TEXT S1 Supplemental Materials and Methods.

Recombinant DNA work

For the construction of the *lcpA* deletion plasmid, the up- and downstream regions (~500 bp) of *lcpA* were amplified using oligonucleotide pairs cg0847-D1/cg0847-D2 and cg0847-D3/cg0847-D4, respectively and chromosomal DNA of *C. glutamicum* ATCC 13032 as template. The resulting PCR products were cloned by Gibson assembly (1) into pK19*mobsacB* cut with EcoRI and BamHI. Transfer of the sequenced plasmid (using primers M13-fw/M13rv) pK19*mobsacB*-Δ*lcpA* into *C. glutamicum* and screening for the first and second recombination event were performed as described (2). Kanamycin-sensitive and sucrose-resistant clones were tested by colony PCR analysis with the oligonucleotide pair cg0847-fw/cg0847-rv for the deletion of *lcpA*. The plasmid pK19*mobsacB*-Δ*lcpB* and the deletion mutants of *lcpB* were constructed analogously.

For the construction of the promoter exchange plasmid pK19-P2732-*lcpA*, four PCR fragments were generated: i) primers Term_fw/Term_rv and plasmid DNA of pEC-XC99E as template; ii) primers Pcg2732-fw/Pcg2732-rv and chromosomal DNA of *C. glutamicum* ATCC 13032 as template; iii) primers cg0847-A-fw/cg0847-B-rv and chromosomal DNA of *C. glutamicum* ATCC 13032 as template; iv) primers P2737-cg0847-C-fw/cg0847-D-rv and chromosomal DNA of *C. glutamicum* ATCC 13032 as template. All four fragments were cloned by Gibson assembly into pK19*mobsacB* cut with EcoRI and BamHI. Transfer of the sequenced plasmid (using primers M13-fw/M13rv) pK19-P2732-*lcpA* into *C. glutamicum* and screening for the first and second recombination event were performed as described (2). Correct integration after the first recombination was checked by colony-PCR using primers cg0847-fw/M13-fw and cg0847-rv/M13-rv. For the second recombination, all growth media were supplemented with 100 mM sodium gluconate to activate the Pcg2732-promoter in front of *lcpA*. Kanamycin-sensitive and sucrose-resistant clones after the second recombination were tested by colony PCR analysis with the oligonucleotide pair cg0847-fw/cg0847-D-rv for the exchange of the promoter. A successful promoter exchange resulted in a longer PCR fragment with these primers (1464 bp vs. 1093 bp). The plasmid pK19-P3323-*lcpA* and the respective promoter mutant were constructed analogously with the exception that gluconate is not required for the second recombination.

For the *lcpA* inactivation plasmid pK18*mob*-inac-*lcpA* the first part of the *lcpA* coding region was amplified using the primer pair given in Table S3 and chromosomal DNA of

C. glutamicum as template. The PCR product was cut with EcoRI/BamHI and ligated into pK18*mob* cut with the same restriction enzymes.

For the construction of plasmid pAN6-*lcpA*, the *lcpA* coding region was amplified using the primer pair given in Table S3 and chromosomal DNA of *C. glutamicum* as template. The PCR-product and the parental plasmid pAN6 were cut with NdeI/NheI and ligated with T4 ligase.

For the construction of plasmid pAN6-venus-*lcpA*, the *lcpA* coding region was amplified using the primer pair CFP_0847_fw/cg0847_pAN6_rv and chromosomal DNA of *C. glutamicum* as template. The venus coding region was amplified using primer pair pAN6_Ven_ fw/CFP_noS_rv and plasmid pJC1-venus-term as template. The resulting PCR products were cloned by Gibson assembly into pAN6 cut with NdeI/NheI. Plasmid pAN6-eyfp-*lcpB* was constructed similarly using pEKEx2-eyfp as PCR template for *eyfp*. pAN6 plasmids encoding shortened variants of LcpA were constructed accordingly using the oligonucleotides given in Table S3.

pAN6 plasmids containing *lcpA* homologs of *M. tuberculosis* or *C. diphtheriae* were constructed accordingly using the oligonucleotides given in Table S3 and the respective chromosomal DNA as template.

For plasmid pET-TEV-*lcpA*ΔTM, two fragments were amplified using the primer pairs 0847-T1-pET-fw/0847-T1-rv and 0847-T2-fw/0847-T2-pET-rv and chromosomal DNA of *C. glutamicum* as template. The resulting PCR products were cloned by Gibson assembly into pET-TEV cut with NdeI/EcoRI.

The insert for plasmid pET-TEV-cgtR10 was amplified using the oligonucleotides given in Table S3 and chromosomal DNA of *C. glutamicum* as template. The PCR-product and the parental plasmid pET-TEV were cut with NdeI/HindIII and ligated with T4 ligase.

Plasmids containing mutated variants of LcpA were constructed using the QuikChange Lightning site-directed mutagenesis kit (Agilent Technologies, Santa Clara, USA) and the oligonucleotides given in Table S3.

The relevant regions of all plasmids were confirmed by sequencing.

Quantitative Real-Time-PCR

Good quality qRT-qPCR results are based on the choice of suitable reference genes which should be expressed at the same level independent of the growth conditions. For our experiment, initially two genes were chosen as reference genes, which appeared to be not regulated based on several experiments in our in-house microarray database. These two genes

were *recF* (cg0005) and *hpt* (cg2985). Primer design was performed with Primer3plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) (3) using the standard settings for qPCR and an annealing temperature of 60°C. One primer pair for each of the reference genes and two primer pairs for the gene of interest (*lcpA*) were designed. PCR-products for the preparation of standard curves were amplified with DreamTaq Polymerase (Thermo Fisher Scientific, Braunschweig, Germany), the respective primer pair (e.g. RT-recF-fw1/RT-recF-rv1) and chromosomal DNA of *C. glutamicum* as template. Purity of the standards was checked by agarose gel electrophoresis.

The optimal annealing temperature for the qPCR was determined using the standards as templates and the following gradient cycling protocol:

Step No:	**°C**	**min:sec**	**go to step**	**loops**
1	95	03:00		
2	95	00:05		
3	55.1-66.9	00:30	2	39
4	Melting curve 60-95°C, 6 s with ΔT = 1°C			

Purity of the PCR-products was checked by agarose gel electrophoresis. The primer pair RT-cg0847-fw2/RT-cg0847-rv2 was chosen for further analysis because the product of primers RT-cg0847-fw1/RT-cg0847-rv1was significantly less pure. An annealing temperature of 59 °C was chosen all for further qPCR runs. The reference genes *hpt* and *recF* performed equally in tests and therefore only *recF* was used for referencing in the final measurements. Standard curves were derived using the generated PCR fragments in concentrations of 1 pg/µl to 100 ag/µl. The evaluation was performed using the software qPCRsoft 2.1 (Analytic Jena) and the ΔΔCt method.

