Supporting Information

The structure of *Acinetobacter* secreted protease CpaA complexed with its chaperone CpaB reveals a novel mode of T2SS chaperone/substrate interaction.

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The file contains 3 Supporting Information figures and 2 Supporting Information tables

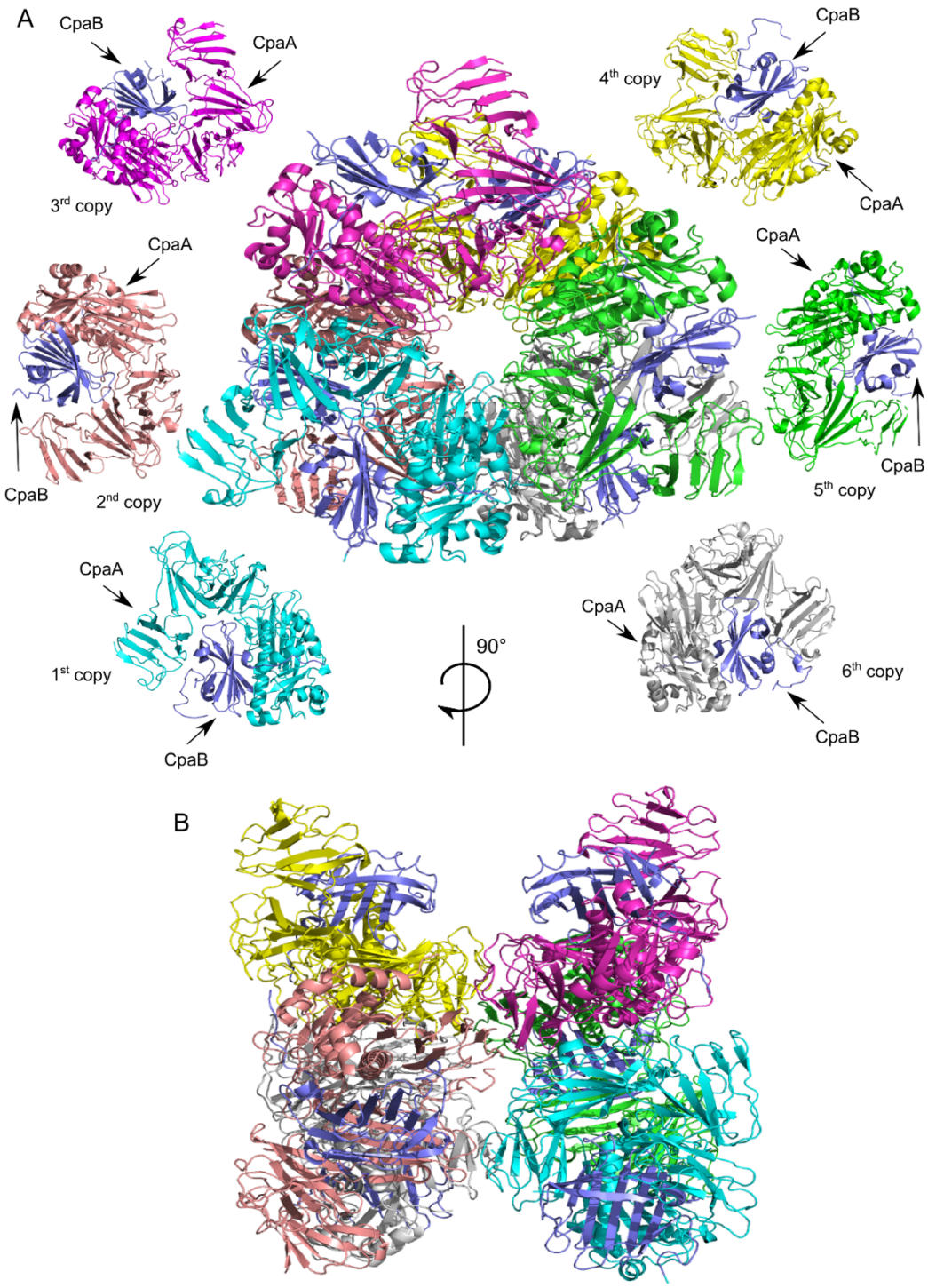

Figure S1. Six identical copies of CpaAB complex in the asymmetric unit.

