

Figure S5 Alignment of nucleotide and amino acid sequences of CPE

(a) Alignment of *cpe* nucleotide sequences of strains CP396 and CP859. Asterisk indicates identical nucleotide. Different nucleotides are shown in red.

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CP396      ATGCTTAGTAACAATTTAAATCCAATGGTGTTCGAAAATGCTAAAGAAGTATTTCTTATT 60
CP859      ATGCTTAGTAACAATTTAAATCCAATGGTGTTCGAAAATGCTAAAGAAGTATTTCTTATT 60
*****

CP396      TCTGAGGATTTAAAAACCAATTAATATTACAACTCTAACTCAAATTTAAGTGATGGA 120
CP859      TCTGAGGATTTAAAAACCAATTAATATTACAACTCTAACTCAAATTTAAGTGATGGA 120
*****

CP396      TTATATGTAATAGATAAAGGAGATGGTTGGATATTAGGGGAACCTCAGTAGTTTCAAGT 180
CP859      TTATATGTAATAGATAAAGGAGATGGTTGGATATTAGGGGAACCTCAGTAGTTTCAAGT 180
*****

CP396      CAAATTCCTTAATCCTAATGAAACAGGTACCTTTAGCCAATCATTAACTAAATCTAAAGAA 240
CP859      CAAATTCCTTAATCCTAATGAAACAGGTACCTTTAGCCAATCATTAACTAAATCTAAAGAA 240
*****

CP396      GTATCTATAAATGTAAATTTTTCAGTTGGATTTACTTCTGAATTTATACAAGCATCTGTA 300
CP859      GTATCTATAAATGTAAATTTTTCAGTTGGATTTACTTCTGAATTTATACAAGCATCTGTA 300
*****

CP396      GAATATGGATTGGAATAACTATAGGAGAACAAAATACAATAGAAAGATCTGTATCTACA 360
CP859      GAATATGGATTGGAATAACTATAGGAGAACAAAATACAATAGAAAGATCTGTATCTACA 360
*****

CP396      ACTGCTGGTCCAAATGAATATGTATATTATAAGGTTTATGCAACTTATAGAAAGTATCAA 420
CP859      ACTGCTGGTCCAAATGAATATGTATATTATAAGGTTTATGCAACTTATAGAAAGTATCAA 420
*****

CP396      GCTATTAGAATTTCTCATGGTAATATCTCTGATGATGGATCAATTTATAAATTAACAGGA 480
CP859      GCTATTAGAATTTCTCATGGTAATATCTCTGATGATGGATCAATTTATAAATTAACAGGA 480
*****

CP396      ATATGGCTTAGTAAAACATCTGCAGATAGCTTAGGAAATATTGATCAAGGTTTCATTAATT 540
CP859      ATATGGCTTAGTAAAACATCTGCAGATAGCTTAGGAAATATTGATCAAGGTTTCATTAATT 540
*****

CP396      GAAACTGGTGAAAGATGTGTTTTAACAGTTCATCTACAGATATAGAAAAAGAAATCCTT 600
CP859      GAAACTGGTGAAAGATGTGTTTTAACAGTTCATCTACAGATATAGAAAAAGAAATCCTT 600
*****

CP396      GATTTAGCTGCTGCTACAGAAAGATTAAATTTAACTGATGCATTAACTCAAATCCAGCT 660
CP859      GATTTAGCTGCTGCTACAGAAAGATTAAATTTAACTGATGCATTAACTCAAATCCAGCT 660
*****

CP396      GGTAATTTATATGATTGGCGTTCTTCTAACTCATACCCTTGGACTCAAAAGCTTAATTTA 720
CP859      GGTAATTTATATGATTGGCGTTCTTCTAACTCATACCCTTGGACTCAAAAGCTTAATTTA 720
*****

CP396      CACTTAACAATTACAGCTACTGGACAAAAATATAGAATCTTAGCTAGCAAAATTGTTGAT 780
CP859      CACTTAACAATTACAGCTACTGGACAAAAATATAGAATCTTAGCTAGCAAAATTGTTGAT 780
*****

