATCAGCCGACAGGAGCCTTGGATACC	This study
Spr0694 _{E170Q} -R	CTGTCGGCTGATCCGCTAGGATAATAGAAGG	This study
Spr0694 _{G173C} -F	CCGACATGTGCCTTGGATACCAAAACAGGTAAC	This study
Spr0694 _{G173C} -R	CCAAGGCACATGTCGGTTCATCCGCTAGG	This study
Spr0695 _{K207A-R208A-K} _{210A} -F	GGAGGCTGCAGCGTCAGCAATATATGGGTTTGGTG	This study
Spr0695 _{K207A-R208A-K} _{210A} -R	CCCATATATTGCTGACGCTGCAGCCTCCGGACTAGTA	This study
pET29b-Spr0693-06	GAAGGAGATATACATATGAAGAAAAAGAATGGTAAAGCT	This study
94-0695-F1	AAAAGTG	
pET29b-Spr0693-06	CCATTCTTTTTCTTCATATGTATATCTCCTTCTTAAAGTTAA	This study
94-0695-R1	ACAAAATTATTTCTAG	
pET29b-Spr0693-06 94-0695-F2	CCTTCGTTATGAACACCACCACCACCACCACTGAGATC	This study
pET29b-Spr0693-06 94-0695-R2	TGGTGGTGGTGTTCATAACGAAGGGCTTCAATTGGATC	This study
pET29b-Spr0693-Hi s-F	TGAAGCAACTAATCACCACCACCACCACCACTGAGATCC	This study
pET29b-Spr0693-Hi s-R	CTCAGTGGTGGTGGTGGTGGTGATTAGTTGCTTCATCAGT CTTCACCTCTTTTCC	This study
pET29b-Spr0693-St	CAACTAATTGGTCTCACCCCCAATTTGAGAAGTGAGATCC	This study
repII-F	GGCTGCTAACAAAGCC	
pET29b-Spr0693-St	GGATCTCACTTCTCAAATTGGGGGGTGAGACCAATTAGTTG	This study
repII-R	CTTCATCAGTCTTCACCTC	
Spr0693 ₃₄₋₃₉₉ -F	GGAGATATACATATGAGACAACCTTCTCAGACTGCTCTAA	This study
Spr0693 ₃₄₋₃₉₉ -R	GTGGTGGTGGTGGTGGTGATTAGTTGCTTCATCAGTCTTC	This study
Spr0693 ₅₉₋₃₂₄ -F	GAAGGAGATATACATATGTCTGTTTTATTGTCAGGGACAGT A	This study
Spr0693 ₅₉₋₃₂₄ -R	CAGTGGTGGTGGTGGTGGTGCTTAGTTTTGCTTTTAACCT	This study

			С		
	Spr06	93 ₇₀₋₂₃₇ -F	GAAGGAGATATACATATGAATGAACAATATGTTTATTTTG	This study	
	Spr06	93 ₇₀₋₂₃₇ -R	CAGTGGTGGTGGTGGTGGTGCTTGACTTGTAAATTTTCAT	This study	
			TGC		
	Spr06	93 _{т197М} -F	GGATGCAATGACTGTTCTCAGTACCCT	This study	
	Spr06	93 _{T197M} -R	GAACAGTCATTGCATCCAATTGACTTTG	This study	
	Spr06	93 _{I317М} -F	CAACATGGAGGTTAAAAGCAAAACT	This study	
	Spr06	93 _{I317M} -R	TAACCTCCATGTTGACAGAAAAACCC	This study	
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