SUPPORTING INFORMATION

Modular Architecture and Unique Teichoic Acid Recognition Features of Choline-Binding Protein L (CbpL) Contributing to Pneumococcal Pathogenesis

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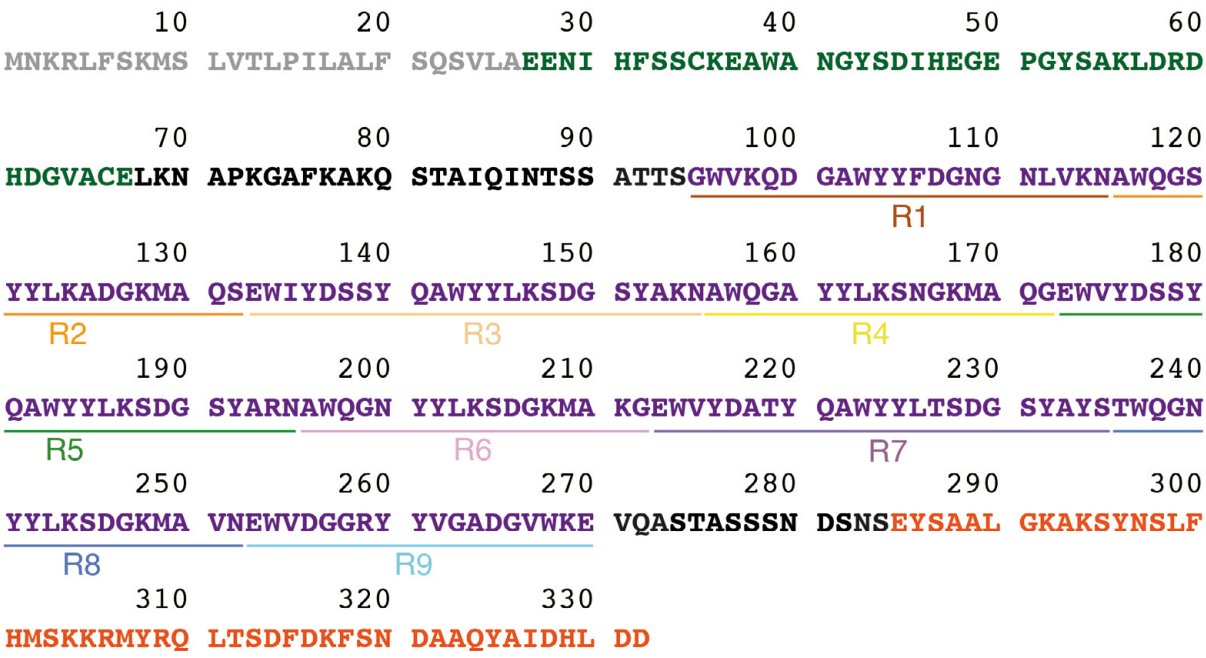

Fig. S1. Amino acid sequence of CbpL.

Color code for the different parts of the protein is as follows: signal peptide (gray), Excalibur domain (green), linkers (black), choline binding domain (CBD, purple), Ltp_Lipoprotein domain (red). Sequence of the choline binding repeats (R1 to R9) integrating the CBD is underlined in different colors.

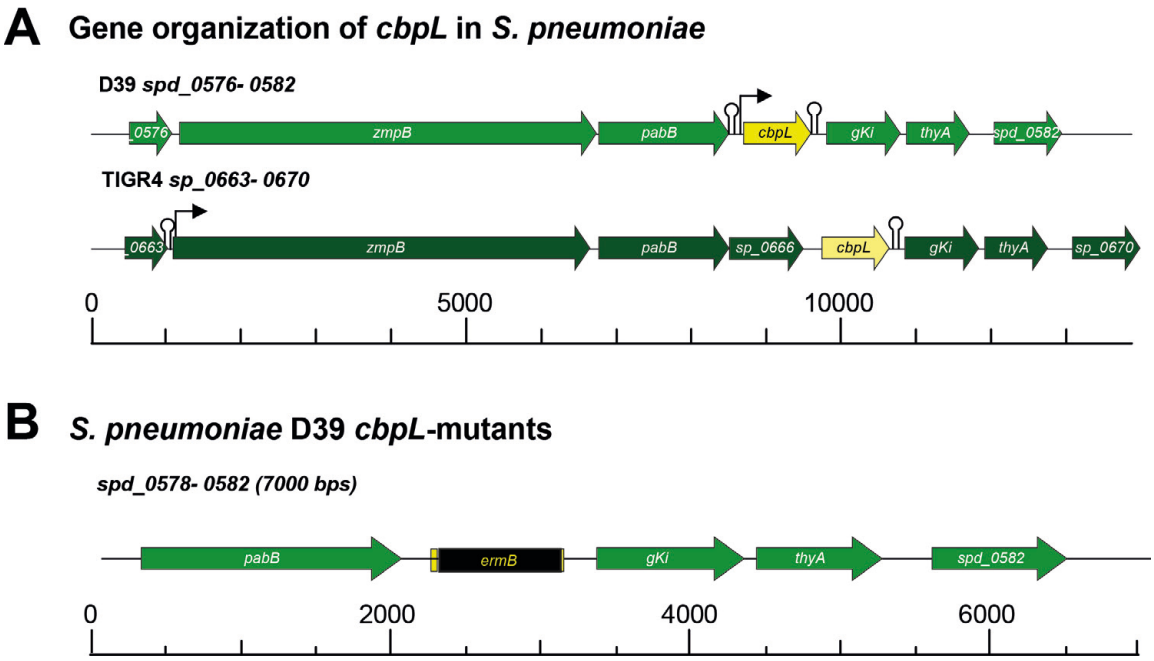

Fig. S2. Molecular organization of the *cbpL*-gene loci in *Streptococcus pneumoniae* D39 and TIGR4.

(**A**) The *in silico* analysis revealed a monocistronic organization of the CbpL-encoding gene in D39 and a polycistronic organization in TIGR4. The protein accession is YP_816078.1. The two genes upstream of *cbpL* in D39 encode a zinc metalloprotease (ZmpB; SPD_0577 in D39) and a *para*-aminobenzoic acid synthetase (PabB; SPD_0578 in D39). The genes downstream of *cbpL* encode a glucokinase (Gki; SPD_0580) and thymidylate synthase (ThyA; SPD_0581), respectively. In TIGR4 the gene *sp_0666* is annotated between *cbpL* and *pabB* and suggested to encode a putative pyrimidine utilization protein [1]. Putative promoters (arrows) were predicted by the Neural Network Promoter Prediction program (http://www.fruitfly.org/seq_tools/promoter.html) and potential rho-independent termination sequences were extracted from the TransTermHPTerminator Prediction list of *S. pneumoniae* D39 (http://transterm.cbcb.umd.edu/tt/Streptococcus_pneumoniae_D39.tt). The sequences

were obtained from the Kyoto Encyclopedia for Genes and Genomes (KEGG). (**B**) Mutants deficient in CbpL-expression were constructed by deletion-insertion mutagenesis. This resulted in a complete deletion of the *cbpL* gene sequence and insertion of an *ermB* gene cassette.