Colony counting

For the estimation of the number of viable cells, ATCC 13032 and SilG-lcpA were cultivated as following. The first preculture and subsequent washing were performed as described in the paragraph “Bacterial strains, plasmids and growth conditions”. The second preculture consisting of 25 ml CGXII with 2% (w/v) glucose as carbon source in a 100 ml baffled shake flask was inoculated to an OD_{600} of 0.5 and incubated at shaking at 130 rpm for 24h at 30°C.

The main culture conditions were identical to those of the second preculture with the only exception that 400 µl of the second preculture were used for inoculation. After 5.5 hours samples for cell counting and OD_{600} measurements were taken. The cells were diluted in PBS and 100 µl of the 10^{-5}-10^{-7} dilutions were spread onto BHI agar plates. After 1-2 days incubation at 30°C the colonies were counted. Due to the different growth behavior of the two strains, CFU were normalized to the OD600 at the time of sampling. The experiment was performed with four biological replicates.

Isolation of material from culture supernatants

For the isolation of material from culture supernatants, the first preculture (20 ml BHI + 100 mM gluconate) was inoculated with a colony from a fresh agar plate and cultivated at 130 rpm and 30°C overnight. The cells were washed with PBS and used to inoculate a second preculture, consisting of 100 ml CGXII with 2% (w/v) glucose, to an OD_{600} of 0.5. This culture was incubated at 130 rpm and 30°C for 24 h. The main culture (2 times 500 ml CGXII with 4% (w/v) glucose in 2l baffled shake flasks) was inoculated with cells from the second preculture to an OD_{600} of 1. After further 24 h the cultures were harvested by centrifugation for 30 min at 4°C and 6.371*g* in a JA10 Rotor. The supernatant was removed and centrifuged again under the same conditions. Then the cell-free supernatants were transferred into ultracentrifuge tubes and centrifuged for 60 min at 4°C and 235.418*g*. The supernatant was discarded and the pellet transferred into an Eppendorf tube and frozen at -80°C. The material was then lyophilized and subsequently stored at room temperature until further analysis.

Analysis of the protein content of the material from the culture supernatant

A few crumbs of the lyophilized material were suspended in 200 µl PBS buffer and delipidated according to (4). The pellet was dissolved in 1x SDS-sample buffer and different concentrations were separated by SDS-PAGE and stained using RAPID Stain™ (G-Biosciences, St. Louis, USA). Visible bands were cut out of the gel, digested with Trypsin and analyzed by MALDI-TOF as described previously (5).

TABLE S1 Strains and plasmids used in this study.

Strain or plasmid	Relevant characteristics	Source or reference
E. coli		
DH5α	F⁻ *endA1* Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169 *recA1 relA1 hsdR17*($r_K^-m_K^+$) *deoR supE44 thi-1 gyrA96 phoA* λ^-; strain used for general cloning procedures	(6)
BL21(DE3)	F⁻ *ompT hsdS*$_B$($r_B^-m_B^-$) *gal dcm* (DE3); host for protein production	(7)
C. glutamicum		
ATCC13032	Biotin-auxotrophic wild type	(8)
ATCC13032 Δ*lcpB*	ATCC13032 with an in-frame deletion of cg3210 (*lcpB*)	This work
SilG-lcpA	Gluconate dependent silencing strain. ATCC13032 with a chromosomal promoter exchange of the native cg0847(*lcpA*)-promoter against the gluconate inducible promoter of cg2732	This work
SilI-lcpA	Inositol dependent silencing strain. ATCC13032 with a chromosomal promoter exchange of the native cg0847(*lcpA*)-promoter against the promoter of cg3323 that is active in the absence of *myo*-inositol	This work
SilG-lcpA Δ*lcpB*	SilG-lcpA with an in-frame deletion of cg3210 (*lcpB*)	This work
C. diphtheriae		
ATCC 27010	wild-type laboratory strain, DNA used as PCR template	DSM 44123
M. tuberculosis		
H37Rv	wild-type laboratory strain, DNA used as PCR template	ATCC 25618
Plasmids		
pK19*mobsacB*	Kan^R.; plasmid for allelic exchange in *C. glutamicum*; (pK18 *ori*V$_{E.c.}$, *sacB*, *lacZ*α)	(9)
pK19*mobsacB*-Δ*lcpA*	Kan^R; pK19*mobsacB* derivative containing a PCR product covering the up- and downstream regions of cg0847	This work
pK19*mobsacB*-Δ*lcpB*	Kan^R; pK19*mobsacB* derivative containing a PCR product covering the up- and downstream regions of cg3210	This work
pK19-P2732-*lcpA*	Kan^R; pK19*mobsacB* derivative containing four PCR products: 500 bp upstream-region of cg0847, a terminator sequence, the promoter of cg2732 and 500 bp of the cg0847 coding region. Used for the exchange of the native cg0847 promoter with the	This work

Strain or plasmid	Relevant characteristics	Source or reference
	promoter of cg2732 to get SilG-lcpA	
pK19-P3323-*lcpA*	Kan^R; pK19*mobsacB* derivative containing four PCR products: 500 bp upstream-region of cg0847, a terminator sequence, the promoter of cg3323 and 500 bp of the cg0847 coding region. Used for the exchange of the native cg0847 promoter with the promoter of cg3323 to get SilI-lcpA	This work
pK18*mob*	Kan^R.; plasmid for targeted gene inactivation in *C. glutamicum*; (pK18 *ori*$V_{E.c.}$, *lacZ*α)	(9)
pK18*mob*-inac-*lcpA*	Kan^R; pK18*mob* derivative containing a PCR product covering the first ~470 bp of the coding region of cg0847	This work
pJC1-venus-term	Kan^R, pJC1 derivative carrying the Venus coding sequence and additional terminators (pCG1 ori_{Cg}, pACYC177 ori_{Ec})	(10)
pEKEx2-eyfp	Kan^R, pEKEx2 containing *eyfp* with artificial RBS, under control of P_{tac}	(11)
pAN6	Kan^R; *C. glutamicum*/*E. coli* shuttle vector for regulated gene expression using P_{tac} (P_{tac} *lacI*^{q} pBL1 *ori*V_{Cg} pUC18 *ori*V_{Ec})	(12)
pAN6-*lcpA*	Kan^R; pAN6-derivative for expression of cg0847 under control of P_{tac}	This work
pAN6-venus-*lcpA*	Kan^R; pAN6-derivative for expression of cg0847 with an N-Terminal fusion to the fluorescent protein venus under control of P_{tac}	This work
pAN6-eyfp-*lcpB*	Kan^R; pAN6-derivative for expression of cg3210 with an N-Terminal fusion to the fluorescent protein EYFP under control of P_{tac}	This work
pAN6-*lcpB*	Kan^R; pAN6-derivative for expression of cg3210 under control of P_{tac}	This work
pAN6-CDC7B0634	Kan^R; pAN6-derivative for expression of *Corynebacterium diphtheriae* CDC7B0634 under control of P_{tac}	This work
pAN6-rv3267	Kan^R; pAN6-derivative for expression of *Mycobacterium tuberculosis* rv3267 under control of P_{tac}	This work
pAN6-rv3484	Kan^R; pAN6-derivative for expression of *Mycobacterium tuberculosis* rv3484 under control of P_{tac}	This work
pAN6-*lcpA*-D88A	Kan^R; pAN6-derivative for expression of cg0847-D88A under control of P_{tac}	This work
pAN6-*lcpA*-R138A	Kan^R; pAN6-derivative for expression of cg0847-R138A under control of P_{tac}	This work
pAN6-*lcpA*-R257A	Kan^R; pAN6-derivative for expression of cg0847-R257A under control of P_{tac}	This work
pAN6-*lcpA*-24-ENDE	Kan^R; pAN6-derivative for expression of cg0847 lacking the first 24 amino acids under control of the P_{tac}	This work
pAN6-*lcpA*-1-477	Kan^R; pAN6-derivative for expression of a shortened cg0847 variant containing only amino acids 1-477 under control of P_{tac}	This work