CP396      TTTAATATTTATTCAAATAATTTTAATAATCTAGTGAAATTAGAACAGTCCTTAGGTGAT 840
CP859      TTTAATATTTATTCAAATAATTTTAATAATCTAGTGAAATTAGAACAGTCCTTAGGTGAT 840
*****

CP396      GGAGTAAAAGATCATTATGTTGATATAAGCTTAGATGCTGGACAATATGTTCTTGTAATG 900
CP859      GGAGTAAAAGATCATTATGTTGATATAAGCTTAGATGCTGGACAATATGTTCTTGTAATG 900
*****

CP396      AAAGCTAATTCATCATATAGTGGAAATTACCCTTATTCAATATTATTTCAAAAATTTTAA 960
CP859      AAAGCTAATTCATCATATAGTGGAAATTACCCTTATTCAATATTATTTCAAAAATTTTAA 960
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Figure S5 (b) Alignment of CPE amino acid sequences of strains CP396 and CP859. Asterisk indicates identical amino acid. Different amino acid is shown in red.

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CP396      MLSNNLPMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGEPSVVSS 60
CP859      MLSNNLPMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGEPSVVSS 60
*****

CP396      QILNPNETGTFSQSLTKSKEVSINVNFSGFTSEFIQASVEYGFGITIGEQTIERSVST 120
CP859      QILNPNETGTFSQSLTKSKEVSINVNFSGFTSEFIQASVEYGFGITIGEQTIERSVST 120
*****

CP396      TAGPNEYVYYKYATYRKYQAIRISHGNI SDDGSIYKLTGIWLSKTSADSLGNIDQSLI 180
CP859      TAGPNEYVYYKYATYRKYQAIRISHGNI SDDGSIYKLTGIWLSKTSADSLGNIDQSLI 180
*****.***

CP396      ETGERCVLTPSTDIEKEILDAAATERLNLTDALNSNPAGNLYDWRSSNSYPWTQKLN 240
CP859      ETGERCVLTPSTDIEKEILDAAATERLNLTDALNSNPAGNLYDWRSSNSYPWTQKLN 240
*****

CP396      HLTITATGQKYRILASKIVDFNIYSNNFNNLVKLEQSLGDGVKDHVVDISLDAGQYVLVM 300
CP859      HLTITATGQKYRILASKIVDFNIYSNNFNNLVKLEQSLGDGVKDHVVDISLDAGQYVLVM 300
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CP396      KANSSYSGNYPYSILFQKF 319
CP859      KANSSYSGNYPYSILFQKF 319
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Figure S5 (c) Alignment of CPE amino acid sequences of *C. perfringens* strains belonging to major and minor lineages. Two strains analyzed in the present study (CP859 and CP396) are shown in yellow. Identical amino acids among all the strains are shown by asterisk at the bottom, while amino acids different from those on the top line (strain 8-6) are indicated in purple. Three domains I, II, III are shown above the sequences. Amino acid residues 44-116 marked in blue are essential for large complex formation and cytotoxicity. Underlined portion (amino acid 290-319) that mediates binding to eukaryotic cell receptor is underlined. Amino acids in red (strain 8-6) are involved in binding to claudin-3/-4.

		II	
8-6	-----	MLSNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	54
B3550. 5	-----	<u>M</u> YNNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	54
F3686	-----	MLSNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	54
CP2	-----	MLSNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	54
CP859	-----	MLSNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	54
F4969	-----	MLSNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	54
F5603	-----	MLSNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	54
SM101	-----	MLSNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	54
JP55	-----	MLSNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	54
VMI/JKSGR/2015	-----	MLSNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	54
D13122	-----	MLSNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	54
CP396	-----	MLSNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	54
3441	MQNTTN	MLSNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	60
CP65	MQNTTN	MLSNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	60
PB-1	MQNTTN	MLSNLNPVMVFENAKEVFLISEDLKTPINITNSNSNSDGLYVIDKGDGWILGE	60
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		III	II	
8-6	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			114
B3550. 5	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			114
F3686	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			114
CP2	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			114
CP859	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			114
F4969	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			114
F5603	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			114
SM101	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			114
JP55	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			114
VMI/JKSGR/2015	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			114
D13122	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			114
CP396	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			114
3441	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			120
CP65	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			120
PB-1	PSVVSSQILNPNETGTFSQSLTKSKEVSINVNFSVGFTSEFIQASVEYGFGITIGEQTNI			120