Pneumococcal CbpL protein sequence alignment

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D39            SPD_0579         MNKRLFLKMS LVTLPILALF SQPVLAEENI HFSSCKEAWA NGYSDIHEGE PGYSAKLDRD   60 aa
R6             SPR_0583         MNKRLFLKMS LVTLPILALF SQPVLAEENI HFSSCKEAWA NGYSDIHEGE PGYSAKLDRD
TIGR4          SP_0667          MNKRLFSKMS LVTLPILALF SQSVLAEENI HFSSCKEAWA NGYSDIHEGE PGYSAKLDRD
OXC141         SPNOXC_06120     MNKRLFLKMS LVTLPILALF SQPVLAEENI HFSSCKEAWA NGYSDIYEGE PGYSAKLDRD
SPN034156      SPN034156_16610  MNKRLFLKMS LVTLPILALF SQPVLAEENI HFSSCKEAWA NGYSDIYEGE PGYSAKLDRD
AP200          SPAP_0656        MNKRLFLKMS LVTLPILALF SQPVLAEENI HFSSCKEAWA NGYSDLHEGE PGYSAKLDRD
CGSP14         SPCG_0623        MNKRLFLKMS LVTLPILALF SQPVLAEENI HFSSCKEAWA NGYSDIHEGE PGYSAKLDRD
INV200         SPNINV200_05890  MNKRLFLKMS LVTLPILALF SQPVLAEENI HFSSCKEAWA NGYSDIHEGE PGYSAKLDRD
Taiwan19f-14   SPT_0691         MNKRLFLKMS LVTLPILALF SQPVLAEENI HFSSCKEAWA NGYSDIHEGE PGYSAKLDRD
Hungary19A6    SPH_0762         MNKRLFLKMS LVTLPILALF SQPVLAEENI HFSSCKEAWA NGYSDIHEGE PGYSAKLDRD
G54            SPG_0608         MNKRLFLKMS LVTLPILALF SQPVLAEENI HFSSCKEAWA NGYSDIHEGE PGYSAKLDRD
SPN994039      SPN994039_06030  MNKRLFLKMS LVTLPILALF SQPVLAEENI HFSSCKEAWA NGYSDIYEGE PGYSAKLDRD
                                ****** *** ********** **.******* ********** *****::*** **********

D39            SPD_0579         HDGVACELKN APKGAFKAKQ STAIQINTSS ATTSGWVKQD GAWYYFDGNG NLVKNAWQGS   120 aa
R6             SPR_0583         HDGVACELKN APKGAFKAKQ STAIQINTSS ATTSGWVKQD GAWYYFDGNG NLVKNAWQGS
TIGR4          SP_0667          HDGVACELKN APKGAFKAKQ STAIQINTSS ATTSGWVKQD GAWYYFDGNG NLVKNAWQGS
OXC141         SPNOXC_06120     HDGVACELKN APKGAFKAKQ STAIQINTSS ATTSGWVKQD GAWYYFDGNG NLVKNAWQGS
SPN034156      SPN034156_16610  HDGVACELKN APKGAFKAKQ STAIQINTSS ATTSGWVKQD GAWYYFDGNG NLVKNAWQGS
AP200          SPAP_0656        HDGVACELKN APKGAFKAKQ STAIQINTSS ATTSGWVKQD GAWYYFDGNG NLVKNAWQGS
CGSP14         SPCG_0623        HDGVACELKN APKGAFKAKQ STAIQINTSS ATTSGWVKQD GAWYYFDGNG NLVKNAWQGS
INV200         SPNINV200_05890  HDGVACELKN APKGAFKAKQ STAIQINTSS ATTSGWVKQD GAWYYFDGNG NLVKNAWQGS
Taiwan19f-14   SPT_0691         HDGVACELKN APKGAFKAKQ AATTQTDTTS STASGWVKQD GSWYYFDGNG NLVKNAWQGN
Hungary19A6    SPH_0762         HDGVACELKN APKGAFKAKQ STAIQINTSS ATTSGWVKQD GAWYYFDGNG NLVKNAWQGS
G54            SPG_0608         HDGVACELKN APKGAFKAKQ STAIQINTSS ATTSGWVKQD GAWYYFDGNG NLVKNAWQGS
SPN994039      SPN994039_06030  HDGVACELKN APKGAFKAKQ STAIQINTSS ATTSGWVKQD GAWYYFDGNG NLVKNAWQGS
                                ********** ********** ::: * :*:* :*:******* *:******** *********.

D39            SPD_0579         YYLKADGKMA QSEWIYDSSY QAWYYLKSDG SYAKNAWQGA YYLKSNGKMA QGEWVYDSSY   180 aa
R6             SPR_0583         YYLKADGKMA QSEWIYDSSY QAWYYLKSDG SYAKNAWQGA YYLKSNGKMA QGEWVYDSSY
TIGR4          SP_0667          YYLKADGKMA QSEWIYDSSY QAWYYLKSDG SYAKNAWQGA YYLKSNGKMA QGEWVYDSSY
OXC141         SPNOXC_06120     YYLKADGKMA QSEWIYDSSY QAWYYLKSDG SYAKNAWQGA YYLKSNGKMV QGEWVYDSSY
SPN034156      SPN034156_16610  YYLKADGKMA QSEWIYDSSY QAWYYLKSDG SYAKNAWQGA YYLKSNGKMV QGEWVYDSSY
AP200          SPAP_0656        YYLKADGKMA QSEWIYDSSY QAWYYLKSDG SYAKNAWQGA YYLKSNGKMA QGEWVYDSSY
CGSP14         SPCG_0623        YYLKADGKMA QSEWIYDSSY QAWYYLKSDG SYAKNAWQGA YYLKSNGKMA QGEWVYDSSY
INV200         SPNINV200_05890  YYLKADGKMA QSEWIYDSSY QAWYYLKSDG SYAKNAWKGA YYLKSNGKMA QGEWVYDSSY
Taiwan19f-14   SPT_0691         YYLKADGKMA QSEWIYDSSY QAWYYLKSDG SYARNAWQGN YYLKSDGKMA KGEWVYDATY
Hungary19A6    SPH_0762         YYLKADGKMA QSEWIYDSSY QAWYYLKSDG SYAKNAWQGA YYLKSNGKMA QGEWVYDSSY
G54            SPG_0608         YYLKADGKMA QSEWIYDSSY QAWYYLKSDG SYAKNAWQGA YYLKSNGKMA QGEWVYDSSY
SPN994039      SPN994039_06030  YYLKADGKMA QSEWIYDSSY QAWYYLKSDG SYAKNAWQGA YYLKSNGKMV QGEWVYDSSY
                                ********** ********** ********** ***:***:*  *****:***. :******::*

D39            SPD_0579         QAWYYLKSDG SYARNAWQGN YYLKSDGKMA KGEWVYDATY QAWYYLTSDG SYAYSTWQGN   240 aa
R6             SPR_0583         QAWYYLKSDG SYARNAWQGN YYLKSDGKMA KGEWVYDATY QAWYYLTSDG SYAYSTWQGN
TIGR4          SP_0667          QAWYYLKSDG SYARNAWQGN YYLKSDGKMA KGEWVYDATY QAWYYLTSDG SYAYSTWQGN
OXC141         SPNOXC_06120     QAWYYLKSDG ---------- -------KMA VNEWVYDATY QAWYYLTSDG SYAYSTWQGN
SPN034156      SPN034156_16610  QAWYYLKSDG ---------- ---------- ---------- ---------- SYARNAWQGN
AP200          SPAP_0656        QAWYYLKSDG SYARNAWQGN YYLKSDGKMA KGEWVYDATY QAWYYLTSDG SYAYSTWQGN
CGSP14         SPCG_0623        QAWYYLKSDG SYARNAWQGN YYLKSDGKMA KGEWVYDATY QAWYYLTSDG SYAYSTWQGN
INV200         SPNINV200_05890  QAWYYLKSDG SYARNAWQGN YYLKSDGKMA KGEWVYDATY QAWYYLTSDG SYAYSTWQGN
Taiwan19f-14   SPT_0691         QAWYYLKSDG SYARNAWQGN YYLKSDGKMA KGEWVYDATY QAWYYLTSDG SYAYSTWQGN
Hungary19A6    SPH_0762         QAWYYLKSDG SYARNAWQGN YYLKSDGKMA KGEWVYDATY QAWYYLTSDG SYAYSTWQGN
G54            SPG_0608         QAWYYLKSDG SYARNAWQGN YYLKSDGKMA KGEWVYVATY QAWYYLTSDG SYAYSTWQGN
SPN994039      SPN994039_06030  QAWYYLKSDG SYARNAWQGN YYLKSDGKMA VNEWVYDATY QAWYYLTSDG SYAYSTWQGN
                                **********                                             ***  .:****