Strain or plasmid	Relevant characteristics	Source or reference
pAN6-*lcpA*-1-388	Kan^R; pAN6-derivative for expression of a shortened cg0847 variant containing only amino acids 1-388 under control of P_{tac}	This work
pAN6-*lcpA*-1-286	Kan^R; pAN6-derivative for expression of a shortened cg0847 variant containing only amino acids 1-286 under control of P_{tac}	This work
pET-TEV	Kan^R; pET28b derivative for overexpression of genes in *E. coli*, adding an N-terminal decahistdine tag and a TEV protease cleavage site to the target protein (pBR322 $oriV_{E.c.}$, P_{T7}, *lacI*)	(13)
pET-TEV-*lcpA*ΔTM	Kan^R; pET-TEV derivative for overproduction of LcpA lacking the transmembrane helix with an N-terminal decahistidine tag which can be cleaved off using TEV protease	This work
pET-TEV-*lcpA*ΔTM-D88A	Kan^R; pET-TEV derivative for overproduction of LcpA-D88A lacking the transmembrane helix with an N-terminal decahistidine tag which can be cleaved off using TEV protease	This work
pET-TEV-*lcpA*ΔTM-R138A	Kan^R; pET-TEV derivative for overproduction of LcpA-R138A lacking the transmembrane helix with an N-terminal decahistidine tag which can be cleaved off using TEV protease	This work
pET-TEV-*lcpA*ΔTM-R257A	Kan^R; pET-TEV derivative for overproduction of LcpA-R257A lacking the transmembrane helix with an N-terminal decahistidine tag which can be cleaved off using TEV protease	This work
pET-TEV-cgtR10	Kan^R; pET-TEV derivative for overproduction of cgtR10 with an N-terminal decahistidine tag which can be cleaved off using TEV protease	This work

TABLE S2 Oligonucleotides used in this study.

Oligonucleotide	Sequence (5' → 3') and properties[a]
Construction of deletion plasmid pK19*mobsacB*-lcpA and PCR-analysis of the resulting mutants	
cg0847-D1	**CAGGTCGACTCTAGAGGATC**CCAGTTGAGAGGAACTCCAC
cg0847-D2	GTGCTGCTAACCCTTCGC
cg0847-D3	**CCGCGAAGGGTTAGCAGCAC**AACCCCGCTTTTCGACGC
cg0847-D4	**AAAACGACGGCCAGTGAATT**TAGAGAGGGTTTTGCGCACG
cg0847-fw	GAAGAACTCCCCATCGATCTC
cg0847-rv	CGCCAAGATCATGCCCATTTC
Construction of deletion plasmid pK19*mobsacB-lcpB* and PCR-analysis of the resulting mutants	
cg3210-D1	**CAGGTCGACTCTAGAGGATC**GTTAATCTTCTACGCCAGTGCTG
cg3210-D2	GGTTAATAGAGTAGCGCCTAGG
cg3210-D3	**GCGCTACTCTATTAACC**AACCCCAGGTAATCGTTCACAG
cg3210-D4	**AAAACGACGGCCAGTGAATT**CGAACTCTTGGTAGGAGACG
cg3210-fw	AGGCCTTGCAGCACTCATTG
cg3210-rv	ATCGTGTTGCGGGCACTGTC
Construction of the promoter exchange plasmid pK19-P2732-*lcpA* and the respective mutant strain	
Term_fw	GTAGCGCCGATGGTAGTG
Term_rv	CATGAGCGGATACATATTTGAATGTATTTAG
Pcg2732-fw	**CAAATATGTATCCGCTCATG**CGATGACATACGAACAAATCGTTG
Pcg2732-rv	GTCTTATCCTTTCTTTGGTGGCG
cg0847-A-fw	**CAGGTCGACTCTAGAGGATC**GGGCGGCAATATTAATAGCCG
cg0847-B-rv	**CACTACCATCGGCGCTAC**GTGTTCTGTATTTATGGAGCGGG
P2737-cg0847-C-fw	**CACCAAAGAAAGGATAAGACA**TGACTGAAAAGTATCGTCCCGTC
cg0847-D-rv	**AAAACGACGGCCAGTGAATT**ATCCTTGTACGCACCGTAAACG
cg0847-fw	GAAGAACTCCCCATCGATCTC
cg0847-rv	CGCCAAGATCATGCCCATTTC
Construction of the promoter exchange plasmid pK19-P3323-*lcpA* and the respective mutant strain	
Term_fw	GTAGCGCCGATGGTAGTG
Term_rv	CATGAGCGGATACATATTTGAATGTATTTAG
Pcg3323_fw	**CAAATATGTATCCGCTCATG**GGAAATCTCCCGAACATCAGAAG
Pcg3323_rv	CTAAAATTTCTCCTCTTAAAAAGATAACGGC
cg0847-A-fw	**CAGGTCGACTCTAGAGGATC**GGGCGGCAATATTAATAGCCG
cg0847-B-rv	**CACTACCATCGGCGCTAC**GTGTTCTGTATTTATGGAGCGGG
P3323-cg0847-C-fw	**TTTAAGAGGAGAAATTTTAGA**TGACTGAAAAGTATCGTCCCGTC
cg0847-D-rv	**AAAACGACGGCCAGTGAATT**ATCCTTGTACGCACCGTAAACG
cg0847-fw	GAAGAACTCCCCATCGATCTC
cg0847-rv	CGCCAAGATCATGCCCATTTC
Construction of pK18*mob*-inac-*lcpA*	
EcoRI_cg0847_fw	GCGCGAATTCACTGAAAAGTATCGTCCCGT
cg0847_BamHI_rv	GCGCGGATCCCACCGTAAACGCCGTT

