

Figure S1. The CspB prodomain can be supplied *in trans* to largely reconstitute CspB function.

(A) Schematic of wild-type CspBA and a construct encoding the prodomain *in trans* (Q66_{TAG}).

“Pro” denotes the prodomain. Q66-TAG encodes a variant in which the CspB prodomain is produced *in trans* from the remainder of CspBA through the introduction of a stop codon after codon 66 and a ribosome binding site and start codon before codon 67. (B) Western blot analyses of CspB(A) and CspC in sporulating cells and purified spores from wild type 630 Δ *erm-p*, Δ *cspBA*, and Δ *cspBA* complemented with either wild-type *cspBA* or the *cspBA* trans-complementation variant. A-P refers to CspB(A) that has undergone autoprocessing to release the CspB prodomain. Δ *spo0A* (Δ 0A) was used as a negative control for sporulating cells. SpoIVA was used as a loading control for sporulating cells, while CotA was used as a loading control for purified spores. An anti-CspB antibody was used to detect full-length CspBA in sporulating cells. A non-specific band in the anti-CspB blot is indicated with an asterisk. The germination efficiency of spores from the indicated strains plated on BHIS media containing 0.1% taurocholate is also shown relative to wild type. The mean and standard deviations shown are based on multiple replicates performed on two independent spore purifications. Statistical significance relative to wild type was determined using a one-way ANOVA and Tukey’s test. (C) Germinant sensitivity of Q66_{TAG} spores plated on BHIS containing increasing concentrations of taurocholate. The number of colony forming units (CFUs) produced by germinating spores is shown. The mean and standard deviations shown are based on multiple replicates performed on two independent spore purifications. Statistical significance relative to wild type was determined using a one-way ANOVA and Tukey’s test. **** p < 0.0001, *** p < 0.001, **p < 0.01.

Figure S2. Restoring CspC's catalytic triad appears to impair protein folding in *E. coli*. (A)

CspC space fill model with jelly roll domain in cyan, prodomain in pink and subtilase domain in grey. Residues identified as being required for *C. difficile* spore germination by Francis et al. [1] in a genetic screen are shown in black. The S443N substitution was identified in combination with V272G. The pseudoactive site residues Thr170 and Gly485 are shown in blue. (B)

Purification of CspC-His₆ variants from the soluble fraction. G171R was included because this substitution had been predicted to destabilize CspC by steric occlusion [2, 3]. Cultures expressing the *cspC* variants were induced with IPTG overnight at 18°C, and aliquots were removed for analysis of the “induced” fraction. Cultures were harvested, and cells were lysed using sonication. Following a high-speed centrifugation, the cleared lysate containing soluble proteins was incubated with Ni²⁺-NTA agarose beads. CspC-His₆ variants were eluted from the beads using imidazole (elution fraction). Equivalent volumes of samples were resolved by SDS-PAGE and analyzed by western blotting (top) and Coomassie staining (bottom).

Figure S3. Purification of CspC variants using the CPD self-cleaving tag and SEC. (A)

Coomassie stains of the CspC-CPD-His₆ purifications. The variants purified are listed above the gels. “+” shows IPTG-induced cell lysates; CL refers to the soluble proteins present in cleared lysates of IPTG-induced cell lysates; E1 and E2 refer to the elutions obtained from the supernatant fraction following inositol hexakisphosphate (InsP₆)-induced cleavage of CspC-CPD-His₆ variants. CspC_{EL} refers to the glutamate and leucine residues that are added to the C-terminus of the CspC variants following InsP₆-mediated cleavage. We note that WT CspC-CPD was present in higher levels in the cleared lysate fraction than the other CspC variants. (B) SEC profiles of CspC_{2xcat} and CspC_{G171R} variants. These traces are identical to those shown in **Figure**

3, except the y-axis has been scaled for these variants, which are purified with much lower yields. The black rectangle and blue and pink arrows identify the fraction that contained these variants. (C) Coomassie stain of fractions taken from the SEC shown in (B). The black rectangle highlights the fraction that was concentrated for the thermal shift assays and Coomassie gels shown in **Figure 3**. Lane 1 in the bottom gel includes SEC-purified CspC_{T170H}, which highlights how CspC_{G171R} runs at a slightly smaller apparent MW relative to CspC_{T170H} (and WT CspC, **Figure 3**). This slight difference in mobility is likely due to CPD-mediated trimming of the partially unfolded CspC_{2xcat} and CspC_{G171R} variants (compare the mobility of these variants in Figure 3 vs. S3). The CPD cleaves after leucine residues [4], and CspC carries a leucine at its C-terminus (residue 557) prior to the LEHHHHHH tag created by the pET22b cloning construct as well as a leucine at position 544.