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R6             SPR_0583         YYLKSDGKMA VNEWVDGGRY YVGADGVWKE GQASTASSSN DSNSEYSAAL GKAKSYNSLF
TIGR4          SP_0667          YYLKSDGKMA VNEWVDGGRY YVGADGVWKE VQASTASSSN DSNSEYSAAL GKAKSYNSLF
OXC141         SPNOXC_06120     YYLKSDGKMA VNEWVDGGRY YVGADGVWKE GQASTASSSN DSNSEYSAAL GKAKSYNSLF
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AP200          SPAP_0656        YYLKSDGKMA VNEWVDGGRY YVGADGVWKE GQASTASSSN DSNSEYSAAL GKAKSYNSLF
CGSP14         SPCG_0623        YYLKSDGKMA VNEWVDGGRY YVGADGVWKE GQASTVSSSN DSNSEYSAAL GKAKSYNSLF
INV200         SPNINV200_05890  YYLKSDGKMA VNEWVDGGRY YVGADGVWKE GQASTVSSSN DSNSEYSAAL GKAKSYNSLF
Taiwan19f-14   SPT_0691         YYLKSDGKMA VNEWVDGGRY YVGADGVWKE GQASTASSSN DSNSEYSAAL GKAKTYNSLF
Hungary19A6    SPH_0762         YYLKSDGKMA VNEWVDGGRY YVGADGVWKE GQASTASSSN DSNSEYSAAL GKAKSYNSLF
G54            SPG_0608         YYLKSDGKMA VNEWVDGGRY YVGADGVWKE GQASTASSSN DSNSEYSAAL GKAKSYNSLF
SPN994039      SPN994039_06030  YYLKSDGKMA VNEWVDGGRY YVGADGVWKE GQASTASSSN DSNSEYSAAL GKAKSYNSLF
                                ********** ********** **********  ****.**** ********** ****:*****

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R6             SPR_0583         HMSKKRMYRQ LTSDFDKFSN DAAQYAIDH- --
TIGR4          SP_0667          HMSKKRMYRQ LTSDFDKFSN DAAQYAIDHL DD
OXC141         SPNOXC_06120     HMSKKTYV-- ---------- ---------- --
SPN034156      SPN034156_16610  HMSKKTYV-- ---------- ---------- --
AP200          SPAP_0656        HMSKKRMYRQ LTSDFDKFSN DAAQYAIDHL DD
CGSP14         SPCG_0623        HMSKKRMYRQ LTSDFDKFSN DAAQYAIDHL DD
INV200         SPNINV200_05890  HMSKKRMYRQ LTSDFDKFSN DAAQYAIDHL DD
Taiwan19f-14   SPT_0691         HMSKKRMYRQ LTSDFDKFSN DAAQYAIDHL DD
Hungary19A6    SPH_0762         HMSKKTYV-- ---------- ---------- --
G54            SPG_0608         HMSKKTYV-- ---------- ---------- --
SPN994039      SPN994039_06030  HMSKKTYV-- ---------- ---------- --
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signal peptide

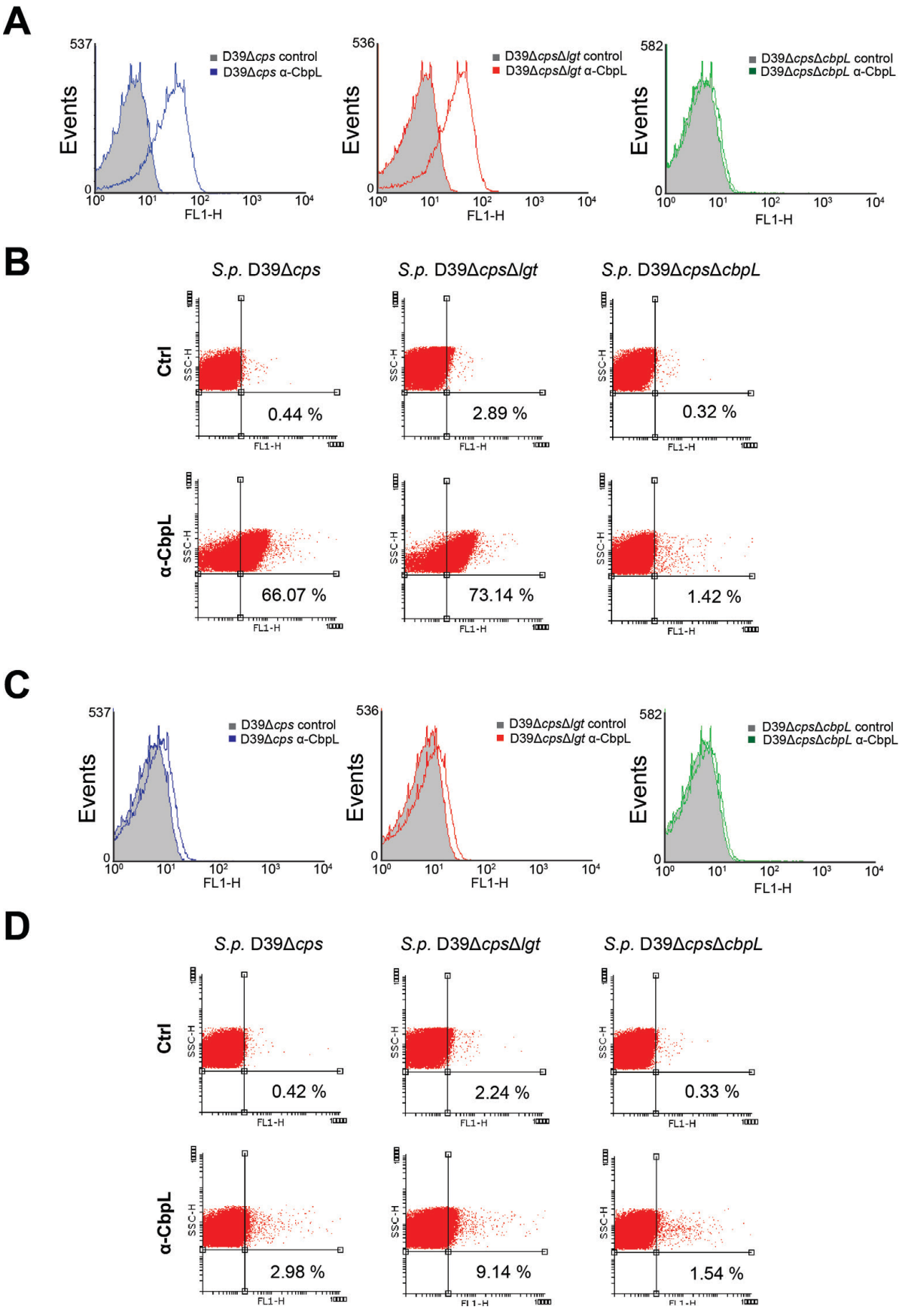
Excalibur domain (DxDXDGXXCE)

Choline Binding Module (CBM)

Ltp-Lipoprotein domain

Fig. S3. Multiple amino acid sequence alignment of pneumococcal CbpL.

Alignment performed with Clustal Omega alignment tool (http://www.ebi.ac.uk/Tools/msa/clustalo/) with *cbpL* sequences as deposited in databases for 12 strains.