Oligonucleotide	Sequence (5' → 3') and properties[a]
Construction of pAN6-*lcpA*	
cg0847-NdeI-fw	GCGCCATATGACTGAAAAGTATCGTCCCGTC
cg0847_rv_NheI_Stop	GCGCGCTAGCTTAGTTAACGCAACGGGGACC
Construction of pAN6-venus-*lcpA*	
pAN6_Ven_ fw	**CTGCAGAAGGAGATATACAT** ATGGTGAGCAAGGGCG
cg0847_pAN6_rv	**CGAACTGTGGGTGGGACCAG** TTAGTTAACGCAACGGGGAC
CFP_noS_rv	CTTGTACAGCTCGTCCATGC
CFP_0847_fw	**CATGGACGAGCTGTACAAG** GTGACTGAAAAGTATCGTCCCG
Construction of pAN6-eYFP-*lcpB*	
pAN6_eYFP_fw	CCTGCAGAAGGAGATATACAATGGTGAGCAAGGGC
eYFP_noS_rv	TCTAGACTTGTACAGCTCGTCC
eYFP_cg3210_fw	GGACGAGCTGTACAAGTCTAGAGATTCACCAGGACAGGG
cg3210_pAN6_rv	GTGGGTGGGACCAGCTAGTTAGCGCATGGAGCTAAATA
Construction of pAN6-*lcpB*	
cg3210_G_fw	**TGCCTGCAGAAGGAGATATACA**TATGGATTCACCAGGACAGGG
cg3210_G_rv_Stop	**GAACTGTGGGTGGGACCAG**TTAGCGCATGGAGCTAAATAGTGC
Construction of pAN6-CDC7B0634	
DIP0680-NdeI-fw	GCGCCATATGTCAGATAATTTTCGCCGTAACCG
DIP0680-NheI-rv	GCGCGCTAGCCTAGTTCACGCAGCGAGGTC
Construction of pAN6-rv3267	
Rv3267-NdeI-fw	GCGCCATATGGTGATGTCTGCGCAACGTGTG
Rv3267-NheI-rv	GCGCGCTAGCTCAGTTGATGCACTCCGGCGCGT
Construction of pAN6-rv3484	
Rv3484-NdeI-fw	GCGCCATATGGCGCGTTCTGAGGGCAATC
Rv3484-NheI-rv	GCGCGCTAGCCTAGTTCACGCAGGGCAC
Construction of mutated LcpA variants by site directed mutagenesis	
cg0847-D88A-fw	GTTGGTGGGTTCTGCTTCCCGTTCCGATGC
cg0847-D88A-rv	GCATCGGAACGGGAAGCAGAACCCACCAAC
cg0847-R138A-fw	CCGCTGTGTCGATTCCTGCCGATACCTATATTCATG
cg0847-R138A-rv	CATGAATATAGGTATCGGCAGGAATCGACACAGCGG
cg0847-R257A-fw	GATGCGTTGTCTTATGTGGCCCAGCGCCACGATCTCC
cg0847-R257A-rv	GGAGATCGTGGCGCTGGGCCACATAAGACAACGCATC
Construction of pAN6 with shortened LcpA variants	
cg0847-NdeI-fw	GCGCCATATGACTGAAAAGTATCGTCCCGTC
cg0847_rv_NheI_Stop	GCGCGCTAGCTTAGTTAACGCAACGGGGACC
cg0847-24-NdeI-fw	GCGCCATATGGCGGGCCATCCTGTGTTC
cg0847-477-NheI-rv	GCGCGCTAGCTTAAGCGTAATCGCCGGCGGATAC

Oligonucleotide	Sequence (5' → 3') and properties[a]
cg0847-388-NheI-rv	GCGCGCTAGCTTATAGGTCAGGGGCCTGATC
cg0847-286-NheI-rv	GCGCGCTAGCTTAAGACAGCACCTGATTAACAAGCG
Construction of pET-TEV-*lcpA*ΔTM	
0847-T1-pET-fw	**ACCTGTATTTTCAGGGCCAT**ATGACTGAAAAGTATCGTCCCGTC
0847-T1-rv	**ACACCATCCACTTTTCCGAC**AGGATGGCCCGCTTGTTTAG
0847-T2-fw	GTCGGAAAAGTGGATGGTGTC
0847-T2-pET-rv	**TTGTCGACGGAGCTCGAATT**TTAGTTAACGCAACGGGGACC
Construction of pET-TEV-cgtR10	
cgtR10_NdeI_fw	TATACATATGATTTCCATTTCCATCGCCGAC
cgtR10_HindIII_rv	TATAAAGCTTCTACAACCAGCCCAATTCCCTG
Primers for RT-qPCR	
RT-recF-fw1	GGCTATCTTGCGCATTTGTC
RT-recF-rv1	TCTCGGCCTTGATTAACAGC
RT-hpt-fw1	ATGTTCCAGCCAACCCATAC
RT-hpt-rv1	TCTTCGGCGTCTTTGAACTC
RT-cg0847-fw1	CAACACCGATACGATCATGG
RT-cg0847-rv1	CGTAAACGCCGTTGATCTTC
RT-cg0847-fw2	TGTTGGTGGGTTCTGATTCC
RT-cg0847-rv2	CCATGATCGTATCGGTGTTG
Standard primers for colony-PCRs and sequencing of plasmids	
M13-fw	CGCCAGGGTTTTCCCAGTCAC
M13-rv	AGCGGATAACAATTTCACACAGGA
pEKEx2 fw	CGGCGTTTCACTTCTGAGTTCGGC
pEKEx2 rv	GATATGACCATGATTACGCCAAGC
pET-Promoter	CGAAATTAATACGACTCACTATAGG
pET-Terminator	TATGCTAGTTATTGCTCAGCGGTG

[a] Overlaps for Gibson assembly are written in bold letters. Restriction sites are underlined.

TABLE S3 Proteins identified by MALDI-TOF analysis in the culture supernatant material of strain SilG-lcpA (compare Fig. S1C).