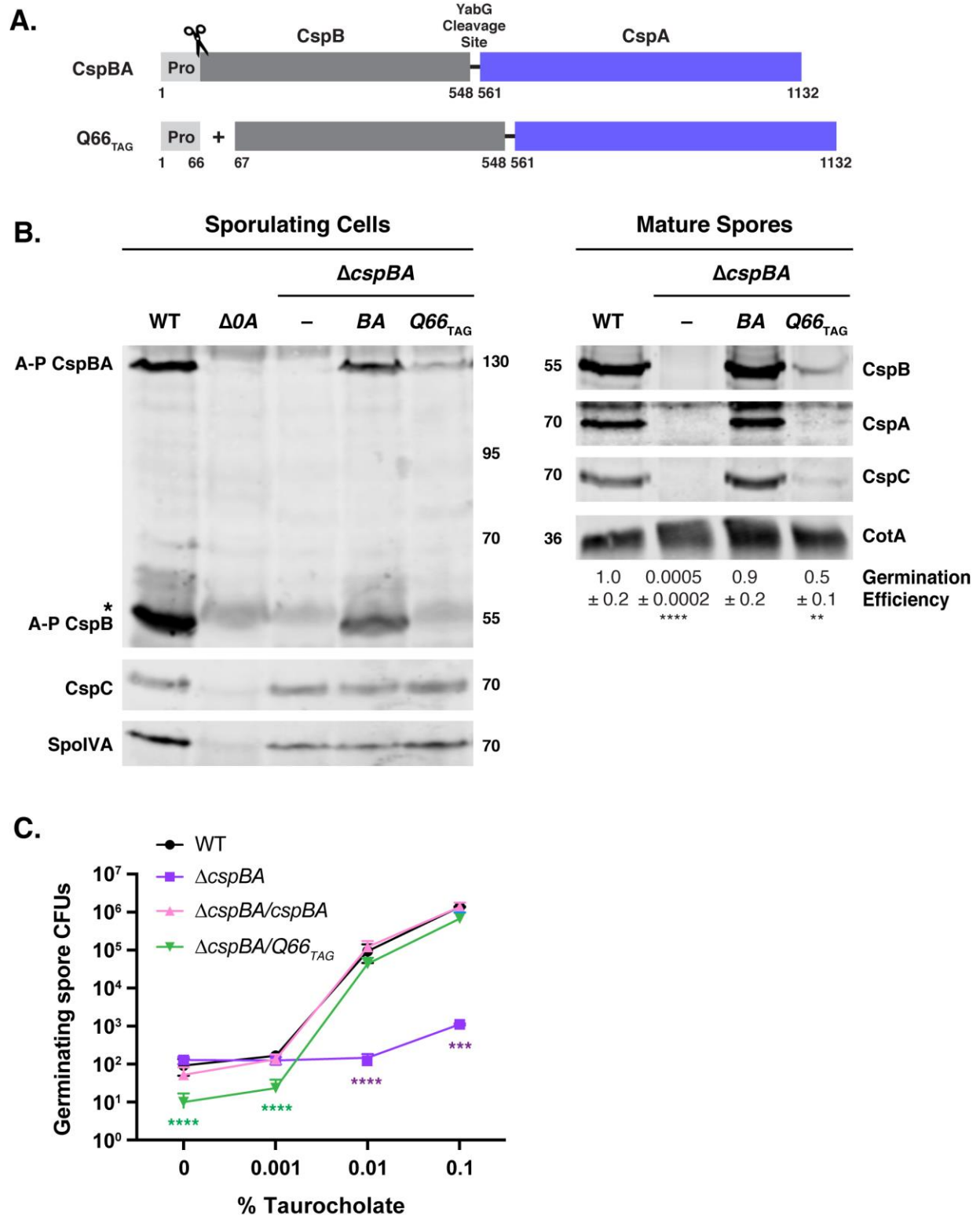


Figure S1. The CspB prodomain can be supplied *in trans* to largely re-constitute CspB function