A
537
Events
0
D39Δcps control
D39Δcps α-CbpL
FL1-H
536
D39ΔcpsΔlgt control
D39ΔcpsΔlgt α-CbpL
582
D39ΔcpsΔcbpL control
D39ΔcpsΔcbpL α-CbpL
B
S.p. D39Δcps
S.p. D39ΔcpsΔlgt
S.p. D39ΔcpsΔcbpL
Ctrl
α-CbpL
SSC-H
0.44 %
2.89 %
0.32 %
66.07 %
73.14 %
1.42 %
C
D
0.42 %
2.24 %
0.33 %
2.98 %
9.14 %
1.54 %

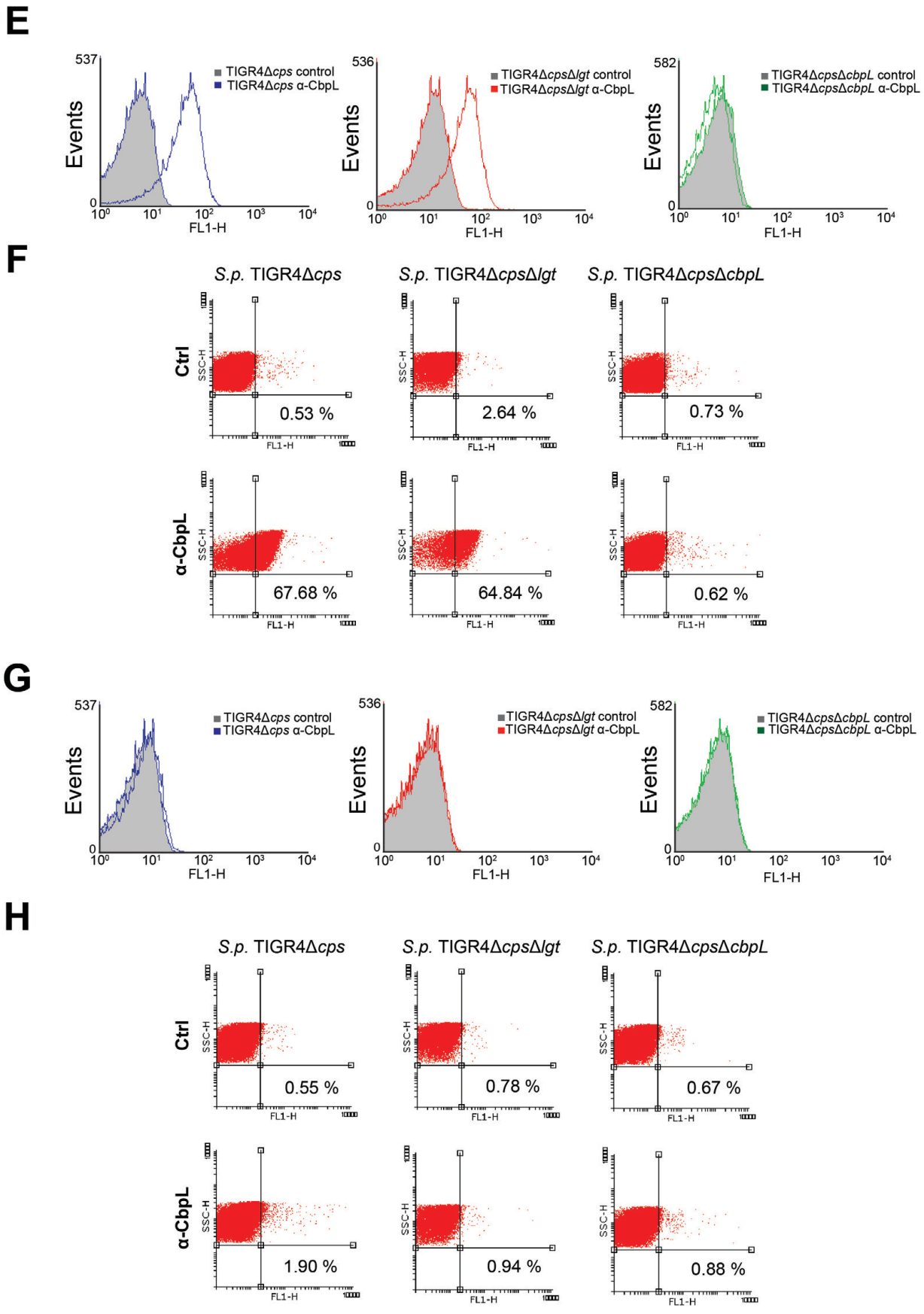


Fig. S4. Surface abundance of CbpL determined by flow cytometry.

The surface abundance of CbpL was measured by flow cytometry using anti-CbpL polyclonal antibodies (1:500) as primary antibodies and secondary goat anti-mouse IgG coupled Alexa-Fluor-488 (1:1000; Invitrogen). The non-encapsulated D39Δ*cps* (**A – D**) or TIGR4Δ*cps* (**E – H**) and their isogenic *lgt*- and *cbpL* mutants were incubated with the antibodies and the fluorescence intensity measured in a FACSCalibur™. The surface abundance of CbpL was measured prior (**A – B** and **E – F**) and post-treatment (**C – D** and **G – H**) of pneumococci with choline chloride (10% ChCl for 30 min). The individual histograms showing the increase in fluorescence intensity (forward scatter: FL1-H) for each strain tested (control: only the secondary anti-mouse IgG Alexa-Fluor conjugate antibody; CbpL detection: incubation with 1st and 2nd antibodies) are shown in **A, C, E**, and **G**. Dot plots of the flow cytometric analyses including the percent of positive events when counting 50000 events are shown in **B, D, F**, and **H**.

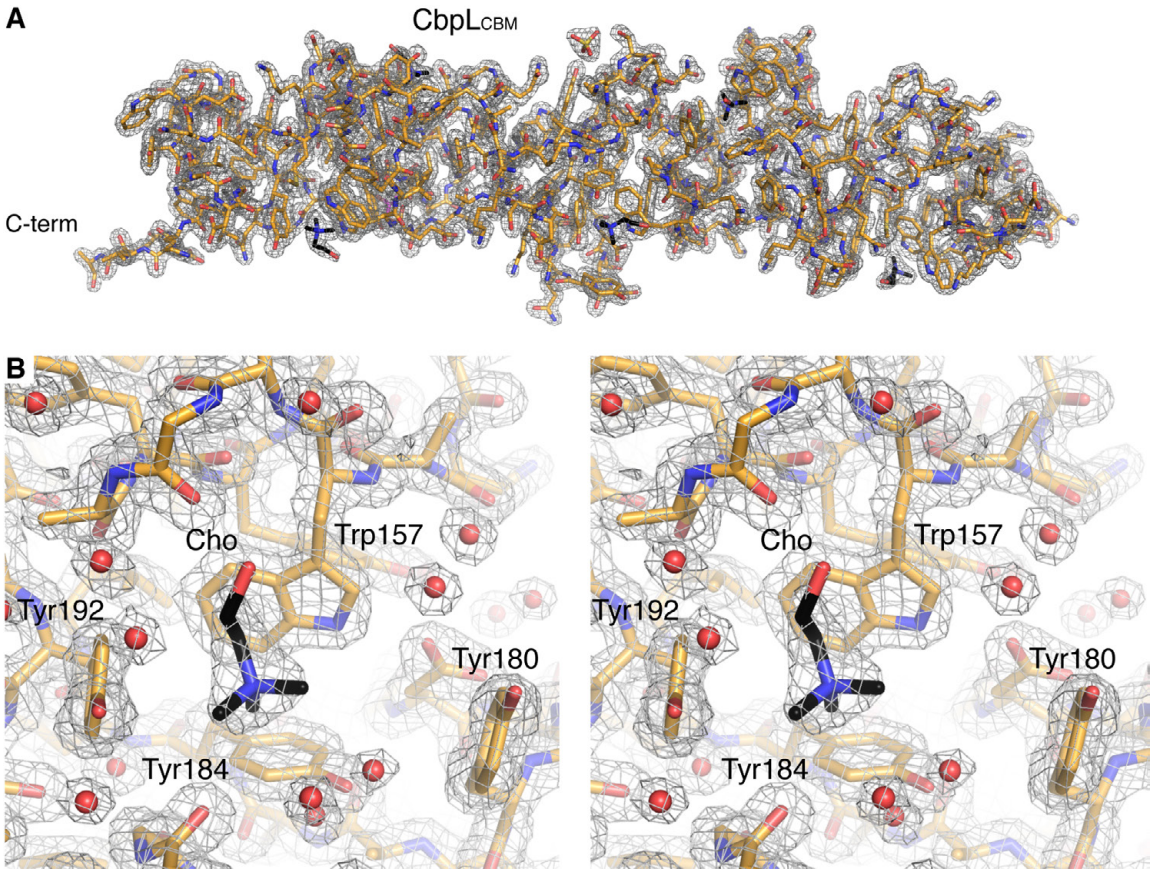


Fig. S5. Electron density in CbpL$_{CBM}$.