No.	Locus tag	Gene name	Annotation/Function
1	cg0576	*rpoB*	DNA-directed RNA polymerase β subunit
2	cg1737	*acn*	aconitase
	cg2963	*clpC*	ATPase subunit of the ATP-dependent ClpC-ClpP1/ClpP2 protease
3	cg0583	*fusA*	elongation factor EF-2/G
	cg2166	*gpsI*	polyribonucleotide nucleotidyltransferase
4	cg1290	*metE*	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase
5	cg0766	*icd*	isocitrate dehydrogenase
	cg1248		putative GTPase involved in stress response
	cg1774	*tkt*	transketolase
6	cg3182	*cop1*	trehalose corynomycolyl transferase
7	cg1451	*serA*	phosphoglycerate dehydrogenase
8	n.i.[a]		
9	n.i.		
10	n.i.		
11	cg0413	*cmt1*	trehalose corynomycolyl transferase
	cg1437	*ilvC*	ketol-acid reductoisomerase
12	cg2833	*cysK*	O-acetylserine thiol-lyase, cysteine synthase
13	cg0842		putative DNA helicase
	cg2409	*ctaC*	cytochrome aa_3 oxidase, subunit 2
14	cg2052		putative secreted protein, CGP3 region
15	cg1911		putative secreted protein, CGP3 region
16	cg0610	*rplE*	50S ribosomal protein L5
17	cg1322		putative protein, conserved, YceI homolog
	cg3071	*pyrE*	orotate phosphoribosyltransferase (EC:2.4.2.10)
	cg0610	*rplE*	50S ribosomal protein L5
18	n.i.		
19	n.i.		
20[b]	cg1109	*porB*	anion-specific porin precursor

[a] not identified

[b] in this measurement the identity of the protein could not be confirmed by MALDI-TOF-MSMS measurements

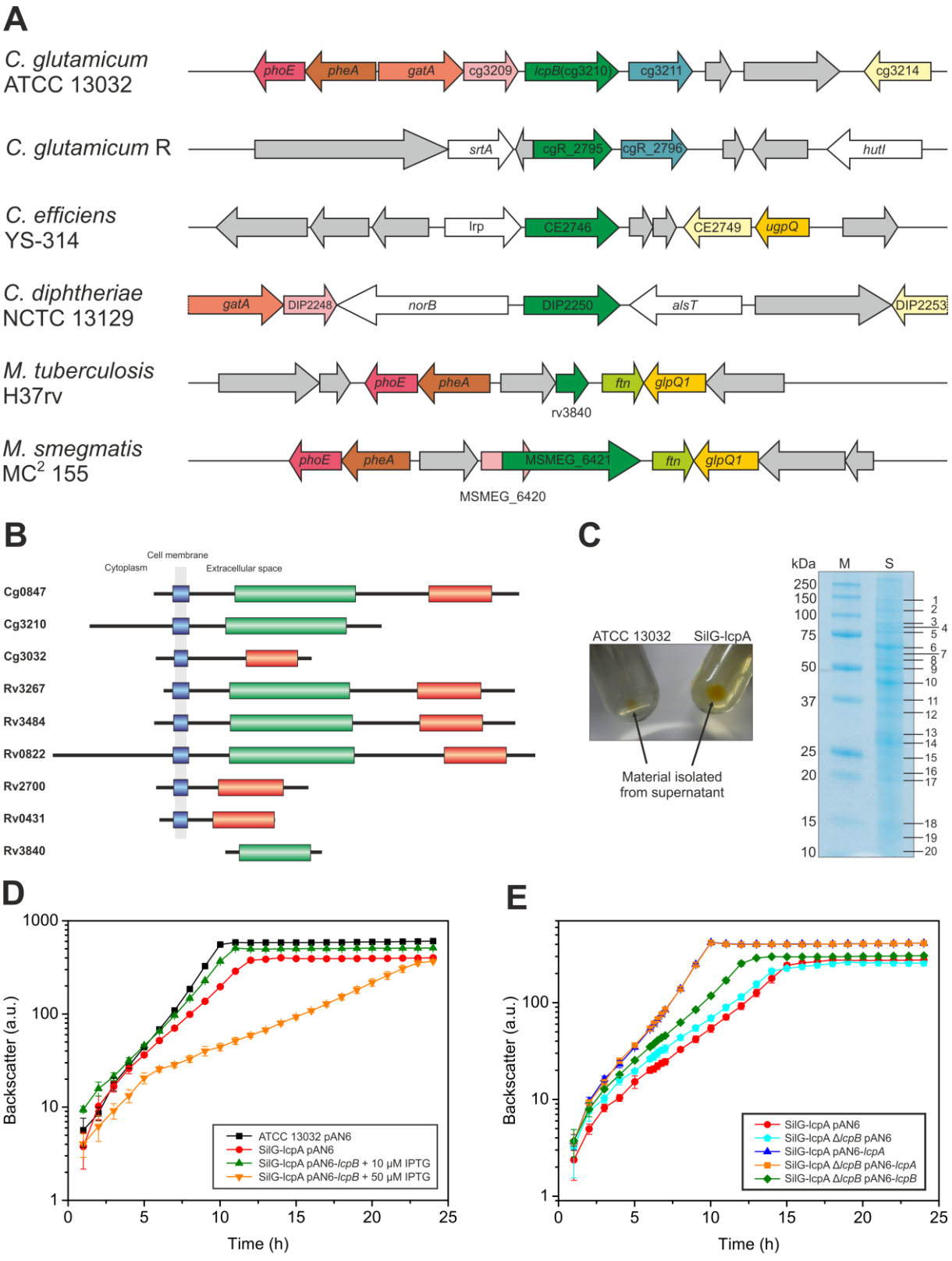

FIG S1 (A) Phylogenetic conservation of *lcpB* among related species (green arrows). Neighboring conserved genes are colored. White indicates other, non-conserved proteins of known function and grey represents hypothetical or uncharacterized proteins. Data were taken from (14). (B) Domain composition of *C. glutamicum* and *M. tuberculosis* LCP proteins. Data

derived from InterPro, Pfam, SMART, and TMPred (blue, transmembrane helix; green, LCP domain; red, LytR_C domain). (C) Isolation of material from culture supernatants and analysis of the protein content. The material was derived from supernatants of stationary phase cultures as described in the supplemental methods section. Presented is the material after ultracentrifugation of the same volume of supernatant of the respective strains. Proteins identified are given in Table S3. M, molecular weight marker; S, material of the SilG-lcpA supernatant. (D) Complementation of the *lcpA* silencing strain with plasmid-encoded *lcpB* and different amounts of IPTG. (E) Complementation of the *lcp* double mutant.