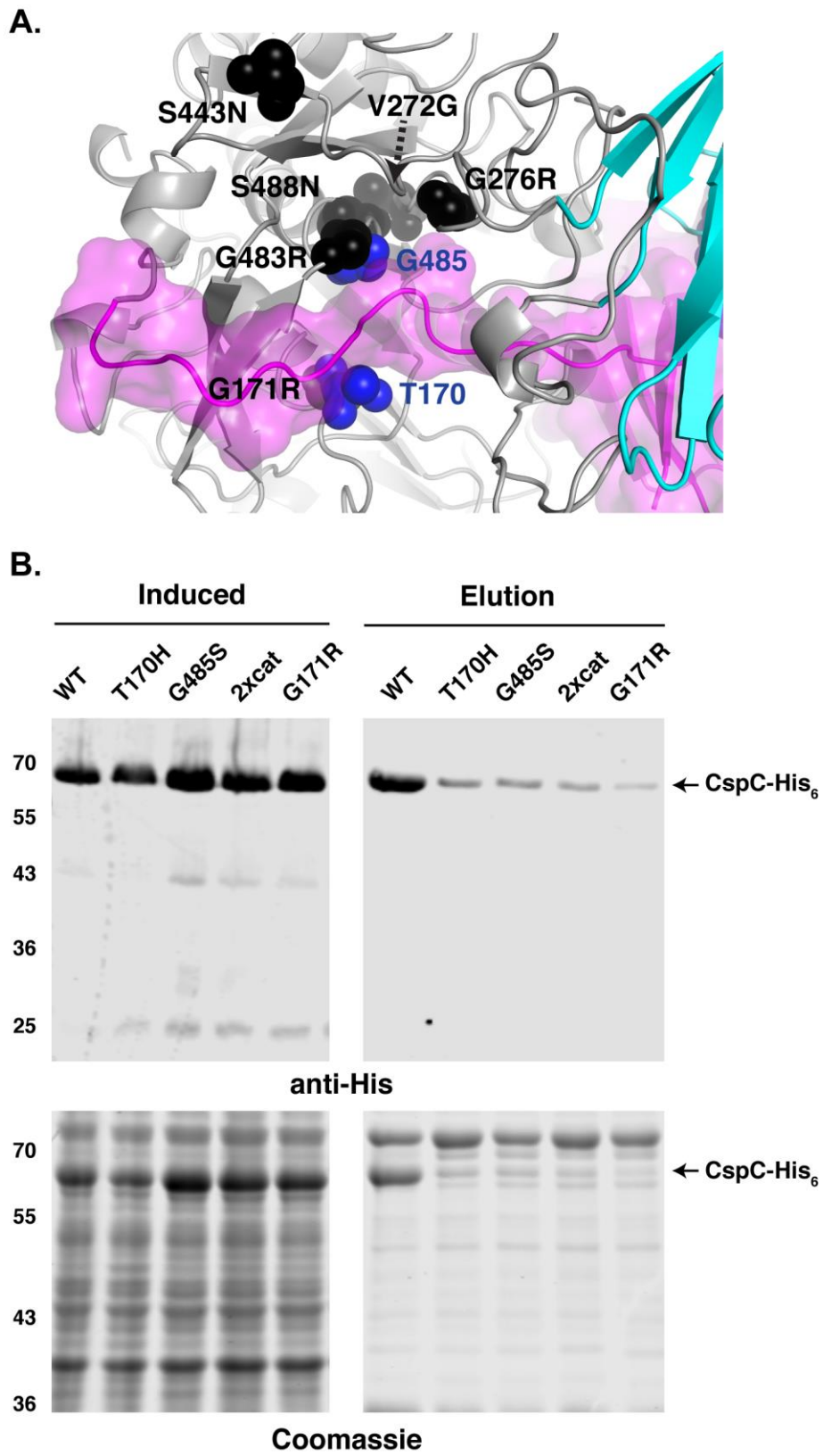


Figure S2. Restoring CspC's catalytic triad appears to impair folding in *E. coli*.