(**A**) Electron density map for the complete CbpL$_{CBM}$:choline complex. (**B**) Stereo view showing a detail of the electron density map in a non-canonical choline-binding site. Residues interacting with choline are represented as capped sticks and labeled. Water molecules represented as red spheres. Electron density maps correspond to the σ-weighted 2Fo–Fc electron density map contoured at 1.0 σ.

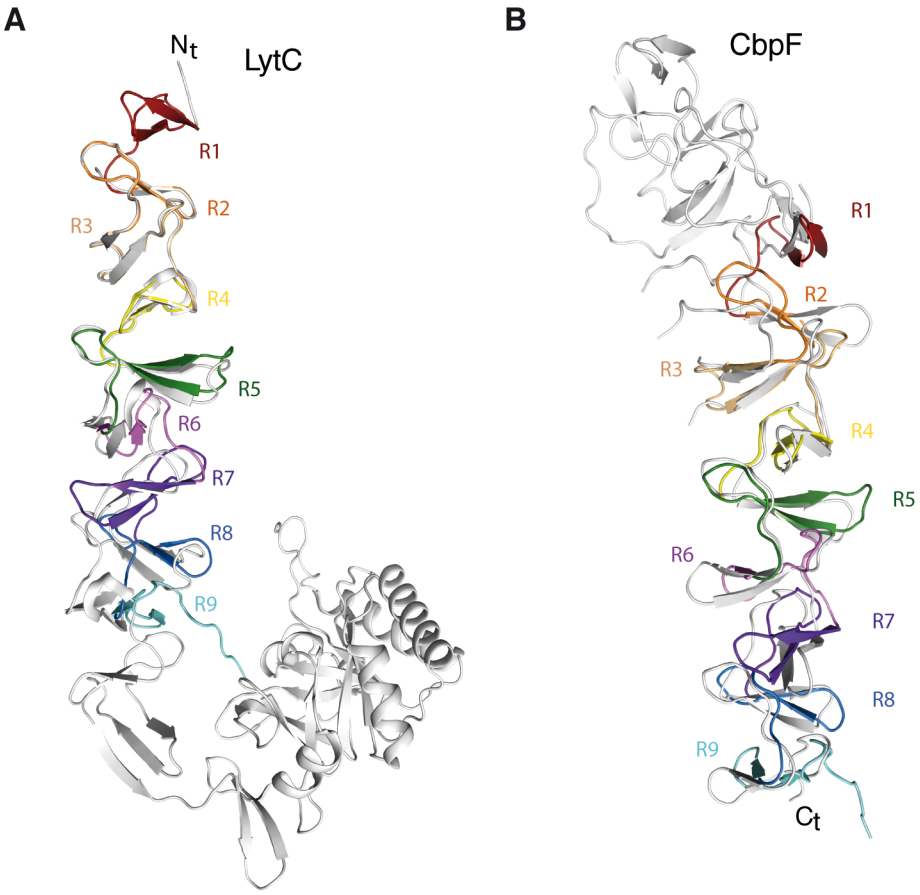


Fig. S6. Structural comparison of CbpL$_{CBM}$ with closest structural homologues.

(**A**) Structural superimposition of pneumococcal autolysin LytC (colored in grey) (PDB code 2WWD) with CbpL$_{CBM}$ (each repeat colored differently) results in a rmsd of 1.9 Å for 155 Cα atoms. (**B**) Structural superimposition of pneumococcal CbpF (colored in grey) (PDB code 2V05) with CbpL$_{CBM}$ (each repeat colored differently) results in a rmsd of 3.1 Å for 173 Cα atoms.

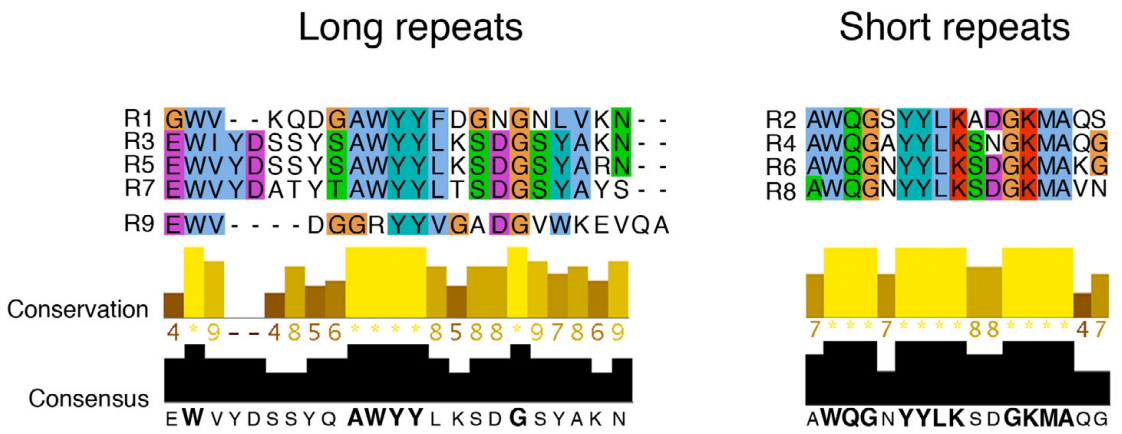


Fig. S7. Sequence comparison of choline-binding repeats in CbpL.

Long choline-binding repeats (left) and short choline-binding repeats (right) in CbpL are compared. Sequence alignments were performed with Clustal Omega and visualized using Jalview. R9 was not considered for the alignments.

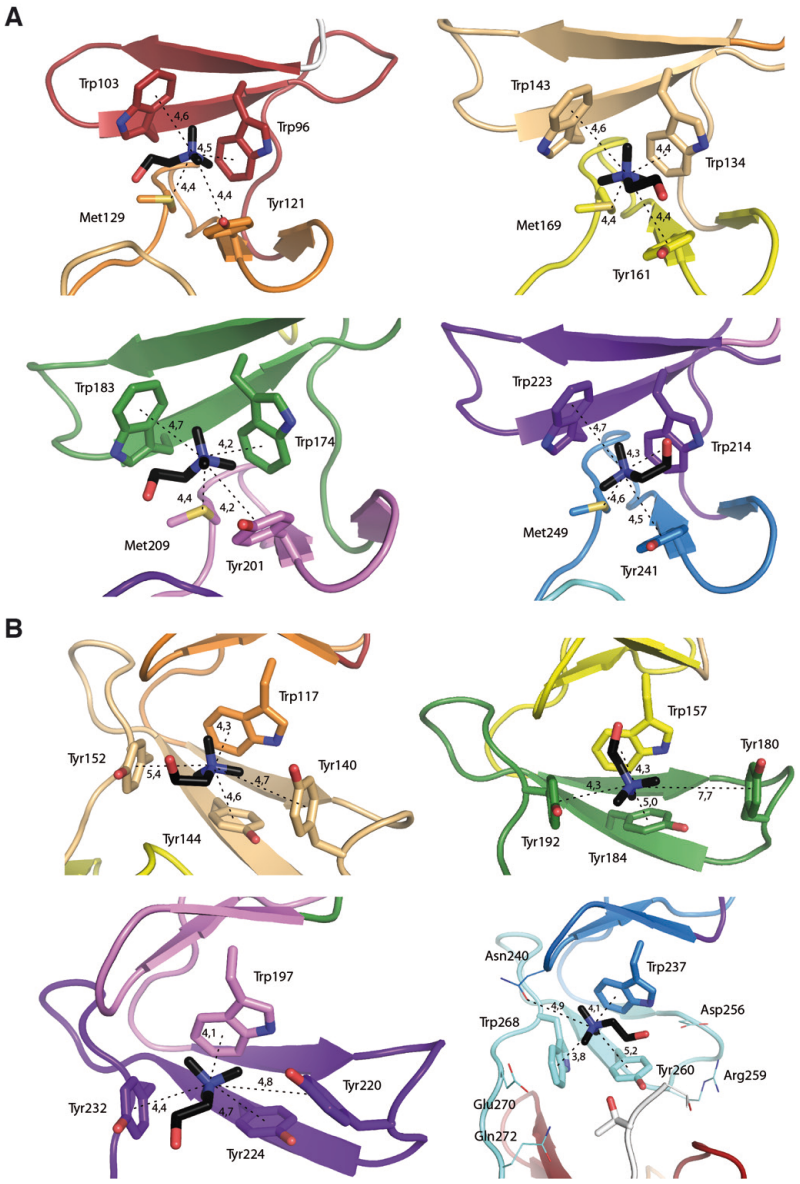


Fig. S8. Structural description of choline-binding sites in CbpL.