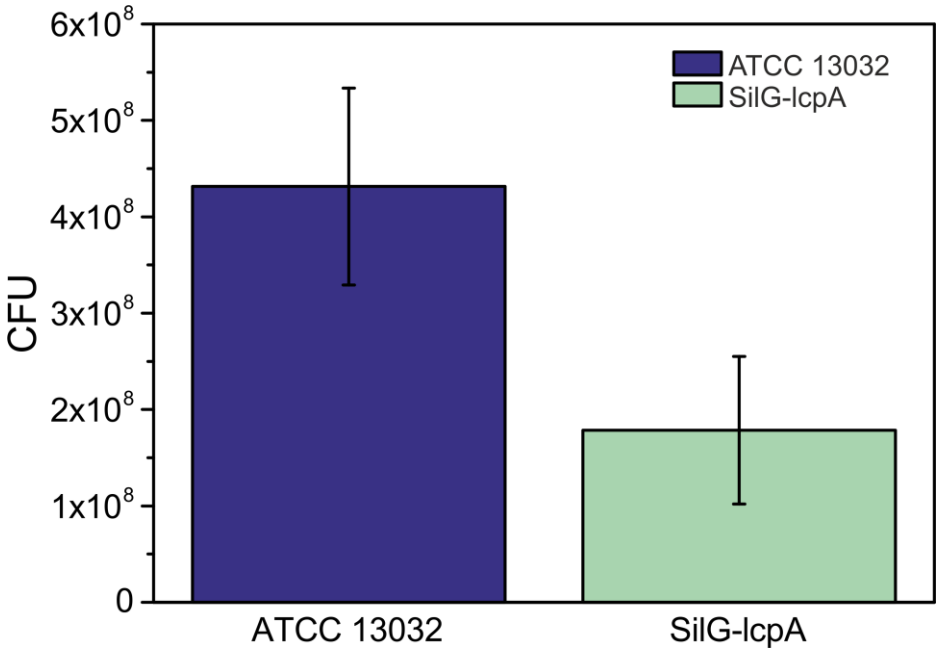

FIG S2 Determination of the CFU of *C. glutamicum* wild-type and SilG-lcpA strains. After precultivation in BHI and subsequently in CGXII with 2% (w/v) glucose the cells were used to inoculate the main culture consisting of 25 ml CGXII with 2% (w/v) glucose in 100 ml baffled shake flasks. After 5.5 hours of cultivation samples for OD measurements and CFU counting were taken. Due to the different growth behavior of the two strains CFUs were normalized to the optical density at 600 nm at the time of sampling (CFU ml^{-1} OD_{600}^{-1}). Presented are the mean and the standard deviation of four biological replicates.

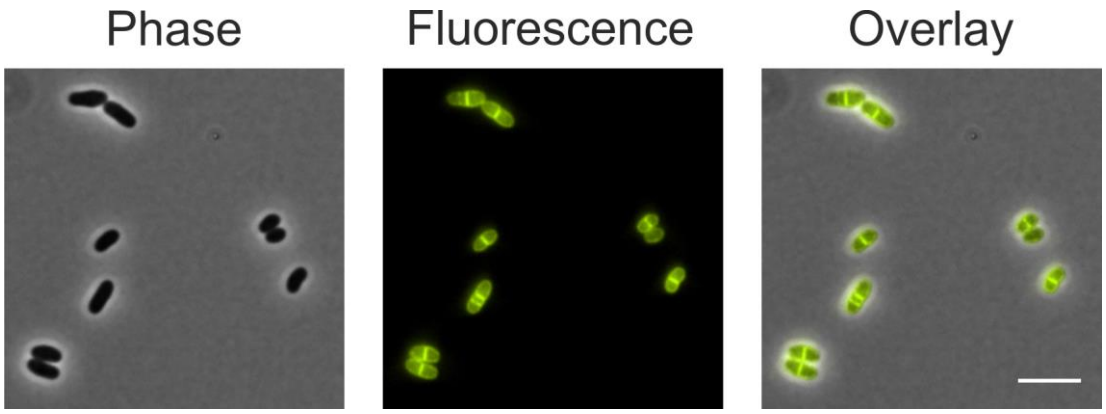

FIG S3 Localization of the membrane protein ChrS fused to *eyfp*. *C. glutamicum* ATCC 13032 was transformed with pJC1-chrS-C-eyfp (Hentschel, unpublished data) and the expression of the sensor kinase was induced with hemin. The picture was taken after 4 hours of induction. Scale bar 5 µm.

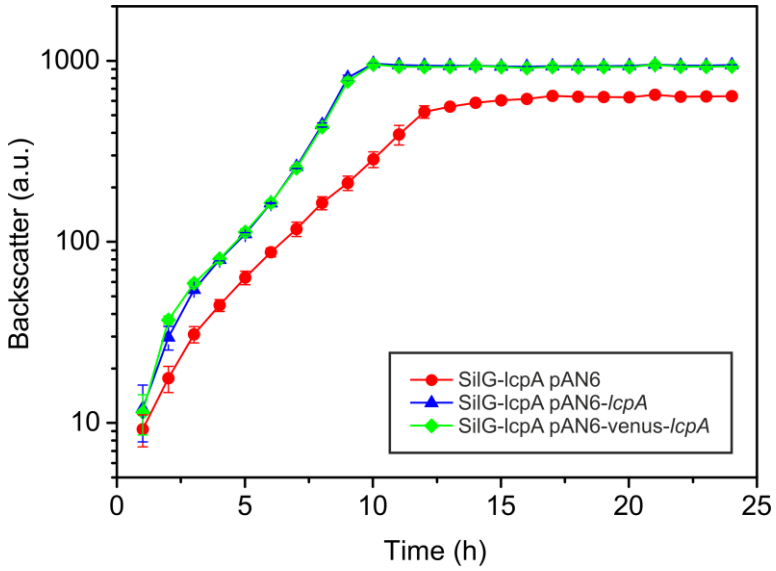

FIG S4 Complementation of the *lcpA* silencing strain with plasmid-encoded *lcpA* as well as the fluorescently labelled variant venus-*lcpA*. Both variants complement equally well proving the full functionality of the fluorescently labelled protein. Cultivation conditions as described for the other complementation experiments in the materials and methods section.