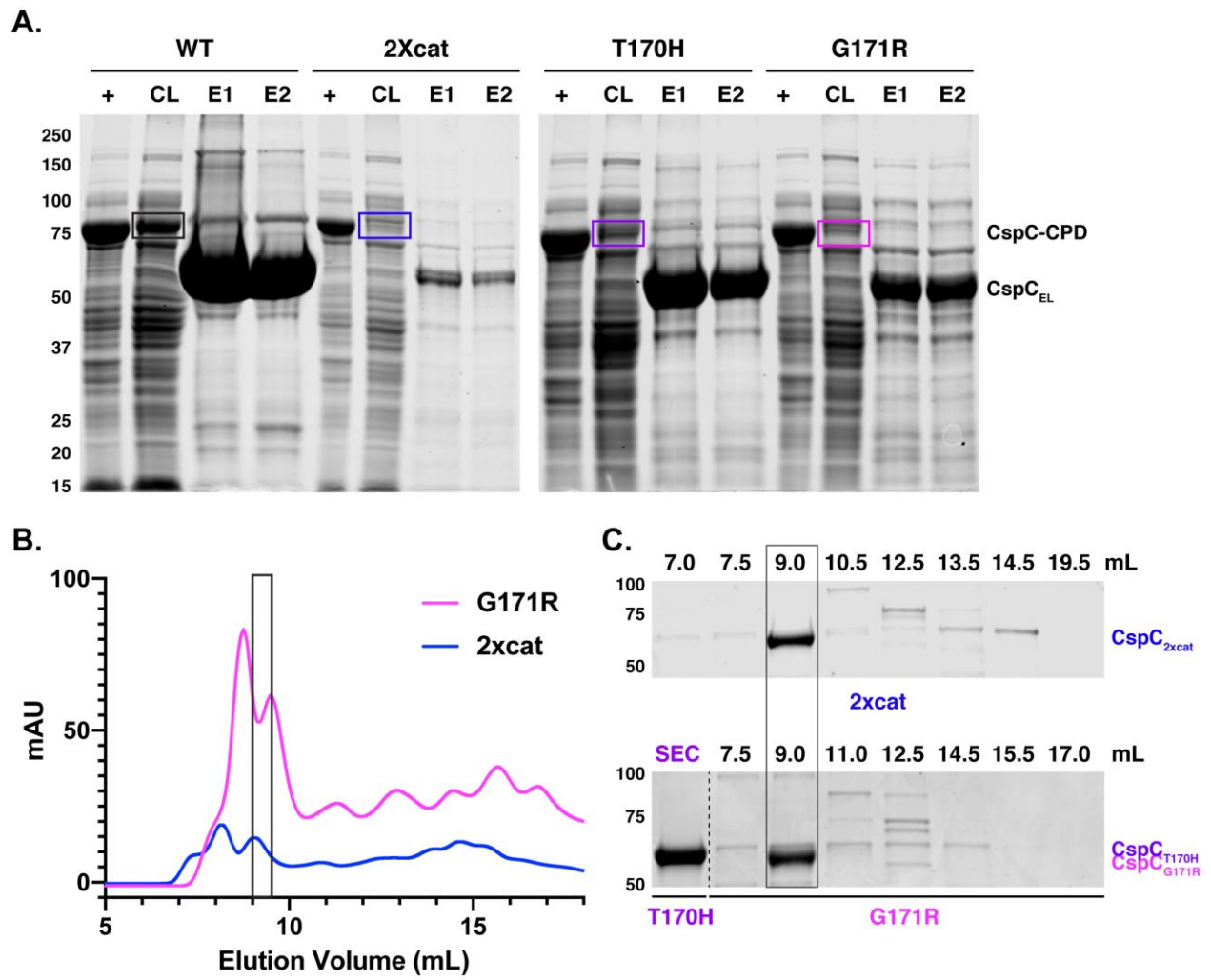


Figure S3. Purification of CspC variants using the CPD self-cleaving tag and SEC.

Supplementary Table S1 – *C. difficile* and *E. coli* strains used in this study.