(A) The structure confirms stoichiometry 1:1. Chains of protease CpaA are shown in colors, CpaB is shown in blue. (B) 90° rotation of the molecule in (A).

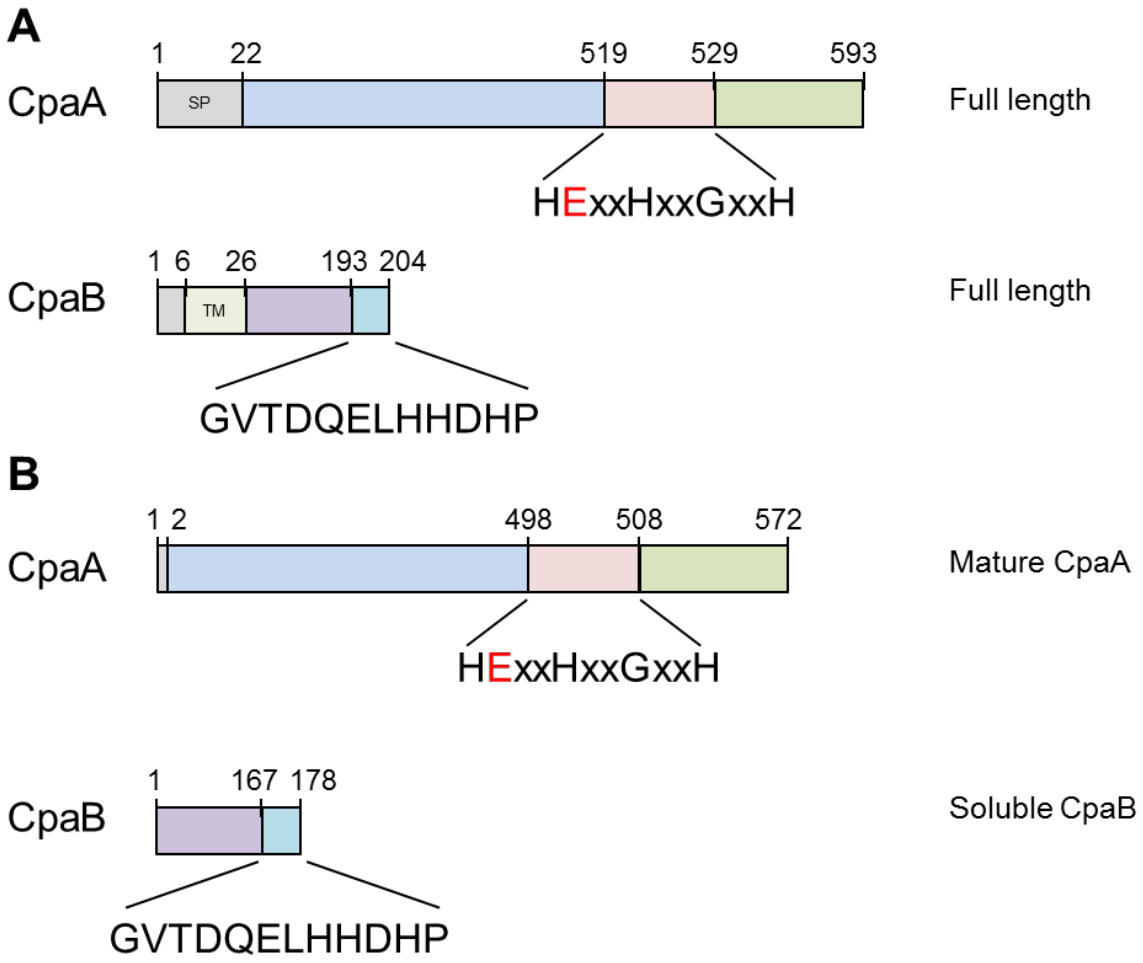

Figure S2. **A**, Schematic domain organization of full length CpaA and CpaB. **B,** Schematic domain organization of the CpaA and CpaB constructs used in this study. Highlighted in red is the general base/acid glutamate (E499 in the mature form) involved in catalysis.