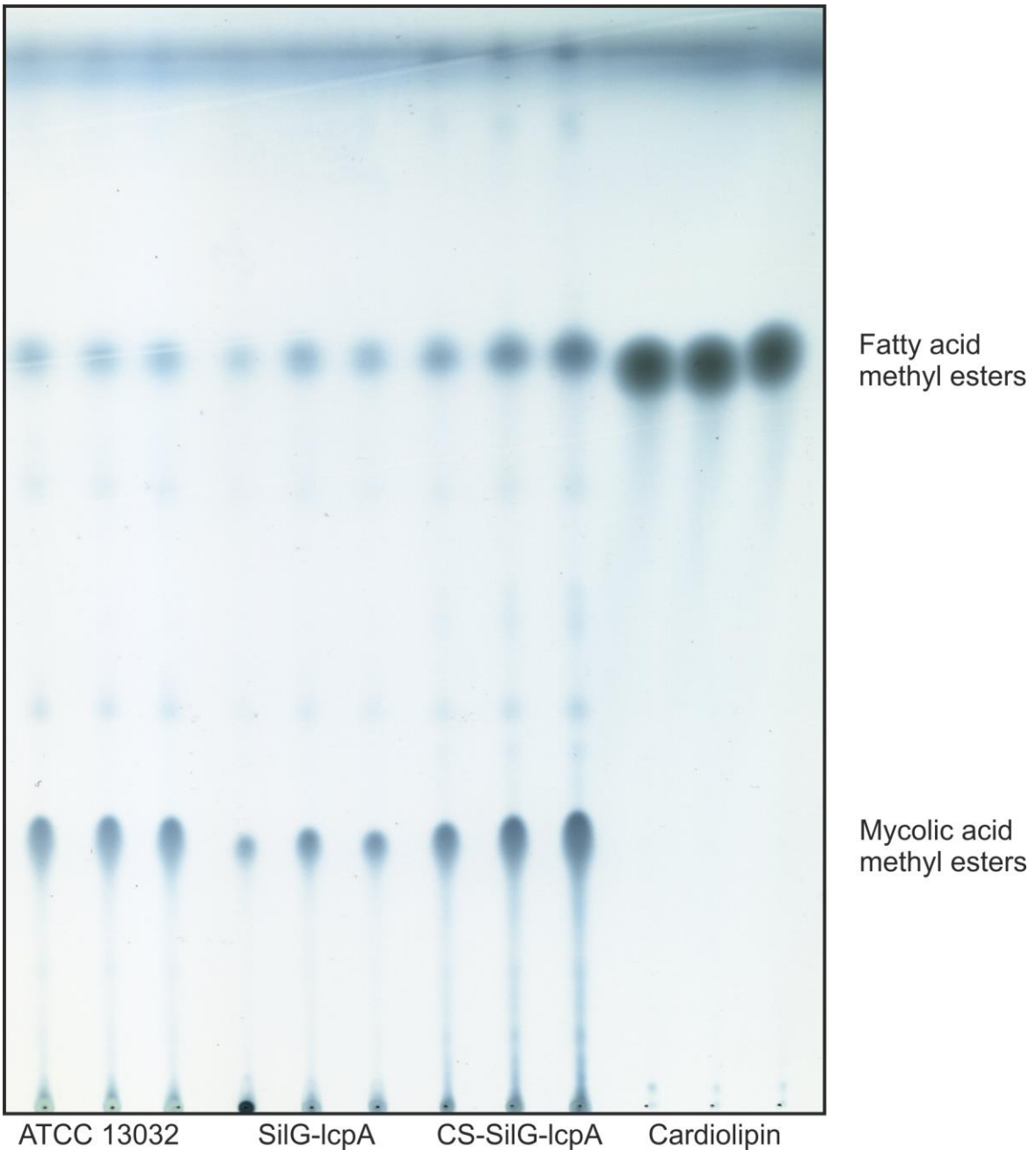

FIG S5 Fatty and mycolic acid methyl esters in the cell walls of *C. glutamicum* ATCC 13032, SilG-lcpA and in the material of the SilG-lcpA supernatant. Mycolic acids and fatty acids from cardiolipin were liberated as methyl esters from purified cell walls by acidic methanolysis. TLC was run in toluene:acetone (97:3, v/v) and developed with phosphomolybdic acid. Cardiolipin from cattle contains C16 and C18 fatty acids and served as standard. CS-SilG-lcpA, material from the culture supernatant of SilG-lcpA. Three technical replicates of one sample each are presented.

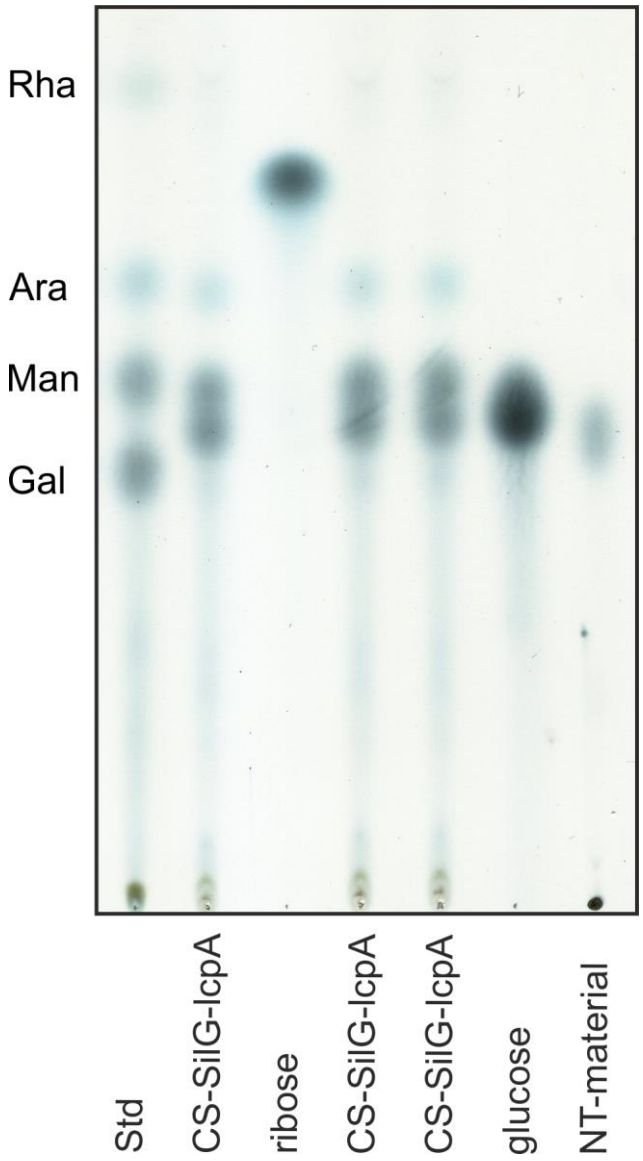

FIG S6 Arabinogalactan analysis in the material of the culture supernatant of SilG-lcpA. CS-SilG-lcpA: Hydrolyzed (2 M HCl, 3 h, 100°C) material from the culture supernatant of SilG-lcpA. NT-material: non-treated material from the culture supernatant. Ribose and glucose served as additional standards. One or three technical replicates of one sample each are shown.