		III	II	
8-6	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLGNI			174
B3550. 5	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLGNI			174
F3686	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLGNI			174
CP2	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLGNI			174
CP859	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLGNI			174
F4969	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLGNI			174
F5603	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLGNI			174
SM101	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLGNI			174
JP55	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLGNI			174
VMI/JKSGR/2015	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLGNI			174
D13122	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLGNI			174
CP396	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLGNI			174
3441	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLANI			180
CP65	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLANI			180
PB-1	ERSVSTTAGPNEYVYKQYATYRKQYAIRISHGNISDGSYKLTGIWLSKTSADSLANI			180
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	II	
III		

[illegible]

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TQKLNHLHTITATGQKYRILASKIVDFNIYSNNFNNLVKLEQSLGDGVKDHYVDISLDAG 294
TQKLNHLHTITATGQKYRILASKIVDFNIYSNNFNNLVKLEQSLGDGVKDHYVDISLDAG 294
TQKLNHLHTITATGQKYRILASKIVDFNIYSNNFNNLVKLEQSLGDGVKDHYVDISLDAG 294
TQKLNHLHTITATGQKYRILASKIVDFNIYSNNFNNLVKLEQSLGDGVKDHYVDISLDAE 294
TQKLNHLHTITATGQKYRILASKIVDFNIYSNNFNNLVKLEQSLGDGVKDHYVDISLDAG 294
TQKLNHLHTITATGQKYRILASKIVDFNIYSNNFNNLVKLEQSLGDGVKDHYVDISLDAG 294
TQKLNHLHTITATGQKYRILASKIVDFNIYSNNFNNLVKLEQSLGDGVKDHYVDISLDAG 294
TQKLNHLHTITATGQKYRILASKIVDFNIYSNNFNNLVKLEQSLGDGVKDHYVDISLDAG 294
TQKLNHLHTITATGQKYRILASKIVDFNIYSNNFNNLVKLEQSLGDGVKDHYVDISLDAG 294
TQKLNHLHTITATGQKYRILASKIVDFNIYSNNFNNLVKLEQSLGDGVKDHYVDISLDAG 294
TQKLNHLHTITATGQKYRILASKIVDFNIYSNNFNNLVKLEQSLGDGVKDHYVDISLDAG 294
TQKLNHLHTITATGQKYRILASKIVDFNIYSNNFNNLVKLEQSLGDGVKDHYVDISLDAG 294
TQKLNHLHTITATGQKYRILASRIVDFNIYSNNFNNLVKLQESLGDGVNDHYDIDISLDAG 300
TQKLNHLHTITATGQKYRILASRIVDFNIYSNNFNNLVKLQESLGDGVNDHYDIDISLDAG 300
TQKLNHLHTITATGQKYRILASRIVDFNIYSNNFNNLVKLQESLGDGVNDHYDIDISLDAG 300

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QYVLVMKANSSYSGNYPYSILFQKF 319
 QYVLVMKANSSYSGNYPYSILFQKF 319
 QYVLVMKANSSYSGNYPYSILFQKF 319
 QYVLVMKANSSYSGNYPYSILFQKF 319
 QYVLVMKANSSYSGNYPYSILFQKF 319
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 QYVLVMKANSSYSGNYPYSILFQKF 319
 QYVLVMKANSSYSGNYPYAILFQKF 325
 QYVLVMKANSSYSGNY----- 316
 QYVLVMKANSSYSGNYPYAILFQKF 325