(**A**) The four canonical choline-binding sites present in CbpL (repeats are colored as in Fig 3). (**B**) The four non-canonical choline-binding sites present in CbpL (colored as in Fig 3) with main residues involved in choline stabilization depicted as capped sticks. Choline moieties attached to the choline-binding sites are represented as capped sticks with C atoms in black.

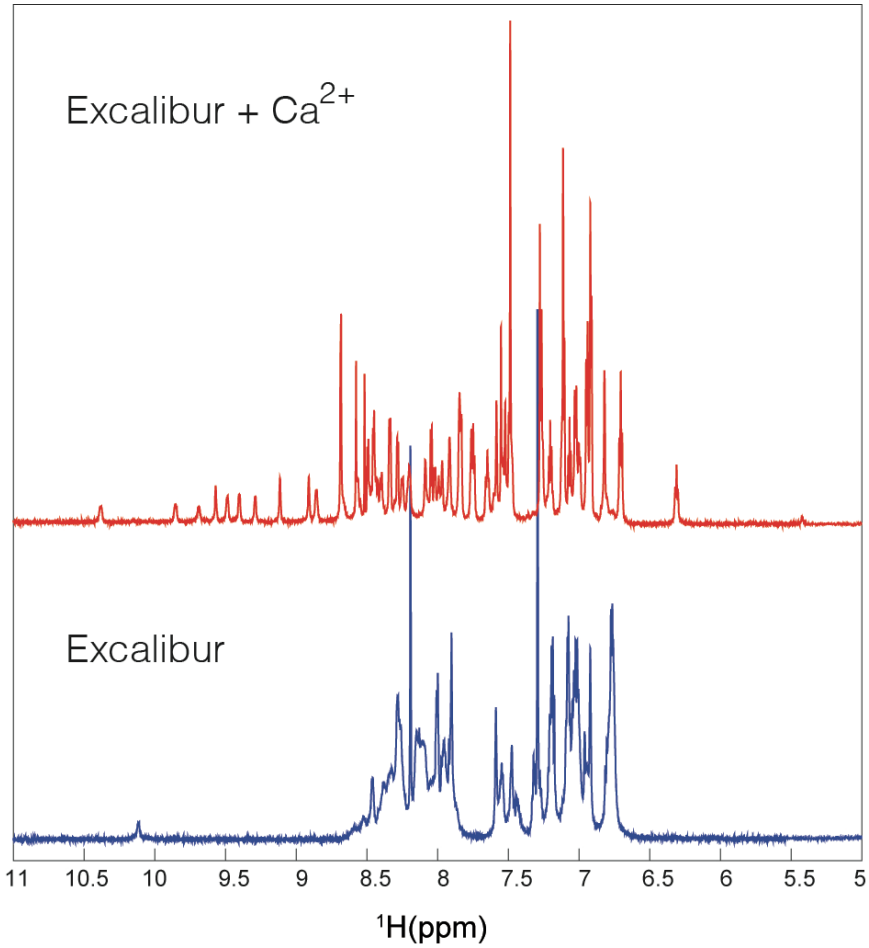


Fig. S9. Folding of the Excalibur domain requires Ca^{2+} binding.

NH region of the ^{1}H NMR spectra of Excalibur, 0.5 mM, pH 5.5, 800 MHz. Without Ca^{2+} the spectrum (colored in blue) shows severe signal overlapping in a narrow chemical shift range compatible with an unfolded peptide conformation. The same spectrum saturated with Ca^{2+} (colored in red) shows a large signal dispersion characteristic of a folded structure.

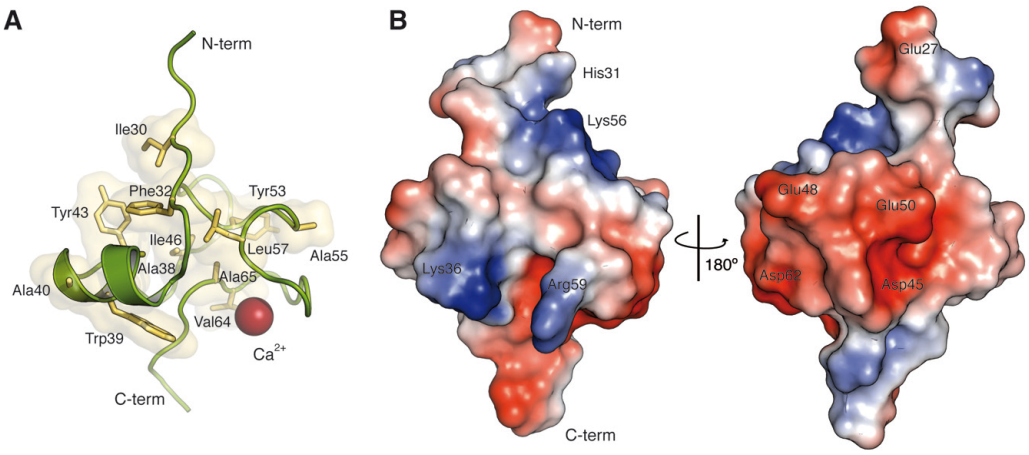


Fig. S10. Structural features of the Excalibur domain.

(**A**) A strong hydrophobic core is built with aromatic and hydrophobic residues, some of them are exposed. (**B**) Electrostatic potential surface of the Excalibur domain presents two faces, one of them with prevalence of positively-charged residues and the other (roughly 180º apart) presenting negatively-charged residues.

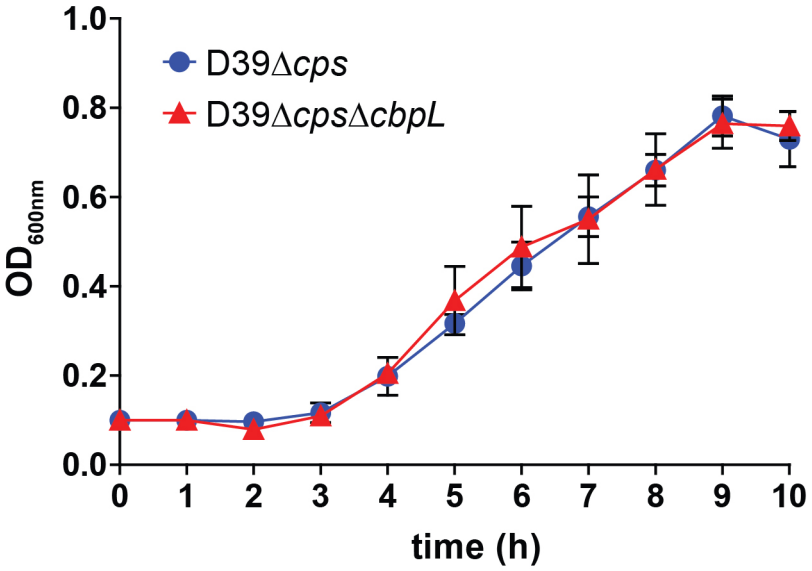


Fig. S11. Growth of pneumococci is not affected in pneumococci lacking CbpL. Pneumococci were cultured in the chemically defined medium $RPMI_{modi}$ [2] under microaerophilic conditions at 37 °C. At each indicated time point the optical density of the culture was measured at 600 nm.