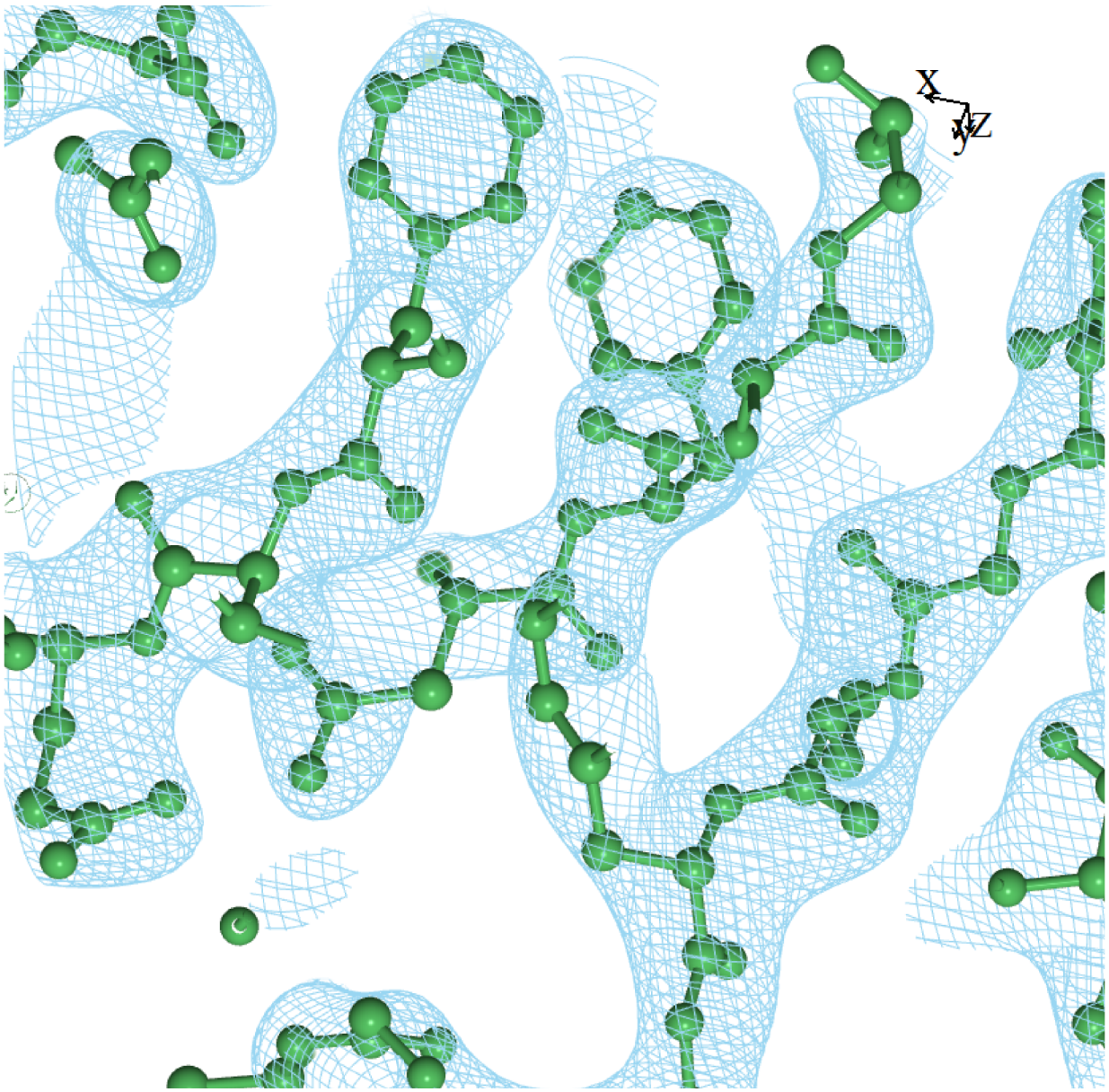

Figure S3. The 2Fo-Fc electron density map at 1σ around CpaAB.