Strain#	Strain name	Relevant genotype or features	Source/reference
<i>C. difficile</i> strains			
789	630 Δ <i>erm</i> Δ <i>pyrE</i> Δ <i>cspBA</i>	630 Δ <i>erm</i> Δ <i>pyrE</i> with <i>cspBA</i> deleted	[5]
799	630 Δ <i>erm</i> Δ <i>pyrE</i> Δ <i>cspC</i>	630 Δ <i>erm</i> Δ <i>pyrE</i> with <i>cspC</i> deleted	[6]
831	630 Δ <i>erm</i> Δ <i>cspC/cspC</i>	630 Δ <i>erm</i> Δ <i>cspC</i> with <i>cspC</i> in the <i>pyrE</i> locus	[6]
846	630 Δ <i>erm</i> -p	<i>erm</i> -sensitive derivate of 630 with <i>pyrE</i> restored	[5]
849	630 Δ <i>erm</i> Δ <i>spo0A</i> -p	630 Δ <i>erm</i> Δ <i>spo0A</i> with <i>pyrE</i> restored	[5]
859	630 Δ <i>erm</i> Δ <i>cspBA</i> -p	630 Δ <i>erm</i> Δ <i>cspBA</i> with <i>pyrE</i> restored	[6]
862	630 Δ <i>erm</i> Δ <i>cspBA/cspBA</i>	630 Δ <i>erm</i> Δ <i>cspBA</i> with <i>cspBA</i> in the <i>pyrE</i> locus	[6]
1238	630 Δ <i>erm</i> Δ <i>cspC</i> -p	630 Δ <i>erm</i> Δ <i>cspC</i> with <i>pyrE</i> restored	[6]
1438	630 Δ <i>erm</i> - Δ <i>cspC/cspC</i> _{T170H}	630 Δ <i>erm</i> Δ <i>cspC</i> with <i>cspC</i> _{T170H} in the <i>pyrE</i> locus	This study
1441	630 Δ <i>erm</i> - Δ <i>cspC/cspC</i> _{G485S}	630 Δ <i>erm</i> Δ <i>cspC</i> with <i>cspC</i> _{G485S} in the <i>pyrE</i> locus	This study
1751	630 Δ <i>erm</i> Δ <i>cspC/cspC</i> _{L64-TAG}	630 Δ <i>erm</i> Δ <i>cspC</i> with <i>cspC</i> _{L64-TAG} in the <i>pyrE</i> locus	This study
1768	630 Δ <i>erm</i> Δ <i>cspC/cspC</i> _{T170H/G485S}	630 Δ <i>erm</i> Δ <i>cspC</i> with <i>cspC</i> _{T170H/G485S} in the <i>pyrE</i> locus	This study
1864	630 Δ <i>erm</i> Δ <i>cspBA/cspBA</i> _{Q66-TAG}	630 Δ <i>erm</i> Δ <i>cspBA</i> with <i>cspBA</i> _{Q55-TAG} in the <i>pyrE</i> locus	This study
2517	630 Δ <i>erm</i> -p Δ <i>cspBA/cspBA</i> _{Q757H}	630 Δ <i>erm</i> Δ <i>cspBA</i> with <i>cspBA</i> Δ <i>C</i> _{Q757H} in the <i>pyrE</i> locus	This study
2520	630 Δ <i>erm</i> -p Δ <i>cspBA/cspBA</i> _{A1064S}	630 Δ <i>erm</i> Δ <i>cspBA</i> with <i>cspBA</i> Δ <i>C</i> _{A1064S} in the <i>pyrE</i> locus	This study
2559	630 Δ <i>erm</i> -p Δ <i>cspBA/cspBA</i> _{Q757H/A1064S}	630 Δ <i>erm</i> Δ <i>cspBA</i> with <i>cspBA</i> Δ <i>C</i> _{Q757H/A1064S} in the <i>pyrE</i> locus	This study
<i>E. coli</i> strains			
Strain#	Strain Background	Plasmid carried	
41	DH5 α	F- Φ 80 <i>lacZ</i> Δ M15 Δ (<i>lacZYA-argF</i>) U169 <i>recA1 endA1 hsdR17</i> (rK ⁻ , mK ⁺) <i>phoA supE44</i> λ - <i>thi-1 gyrA96 relA1</i>	D. Cameron
455	BL21	pET28a- <i>cspB</i> (548aa)	[7]
471	BL21	pET28a- <i>cspB</i> (548aa)-S461A	[7]
531	HB101/pRK24	F- <i>mcrB mrr hsdS20</i> (rB ⁻ mB ⁻) <i>recA13 leuB6 ara-13 proA2 lavYI galK2 xyl-6 mtl-1 rpsL20</i> carrying pRK24	C. Ellermeier
981	BL21(DE3)	pET22b <i>cspC</i> -His ₆ codon-optimized	This study
982	BL21	pET22b <i>cspC</i> -CPD codon-optimized	This study
1185	BL21	pET22b <i>cspC</i> _{T170H} -CPD codon-optimized	This study
1169	BL21	pET22b <i>cspC</i> _{G171R} -CPD codon-optimized	This study
1281	BL21(DE3)	pET28a <i>cspBA</i> codon-optimized	This study
1889	HB101	pMTL-YN1C Δ <i>cspBA-cspC</i> _{T170H}	This study
1890	HB101	pMTL-YN1C Δ <i>cspBA-cspC</i> _{G485S}	This study
2018	HB101	pMTL-YN1C Δ <i>cspBA-cspC</i> _{T170H/G485Sr}	This study
2062	HB101	pMTL-YN1C Δ <i>cspBA-cspC</i> _{L64-TAG}	This study
2076	HB101	pMTL-YN1C <i>cspBA</i> _{Q66-TAG} - Δ <i>cspC</i>	This study
2098	BL21(DE3)	pET22b <i>cspC</i> _{T170H/G485S} -His ₆ codon-optimized	This study
2099	BL21(DE3)	pET22b <i>cspC</i> _{G485S} -His ₆ codon-optimized	This study
2126	BL21(DE3)	pET22b <i>cspC</i> _{T170H} -His ₆ codon-optimized	This study
2127	BL21(DE3)	pET22b <i>cspC</i> _{G171R} -His ₆ codon-optimized	This study
2368	HB101	pMTL-YN1C <i>cspBA</i> Δ <i>C</i> _{A1064S}	This study
2370	HB101	pMTL-YN1C <i>cspBA</i> Δ <i>C</i> _{Q757H}	This study
2382	HB101	pMTL-YN1C <i>cspBA</i> Δ <i>C</i> _{Q757H/A1064S}	This study