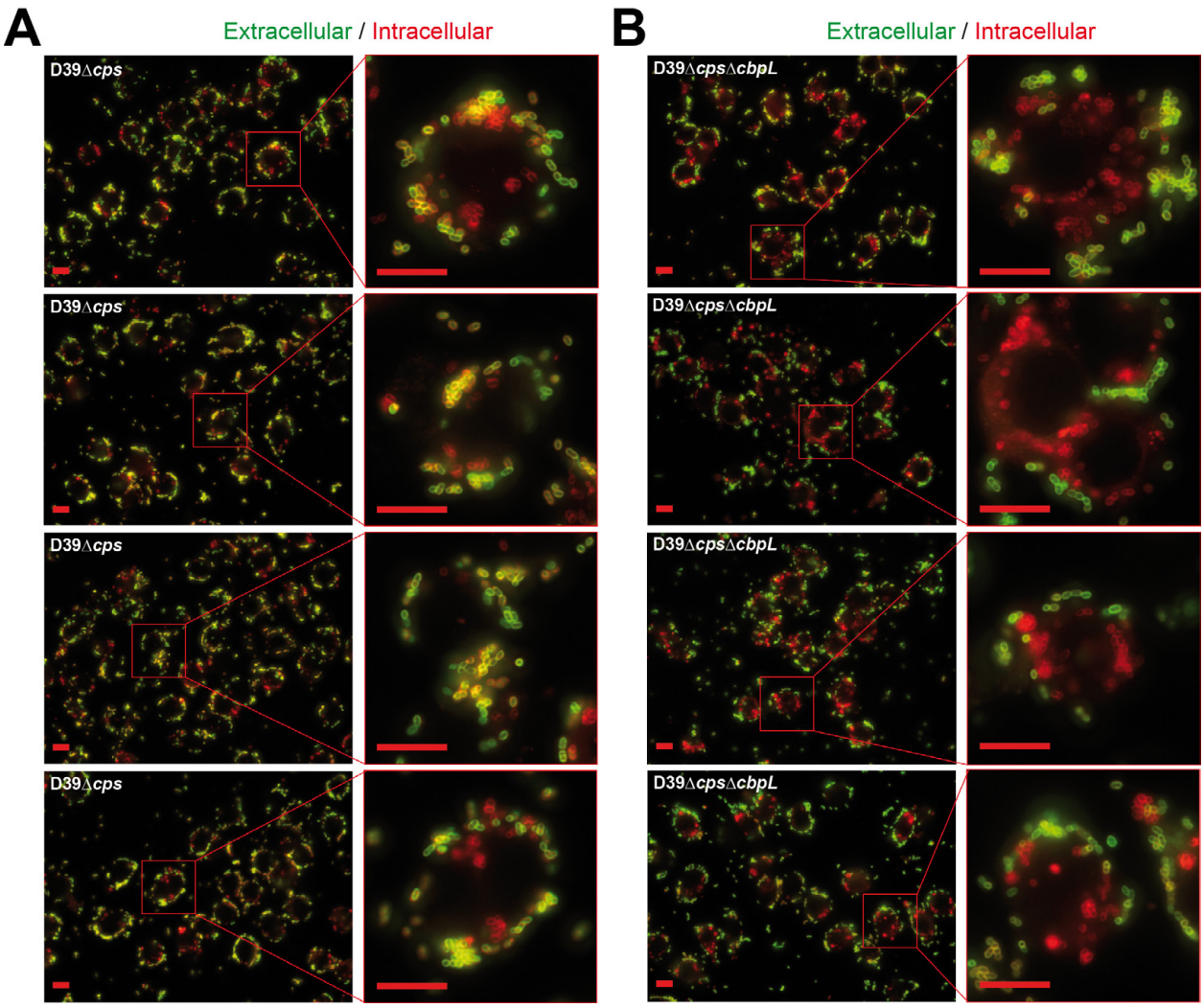


Fig. S12. Immunofluorescence microscopy of macrophage-associated and phagocytosed pneumococci.

Representative immunofluorescence microscopic images of D39Δ*cps* (**A**) and CbpL-deficient D39Δ*cps*Δ*cbpL* (**B**) pneumococci attached (green) to J774 macrophages and internalized by J774 macrophages (red) 30 min post infection with an MOI of 50 bacteria per macrophage. Extracellular pneumococci were stained with goat anti-rabbit Alexa Fluor 488 (green) after using anti-pneumococcal polyclonal antibodies as the first antibody, while intracellular pneumococci were stained after Triton™ X-100 permeabilization with goat anti-rabbit Alexa Fluor 568 (red) post Triton X-100

treatment using again rabbit anti-pneumococcal polyclonal antibodies as the first antibody. Bar represents 10μm.

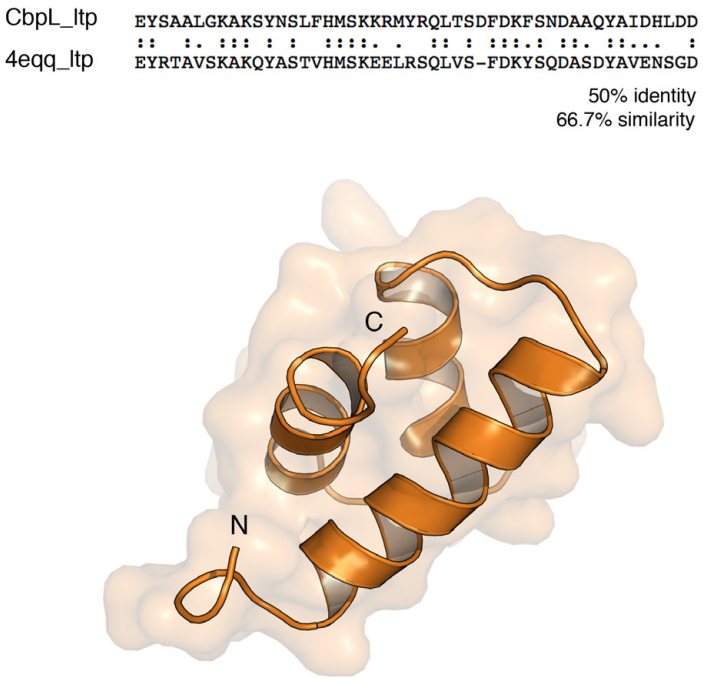


Fig. S13. Homology modeling of CbpL Ltp_Lipoprotein domain.

Homology modeling was performed using the online Swiss-Prot server using $Ltp_{TP\text{-}J34}$ protein (PDB code 4EQQ) from temperate phage TP-J34 of *Streptococcus thermophilus* as template [3-5]. Sequence alignment was performed using ClustalW.