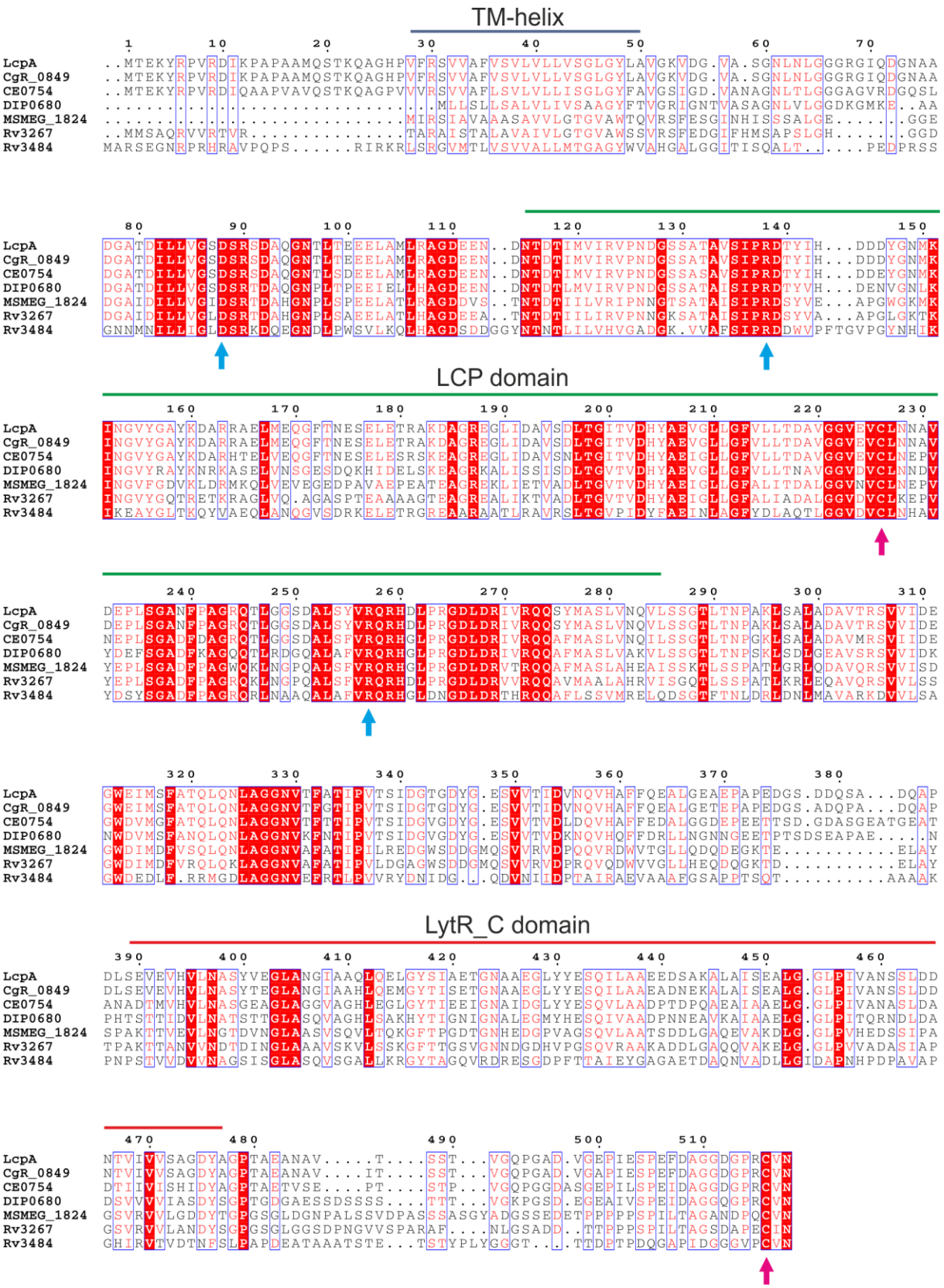

FIG S7 Alignment of LcpA and homologous proteins of related species. *C. glutamicum* ATCC 13032 LcpA, *C. glutamicum* R CgR_0849, *Corynebacterium efficiens* YS-314 CE0754, *Corynebacterium diphtheriae* NCTC 13129 DIP0680, *Mycobacterium smegmatis*

MC^2 155 MSMEG_1824, *Mycobacterium tuberculosis* H37rv Rv3267 and Rv3484. Cyan arrows indicate positions that were mutated in this study. Pink arrows indicate conserved cysteines. Residues highlighted with a red background are strictly conserved, residues printed in red are partially conserved.

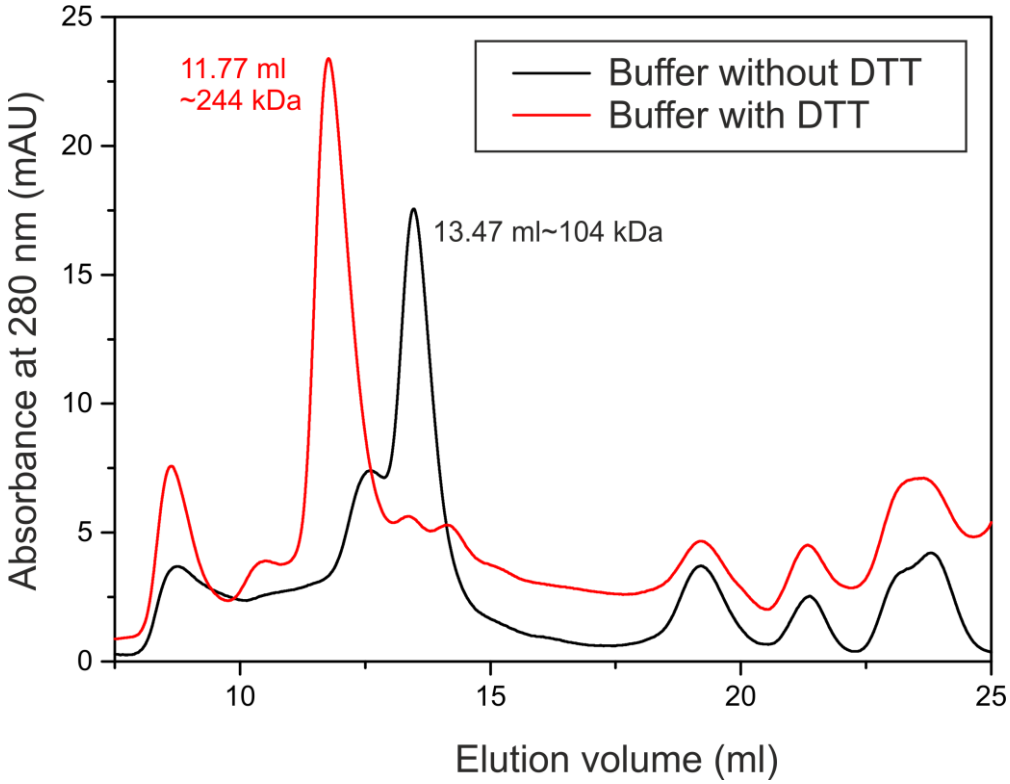

FIG S8 Size exclusion chromatography of LcpAΔTM in the presence and absence of 1 mM DTT. The chromatography was performed in GF-buffer (20 mM Tris-HCl pH 8.0, 200 mM NaCl and optional 1 mM DTT) at a flow rate of 0.5 ml/min using a Superdex 200 Increase 10/300 column, connected to an Äkta Pure25 system. The molecular weight of the protein was estimated by comparison with standard proteins of known molecular weight (Cytochrome C, 12.4 kDa; Carbonic Anhydrase, 29 kDa; Albumin, 66 kDa; Alcohol Dehydrogenase, 150 kDa; β-Amylase, 200 kDa) (Molecular weight markers MWGF200, Sigma-Aldrich, Munich, Germany).

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