2372	BL21(DE3)	pET28a <i>cspBA</i> _{Q757H} codon-optimized	This study
2384	BL21(DE3)	pET28a <i>cspBA</i> _{A1064S} codon-optimized	This study
2385	BL21(DE3)	pET28a <i>cspBA</i> _{Q757H/A1064S} codon-optimized	This study
2646	BL21	pET22b <i>cspC</i> _{T170H/G485S} -CPD codon-optimized	This study

Plasmids

Plasmids	Relevant features	Source/reference
pMTL-YN1C	Unstable plasmid for restoring <i>pyrE</i> locus in 630Δ <i>erm</i> <i>pyrE</i>	[8]
pET22b	Expression vector for IPTG-inducible production of C-terminally His ₆ -tagged proteins	Novagen
pET28a	Expression vector for IPTG-inducible production of His ₆ -tagged proteins	Novagen
pET22b-CPD _{SacI}	Expression vector for IPTG-inducible production of C-terminally CPD-His ₆ -tagged proteins	[9]

3034	5' NcoI <i>cspBA</i> CO pET28a Gibson 3' XhoI <i>cspBA</i> CO	CTTTAAGAAGGAGATATA <u>CCATGG</u> CTATTATCATTAATTACGAACTGATTGTGAA GTAC
3035	pET28a Gibson 5' Q757H SOE <i>cspBA</i>	CAGTGGTGGTGGTGGTGGT <u>GCTCGAGG</u> CGCAGTACATCAAACATGCCACG
3036	codon opt 3' Q757H rev eos <i>cspBA</i>	CTCTCTCAGGATGAGGTGCGGTCACGGCACCATGCTGTCCGGCATCTGCTC
3037	codon opt 5' A1064S SOE <i>cspBA</i>	GAGCAGATGCCGGACAGCATGGTGCCGTGACCGACCTCATCCTGAGAGAG
3038	codon opt 3' A1064S rev eos	CAATACATATGCAACCATTACTGGGACCTCAGCGGCGAGCGCGCATGCGGCTG
3039	<i>cspBA</i> codon opt 5' Q757H SOE <i>cspBA</i>	CAGCCGCATGCGCGCTCGCCGCTGAGGTCCCAGTAATGGTTGCATATGTATTG
3040	630 3' Q757H rev eos <i>cspBA</i>	CTAGTCTATCTCAGGATGAAGTTGGTCATGGA ACTATGTTGAGTGGGATATGTGC
3041	630	GCACATATCCCACTCAACATAGTTCATGACCAACTTCATCCTGAGATAGACTAG

^aRestriction sites are underlined

Supplementary Table S3– Peptostreptococcaceae CspBA homologs

Organism genus and species	Accession		CspB			CspA		
<i>Clostridioides difficile</i> 630	CAJ69133.1	DSG	HGTH	GTSMATP	DTG	HGTM	GTSAAAA	
<i>Clostridioides difficile</i> R20291	CDR20291_2147	DSG	HGTH	GTSMATP	DSG	RGTM	GTAASA	
<i>Clostridioides difficile</i> Y165	EQI24072.1	DSG	HGTH	GTSMATP	GSG	YGTI	GTAASAS	
<i>Romboutsia maritimum</i>	WP_095405957.1	DSG	HGTH	GTSMATP	DTG	SGTM	GTAVAAA	
<i>Romboutsia weinsteini</i>	WP_094368045.1	DSG	HGTH	GTSMATP	DSG	NGTM	GTAPAAA	
<i>Romboutsia sp. Marseille-P6047</i>	WP_122638576.1	DSG	HGTH	GTSMATP	DSG	NGTM	GTAPAAA	
<i>Romboutsia lituseburensis</i>	WP_092722645.1	DSG	HGTH	GTSMATP	DSG	NGTM	GTAPAAA	
<i>Paeniclostridium sordellii</i>	CEN89310.1	DSG	HGTH	GTSMATP	DSG	TGTM	GTAASAA	
<i>Paeniclostridium sordellii</i>	WP_081015910.1	DSG	HGTH	GTSMATP	DSG	SGTM	GTAPAAA	
<i>Paeniclostridium sordellii</i>	CEQ11710.1	DSG	HGTH	GTSMATP	DSG	HGTM	GTSAAAGA	
<i>Romboutsia hominis</i>	CEI72315.1	DSG	HGTH	GTSMATP	DSG	SGTM	GTAPAAA	
<i>Romboutsia timonensis</i>	WP_071121078.1	DSG	HGTH	GTSMATP	DSG	HGTM	GTSAAAGA	
<i>Paraclostridium bifermentans</i>	WP_142730225.1	DSG	HGTH	GTSMATP	DSG	TGTL	GTSAAAA	
<i>Romboutsia ilealis</i>	CED93323.1	DSG	HGTH	GTSMATP	DTG	HGTM	GSSAAGA	
<i>Paraclostridium bifermentans</i>	WP_025162001.1	DSG	HGTH	GTSMATP	DTG	HGTM	GSSAAGA	
<i>Peptostreptococcaceae bacterium</i>	WP_148487629.1	DSG	HGTH	GTSMATP	DTG	HGTM	GSSAAGA	