Table S1. Pairwise overlay rmsd between CpaA repeats				
Domain	1st Repeat	2nd Repeat	3rd Repeat	4th Repeat
1st Repeat	0	1.378	0.605	2.539
2nd Repeat	1.378	0	0.864	2.054
3rd Repeat	0.605	0.864	0	1.157
4th Repeat	2.539	2.054	1.157	0

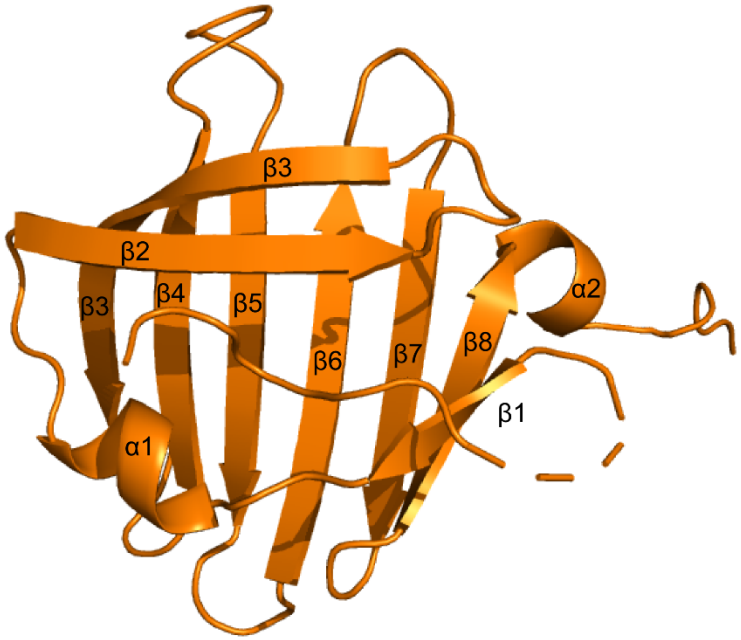

Figure S4. Topology of CpaB. A β-sandwich structure comprises vertical antiparallel β-sheet formed by strands β1 and β3–β8 (arrows) and helix α1, and a small two stranded β-sheet orthogonal to the large β-sheet made of β2 and β3 and α2.

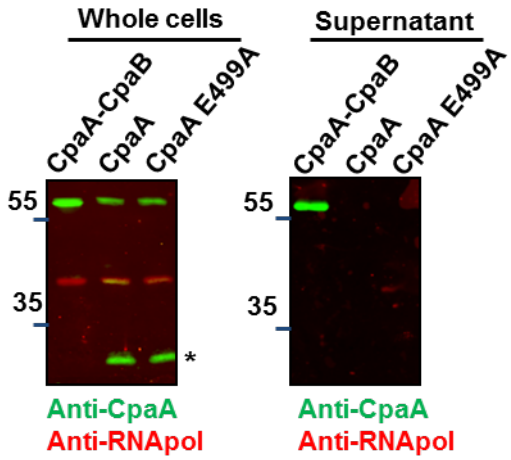

Figure S5. CpaB does not protect CpaA from self-proteolysis. Whole cells and supernatant preparations of *A. nosocomialis* Δ*cpaAB* cells expressing CpaA-CpaB, CpaA or CpaAE499A were analyzed by Western blot using anti-CpaA polyclonal antibody. RNA polymerase was used as a lysis control (anti-RNApol antibody). The asterisk indicated the proteolytic fragment derived from CpaA.