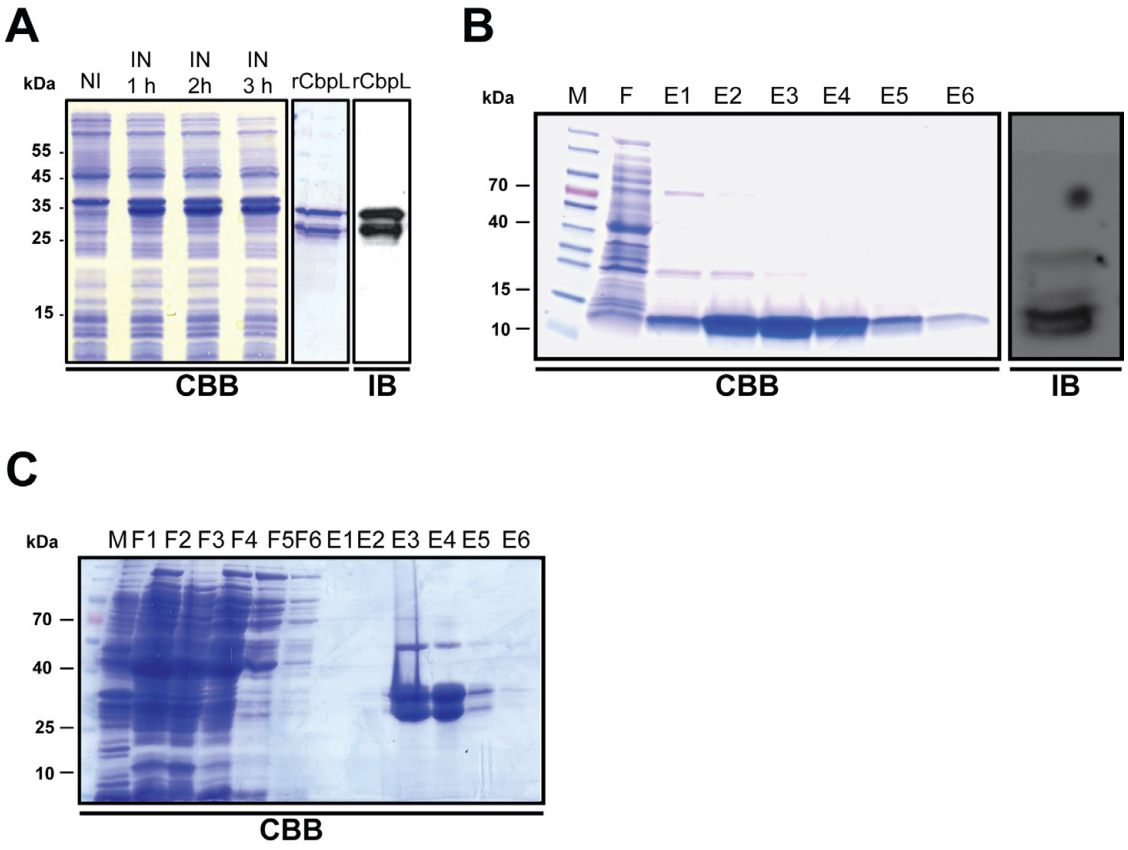


Fig. S14. Heterologous expression und purification of His_6-tagged CbpL, His_6-tagged Excalibur domain, and His_6-tagged Excalibur-CBM domain of CbpL.

(A) Heterologous expression of CbpL lacking the signal peptide (aa 27-332), (**B**) Excalibur domain (aa 27-70), and (**C**) Excalibur-CBM (aa 27- 270) cloned into expression vector pTP1 and induced with 1 mM IPTG. The His_6-tag of the purified proteins was removed by cleavage with the TEV protease. The proteins were stained with Comassie brilliant blue (CBB) and the immunoblots (IB) were performed with anti-CbpL polyclonal antibodies generated against rCbpL. NI, non-induced; IN, induced; rCbpL, recombinant without His_6-tag. F: flow through and E: protein elution fractions.

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Table S1. Strain and plasmid list

Strain or plasmid	Serotype and relevant Genotype[a]	Resistance	Source or Reference
Streptococcus pneumoniae			
SP37	35A	None	NCTC10319
SP39	3	None	ATCC 6303
SP70	12F	None	MUD[b] [6]
SP129	19F	None	MUD[b] [6]
SP173 (R800)	NC[c]	None	[7]
SP257 (D39)	2	None	NCTC7466
SP261 (TIGR4)	4	None	[8]
SP309 (G54)	19F	None	[9]
SP313 (R6)	NC[c]	None	[10]
SP332	1	None	[11]
PN111	D39*Δcps*	Km^r	[12]
PN149	D39*lux*	Km^r	[13]
PN220	D39*ΔcpsΔlgt*	Km^r, Erm^r	[14]
PN319	D39Δ*cps*Δ*cbpL*	Km^r, Erm^r	This work
PN329	D39*lux*Δ*cbpL*	Km^r, Erm^r	This work
PN259	TIGR4*Δcps*	Km^r, Erm^r	[15]
PN443	TIGR4*ΔcpsΔlgt*	Km^r, Erm^r	This work
PN249	TIGR4Δ*cps*Δ*cbpL*	Km^r, Erm^r	This work
Escherichia coli			
DH5α	*Δ(lac)U169, endA1, gyrA46, hsdR17, Φ80Δ(lacZ)M15, recA1, relA1, supE44, thi-1*	None	Novagen
BL21 (DE3)	*E.coli B, F- dcm ompT hsdS gal λ (DE3), T7 polymerase gene under control of the lacUV5 promoter*	None	Stratagene
Plasmids			
pGEM-T Easy	TA cloning vector for PCR products; Ap^r	Ap^r	Promega
pE89	pCR2.1Topo derivative with erythromycin cassette	Ap^r, Km^r , Erm^r	[16]
p562	pGEM-T derivative with *sp_0667* + 5´and 3´ flanking region for mutagenesis (TIGR4-derived strains)	Ap^r	This work
p569	pGEM-T derivative with *sp_0667* interrupted by Erm resistance cassette (TIGR4-derived strains)	Ap^r, Erm^r	This work
p716	pGEM-T derivative with *spd_0579* + 5´and 3´ flanking region for mutagenesis (D39-derived strains)	Ap^r	This work
p717	pGEM-T derivative with *spd_0579* interrupted by Erm resistance cassette (D39-derived strains)	Ap^r, Erm^r	This work
pET28a	Protein expression vector	Km^r	Novagen
pTP1	pET28a derivative expression vector	Km^r, Erm^r	[6]
p630	pET28TEV derivative with TIGR4 *sp_0667* (*cbpL*) for protein production and mice immunization	Km^r	This work
p718	pET28TEV derivative with TIGR4 *sp_0667* (*cbpL*) for protein production	Km^r	This work
p1025	pET28TEV derivative with TIGR4 *sp_0667* w/o Ltp for protein production	Km^r	This work
p1070	pET28TEV derivative with TIGR4 Excalibur_*sp_0667* for protein production	Km^r	This work

[a]Ap, ampicillin; Km, kanamycin; Erm, erythromycin; r, resistant

[b]MUD, Medical University of Düsseldorf, Germany

[c]NC, nonencapsulated strain

Table S2. Primer list

Primer intended use		Primer name	Sequence (5'-3')
Insertion-deletion mutagenesis			
Amplification of *sp_0667* + 5´and			
3´ flanking region (D39-derived strains)		CbpL_632	5`-ACTAGAATTCGACAAAAGATAGAGGCGGA-3`
		CbpL_409	5`-AACCTTCCAAGCTGCAGCTGCTGCACCAGCAACA-3`
Amplification of *sp_0667* + 5´and			
3´ flanking region (TIGR4-derived strains)		CbpL_406	5`-CTACTACTAGAATTCTGCATGGGTTAGGGCAGT-3`
		CbpL_409	5`-AACCTTCCAAGCTGCAGCTGCTGCACCAGCAACA-3`
Inverse PCR of *sp_0667* + 5´and			
3´ flanking region (pGEMTeasy)		CbpL _408	5`-ACTCACTCACTGCTCGAGTGCAGCTCAATATGCCATT-3`
		CbpL _407	5`-ATCATCATCATCGGGTACCCGTTTATTCATTTCTTTCTCCCATA-3`
Antibiotic cassette amplification			
erythromycin (*ermB*)		ermB_105	5`-GATGATGATGATCCCGGGTACCAAGCTTGAATTCACG GTTCGTGTTCGTGCTG-3`
		ermB_106	5`-AGTGAGTGAGTCCCGGGCTCGAGAAGCTTGA ATTCGTAGGCGCTAGGGACCTC-3`
Recombinant protein production and vector modification			
sp_0667 (TIGR4)	His-tag-protein rCbpL	CbpL _461	5`-GCGCGCTAGCGAAGAAAACATCCATTTTTC-3`
		CbpL _462	5`-GGCCGAGCTCTTAATCATCTAAATGATCAATGG-3`
sp_0667 (TIGR4)	untagged protein rCbpL	CbpL _598	5`-GCGCCCATGGAAGAAAACATCCATTTTTC-3`
		CbpL _462	5`-GGCCGAGCTCTTAATCATCTAAATGATCAATGG-3`
sp_0667 (TIGR4)		CbpL _461	5`-GCGCCCATGGAAGAAAACATCCATTTTTC-3`
Excalibur domain	His-tag-protein	CbpL _1156	5`- GGCCGAGCTCTAATTTTTCAATTCGCAAGCCAC -3`
sp_0667 (TIGR4)		CbpL _461	5`-GCGCCCATGGAAGAAAACATCCATTTTTC-3`
Excalibur-CBM (w/o Ltp)	His-tag-protein	CbpL _1321	5`- GCGCGCGAGCTCGCTATCATTACTAGAAGAAGCT -3`
sp_0667 (TIGR4)		CbpL _1470	5`-GCGCGCGCTAGCGCTCCTAAGGGTGCTTTTAA-3`

[a] Restriction sites are underlined