<i>Paraclostridium bifermentans</i>	WP_148550927.1	DSG	HGTH	GTSMATP	DSG	SGTL	GTAASSA
<i>Paraclostridium benzoelyticum</i>	OXX84749.1	DSG	HGTH	GTSMATP	DSG	SGTL	GTAASSA
<i>Paraclostridium benzoelyticum</i>	WP_046823062.1	DSG	HGTH	GTSISAA	DSG	SGTM	GTAVAAA
<i>Terrisporobacter glycolicus</i>	WP_148557837.1	DSG	HGTH	GTSISAA	DSG	SGTM	GTAVAAA
<i>Terrisporobacter glycolicus</i>	WP_083399335.1	DSG	HGTH	GTSMATP	DSG	TGTL	GTAAAAA
<i>Romboutsia lituseburensis</i>	WP_092722648.1	DSG	HGTH	GTSMATP	DSG	TGTL	GTAAAAA
<i>Romboutsia lituseburensis</i>	SDL34486.1	DSG	HGTH	GTSMATP	DSG	SGTL	GTAASAA
<i>Terrisporobacter othiniensis</i>	KHS58529.1	DSG	HGTH	GTSMATP	DTG	SGTL	GTSAAAA
<i>Terrisporobacter othiniensis</i>	WP_082007783.1	DSG	HGTH	GTSMATP	DTG	SGTL	GTSAAAA
<i>Terrisporobacter glycolicus</i>	WP_018589409.1	DSG	HGTH	GTSMATP	DTG	SGTL	GTSAAAA
<i>Intestinibacter bartlettii</i>	WP_055088383.1	DSE	NNED	-----	DSG	HGTM	GTAPAAA
<i>Intestinibacter bartlettii</i>	WP_078687908.1	DSG	HGTH	GTSMSAP	DSG	HGTL	GTSAGA
<i>Intestinibacter bartlettii</i>	WP_147616711.1	DSG	HGTH	GTSMAAP	DSG	HGTR	GTSASGA
<i>Asaccharospora irregularis</i>	WP_073125354.1	DSG	HGTH	GTSMAAP	DSG	HGTK	GTSASAA
<i>Tepidibacter mesophilus</i>	WP_099188795.1	DSG	HGTH	GTSMAAP	DSG	HGTM	GTSVAAA
<i>Tepidibacter formicigenes</i>	WP_072888171.1	DSG	HGTH	GTSMAAP	DSG	HGTM	GTSVAAA
<i>Tepidibacter thalassicus</i>	WP_084601981.1	DSG	HGTH	GTSMAAP	DSG	HGTM	GTSVAAA
<i>Clostridium paradoxum</i>	WP_083528235.1	DSG	HGTH	GTSMATP	DTG	QGTM	GTAASA
<i>Clostridium paradoxum</i>	KXZ39276.1	DSG	HGTH	GTSMATP	DTG	QGTM	GTAASA
<i>Clostridium thermoalcaliphilum</i>	WP_079411776.1	DSG	HGTH	GTSMATP	DTG	QGTM	GTAASA

Supplementary Table S4. Peptostreptococcaceae CspC homologs.