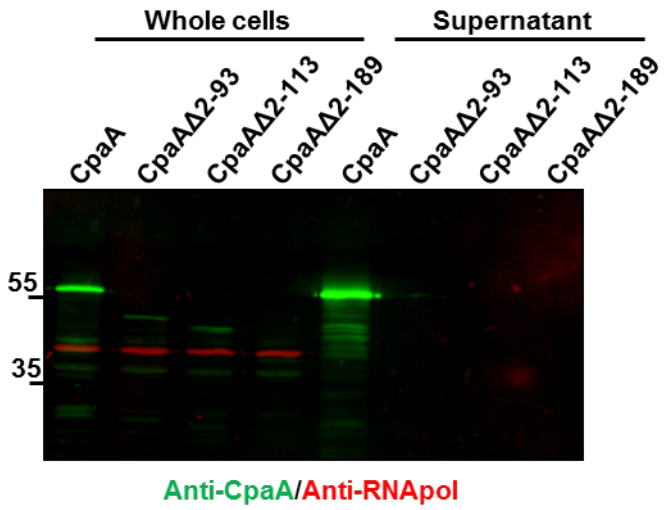

Figure S6. CpaA lectin domains are required for its stability. Whole cells and supernatant preparations of *A. nosocomialis* Δ*cpaAB* cells expressing CpaA-CpaB, CpaAΔ2-93-CpaB or CpaAΔ2-113-CpaB or CpaAΔ2-189-CpaB were analyzed by Western blot using anti-CpaA polyclonal antibody. RNA polymerase was used as a lysis control (anti-RNApol antibody). CpaA expression and secretion was detected only in the cells carrying full length variant.

Table S2. Hydrogen bonds and salt bridge contacts for CpaA and CpaB			
Hydrogen bonds		Salt Bridges	
CpaA	**CpaB**	**CpaA**	**CpaB**
Asp^{B61}(OD1)	Asn^{H96}(ND2)	Lys^{B34}(NZ)	Glu^{H92}(OE2)
Glu^{B70}(OE2)	Asn^{H96}(N)	Glu^{B70}(OE1)	Arg^{H97}(NH1)
Glu^{B70}(OE2)	Asn^{H96}(O)	Glu^{B70}(OE2)	Arg^{H97}(NE)
Glu^{B70}(OE1)	Arg^{H97}(NH1)	Glu^{B70}(OE2)	Arg^{H97}(NH1)
Gln^{B266}(O)	Asn^{H124}(ND2)	Lys^{B309}(NZ)	Glu^{H103}(OE1)
Ser^{B282}(OG)	Asn^{H124}(ND2)	Lys^{B309}(NZ)	Glu^{H103}(OE2)
Ser^{B282}(OG)	Asp^{H122}(OD1)	Arg^{B397}(NE)	Glu^{H153}(OE1)
Lys^{B309}(NZ)	Glu^{H103}(OE1)	Arg^{B397}(NE)	Glu^{H153}(OE2)
Leu^{B389}(O)	Lys^{H155}(NZ)	Glu^{B400}(OE2)	Arg^{H113}(NH1)
Ser^{B392}(OG)	Asp^{H110}(OD2)	Glu^{B400}(OE1)	Arg^{H113}(NH1)
Ser^{B392}(N)	Asp^{H136}(OD2)	His^{B498}(NE2)	Asp^{H170}(OD1)
Ala^{B393}(N)	Asp^{H136}(OD2)	His^{B498}(NE2)	Asp^{H170}(OD2)
Arg^{B397}(NE)	Glu^{H153}(OE1)	His^{B502}(NE2)	Asp^{H170}(OD2)
Glu^{B400}(OE2)	Arg^{H113}(NH1)	His^{B508}(NE2)	Asp^{H170}(OD1)
Glu^{B400}(OE2)	Thr^{H131}(OG1)		
Glu^{B400}(OE1)	Gln^{H142}(NE2)		
Cys^{B470}(N)	Thr^{H169}(O)		
Ser^{B492}(N)	Gly^{H167}(O)		
His^{B498}(NE2)	Asp^{H170}(OD1)		
His^{B508}(N)	Glu^{H172}(OE2)		
His^{B508}(NE2)	Asp^{H170}(OD1)		
Asn^{B531}(O)	Asn^{H149}(ND2)		