Organism genus and species	Accession		CspC	
<i>Clostridium difficile</i> 630	CAJ69132.1	DSG	TGTM	GTGVSAS
<i>Clostridioides difficile</i> R20291	6MW4_A	DSG	TGTI	GTGISSS
<i>Clostridioides difficile</i> Y165	EQF98486.1	DSG	TGTI	GTGISSS
<i>Romboutsia weinsteini</i>	WP_094368046.1	DSG	TGTI	GTGVSSS
<i>Romboutsia maritimum</i>	WP_115975881.1	DSG	TGTI	GTGVSSS
<i>Romboutsia maritimum</i>	WP_115975890.1	DSG	TGTI	GTGVSSS
<i>Paeniclostridium sordellii</i>	WP_055341573.1	DSG	TGTM	GTGVSSS
<i>Paeniclostridium sordellii</i>	WP_057564142.1	DSG	TGTT	GTGVSSS
<i>Paeniclostridium sordellii</i>	CEK30389.1	DSG	TGTT	GTGVSSS
<i>Clostridium dakareense</i>	WP_042275205.1	DSG	TGTM	GTGVSSS
<i>Clostridiales</i>	WP_053830978.1	DSG	TGTT	GTGVSSS
<i>Paraclostridium bifermentans</i>	WP_148550882.1	DSG	TGTT	GTGVSSS
<i>Romboutsia</i> sp. MT17	WP_092926789.1	DSG	TGTM	GTGVSSS
<i>Paraclostridium bifermentans</i>	WP_142730224.1	DSG	TGTT	GTGVSSS
<i>Paraclostridium bifermentans</i>	WP_025162002.1	DSG	TGTI	GTGVSSS
<i>Romboutsia hominis</i>	CEI72316.1	DSG	TGTT	GTGVSSS
<i>Paraclostridium benzoelyticum</i>	WP_046821862.1	DSG	TGTI	GTGVSTS
<i>Romboutsia weinsteini</i>	WP_094369213.1	DSG	TGTM	GTGISSS
<i>Paraclostridium benzoelyticum</i>	OXX84577.1	DSG	TGTI	GTGVSSS
<i>Romboutsia lituseburensis</i>	WP_092722643.1	DSG	TGTM	GTGISSS
<i>Clostridioides manganotii</i>	WP_024620345.1	DSG	TGTM	GTGVSSS
<i>Romboutsia</i> sp. Marseille-P6047	WP_122638577.1	DSG	TGTM	GTGISSS
<i>Clostridioides manganotii</i>	WP_027702696.1	DSG	TGTI	GTGISSS
<i>Peptostreptococcaceae bacterium</i> VA2	WP_026901720.1	DSG	TGTM	GTGISSS
<i>Clostridioides manganotii</i>	WP_024620469.1	DSG	TGTI	GTGVSSS
<i>Romboutsia timonensis</i>	WP_071121077.1	DSG	TGTI	GTGISAS
<i>Asaccharospora irregularis</i>	WP_073125363.1	DSG	TGTI	GTGVSSS
<i>Romboutsia ilealis</i>	CED93324.1	DSG	TGTI	GTGVSSS
<i>Terrisporobacter glycolicus</i>	WP_148557830.1	DSG	TGTL	GTGVSSS
<i>Terrisporobacter glycolicus</i>	WP_018589415.1	DSG	TGTL	GTGVSSS

References

- 1 Francis, M. B., Allen, C. A., Shrestha, R. and Sorg, J. A. (2013) Bile acid recognition by the *Clostridium difficile* germinant receptor, CspC, is important for establishing infection. PLoS Pathog. **9**, e1003356
- 2 Kochan, T. J., Foley, M. H., Shoshiev, M. S., Somers, M. J., Carlson, P. E. and Hanna, P. C. (2018) Updates to *Clostridium difficile* Spore Germination. J Bacteriol. **200**
- 3 Rohlfing, A. E., Eckenroth, B. E., Forster, E. R., Kevorkian, Y., Donnelly, M. L., Benito de la Puebla, H., Doublie, S. and Shen, A. (2019) The CspC pseudoprotease regulates germination of *Clostridioides difficile* spores in response to multiple environmental signals. PLoS Genet. **15**, e1008224
- 4 Shen, A., Lupardus, P. J., Albrow, V. E., Guzzetta, A., Powers, J. C., Garcia, K. C. and Bogyo, M. (2009) Mechanistic and structural insights into the proteolytic activation of *Vibrio cholerae* MARTX toxin. Nat Chem Biol. **5**, 469-478
- 5 Donnelly, M. L., Li, W., Li, Y. Q., Hinkel, L., Setlow, P. and Shen, A. (2017) A *Clostridium difficile*-Specific, Gel-Forming Protein Required for Optimal Spore Germination. mBio. **8**
- 6 Kevorkian, Y. and Shen, A. (2017) Revisiting the Role of Csp Family Proteins in Regulating *Clostridium difficile* Spore Germination. J Bacteriol. **199**
- 7 Adams, C. M., Eckenroth, B. E., Putnam, E. E., Doublie, S. and Shen, A. (2013) Structural and functional analysis of the CspB protease required for *Clostridium* spore germination. PLoS Pathog. **9**, e1003165
- 8 Ng, Y. K., Ehsaan, M., Philip, S., Collery, M. M., Janoir, C., Collignon, A., Cartman, S. T. and Minton, N. P. (2013) Expanding the repertoire of gene tools for precise manipulation of the *Clostridium difficile* genome: allelic exchange using *pyrE* alleles. PLoS One. **8**, e56051
- 9 Shen, A., Lupardus, P. J., Morell, M., Ponder, E. L., Sadaghiani, A. M., Garcia, K. C. and Bogyo, M. (2009) Simplified, enhanced protein purification using an inducible, autoprocessing enzyme tag. PLoS One. **4**, e8119