Table S3. Strains and plasmids used in this study		
Strain	**Genotype**	**Reference/source**
Stellar	*F–, endA1, supE44, thi-1, recA1, relA1, gyrA96, phoA, Φ80d lacZΔ M15, Δ (lacZYA - argF) U169, Δ (mrr - hsdRMS - mcrBC), ΔmcrA, λ–*	Clontech
Rosetta 2	F- ompT hsdSB(rB- mB-) gal dcm (DE3) pRARE2 (CamR)	Novagen
Δ*cpaAB*	*A. nosocomialis* M2 Δ *cpaAB::frt*	(1)
Plasmid	**Description**	**Reference/source**
pETDUET -cpaA -his -CpaB	*cpaA-6xhis-cpaB* cloned into pETDUET. This construct encodes the mature form of CpaA and a soluble form of CpaB lacking its transmembrane domain.	(1)
pWH-*cpaA-his-cpaB*	*cpaA-6xhis-cpaB* cloned into pWH1266 with its own promoter region	(2)
pWH-*cpaA-cpaB-his*	*cpaA-cpaB-6xhis* cloned into pWH1266 with its own promoter region	(1)
pMFH23	CpaB D170A using pWH-*cpaA-cpaB-his* as template	This study
pMFH24	CpaB D170E using pWH-*cpaA-cpaB-his* as template	This study
pMFH25	CpaB D170N using pWH-*cpaA-cpaB-his* as template	This study
pMFH26	CpaB Δ167-178 using pWH-*cpaA-cpaB-his* as template	This study
pMFH30	CpaB D170H using pWH-*cpaA-cpaB-his* as template	This study
pMFH31	CpaB D170A E172A using pMFH23 as template	This study
pMFH32	CpaA E499A using pWH-*cpaA-his-cpaB* as template	This study
pMFH40	CpaA Δ2-93 using pWH-*cpaA-his-cpaB* as template, CpaA without the 1st lectin domain	This study
pMFH41	CpaA Δ2-113 using pWH-*cpaA-his-cpaB* as template, CpaA without the 1st lectin domain and loop region	This study
pMFH42	CpaA Δ2-189 using pWH-*cpaA-his-cpaB* as template, CpaA without the 1st and 2nd lectin domains and loop region between them	This study

Table S4. Primers used in this study	
Description	**Sequence**
CpaB D170A 1	catgatgtaactcctgagctgtaacaccctcatcc
CpaB D170A 2	ggatgagggtgttacagctcaggagttacatcatg
CpaB D170E 1	atcatgatgtaactcctgctctgtaacaccctcatcc
CpaB D170E 2	ggatgagggtgttacagagcaggagttacatcatgat
CpaB D170N 1	ctatggatgagggtgttacaaatcaggagttacatcatgat
CpaB D170N 2	atcatgatgtaactcctgatttgtaacaccctcatccatag
CpaB Δ167-178 Inf fw	gcaaactatggatgagcatcatcatcatcactagaggaataaatgattttgg
CpaB Δ167-178 Inf rv	ctcatccatagtttgcaccaacccaacacc
CpaB D170H 1	catgatgtaactcctgatgtgtaacaccctcatccat
CpaB D170H 2	atggatgagggtgttacacatcaggagttacatcatg
CpaB D170AE172A 1	gatgatcatgatgtaacgcctgagctgtaacaccc
CpaB D170AE172A 2	gggtgttacagctcaggcgttacatcatgatcatc
CpaA E499A 1	tgcagccatgcgtcatgcagttgggcataatcttg
CpaA E499A 2	caagattatgcccaactgcatgacgcatggctgca
CpaA Δlectins Inphusion rv	agcatatacaagtgaagatgcaacaattgcacc
CpaA Δ2-93 Inphusion fw	gcatcttcacttgtatatgctcagctagcaacggttagtcctactactggg
CpaA Δ2-113 Inphusion fw	gcatcttcacttgtatatgctgcaaccatgcaaaaagtacttattcaagatgacaaatgg
CpaA Δ2-189 Inphusion fw	gcatcttcacttgtatatgctaaacctcagaagttaaatgttcagcaaattgg

REFERENCES

1. Kinsella, R. L., Lopez, J., Palmer, L. D., Salinas, N. D., Skaar, E. P., Tolia, N. H., and Feldman, M. F. (2017) Defining the interaction of the protease CpaA with its type II secretion chaperone CpaB and its contribution to virulence in Acinetobacter species. *Journal of Biological Chemistry* **292**, 19628-19638
2. Harding, C. M., Kinsella, R. L., Palmer, L. D., Skaar, E. P., and Feldman, M. F. (2016) Medically Relevant Acinetobacter Species Require a Type II Secretion System and Specific Membrane-Associated Chaperones for the Export of Multiple Substrates and Full Virulence. *PLoS pathogens* **12**, e1005391