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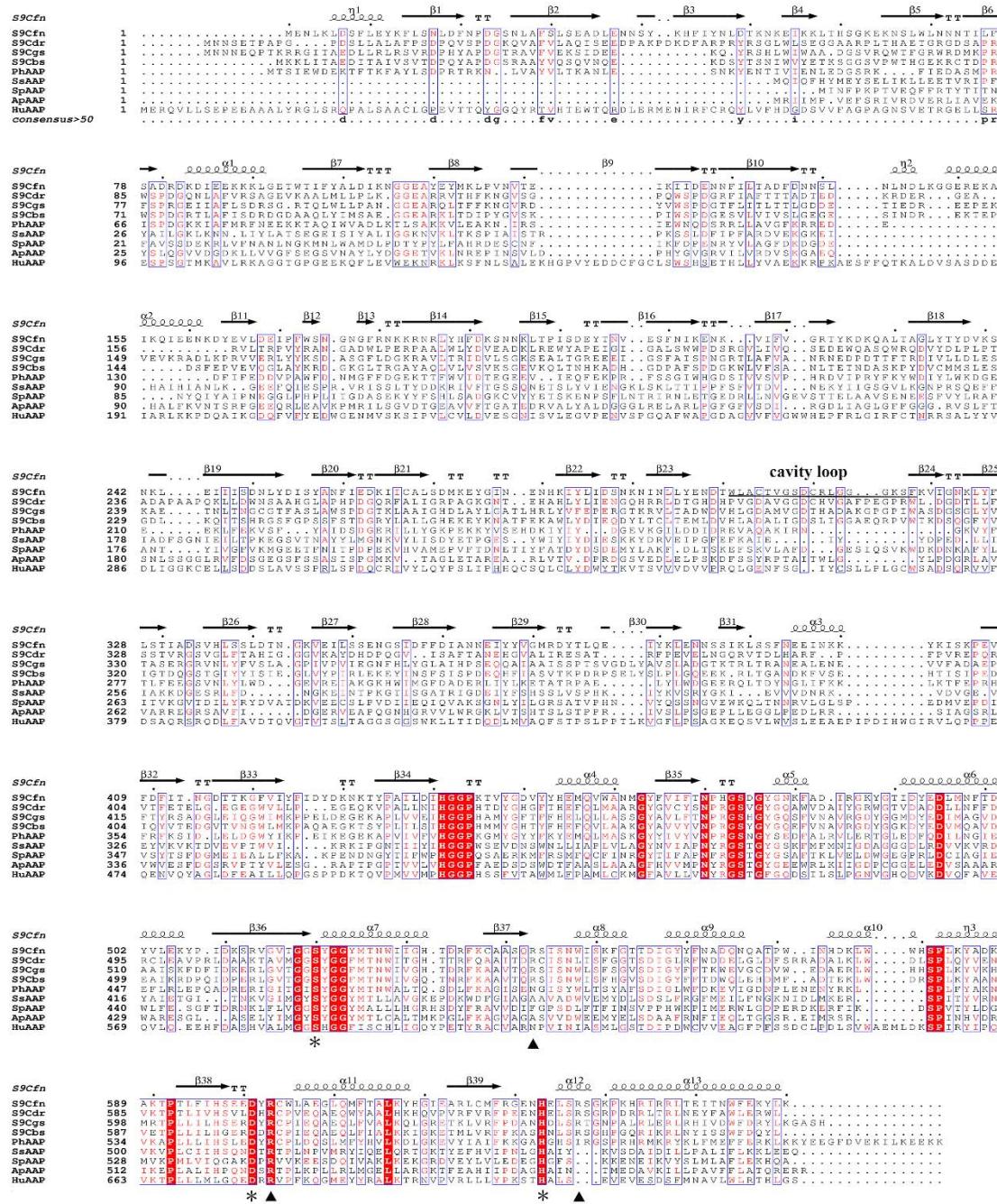
Supporting information for article:

Structural and biochemical analyses of the tetrameric carboxypeptidase S9Cfn from *Fusobacterium nucleatum*

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Table S1 Sequences of the primers used in this study.

Primer name	Nucleotide sequence (5' to 3')
S9Cfn-F	ACGACGACAAGCATATGTGGAAAACCTGAAACTGGATAG
S9Cfn-R	GCAGCCGGATCCTCGAGCTTCAGGTATTTCAAACCA
S9Cfn(G148C)-F	TCTGAAATGTGGTGAACCGCGAAAAAG
S9Cfn(G148C)-R	GTTCACCACATTCAGATCGTTCAGA
S9Cfn(S520A)-F	ACCGGTGGCGCTTATGGCGGCTATATGAC
S9Cfn(S520A)-R	GCCGCCATAAGCGCCACCGGTACACCC
S9Cfn(R543A)-F	GCCAGTCAGGCAAGCATTAGCAATTGG
S9Cfn(R543A)-R	GCTAATGCTTGCTGACTGGCTGCGCATT
S9Cfn(D601N)-F	AGCGAAGAAAATTATCGTTGCTGGCT
S9Cfn(D601N)-R	CAGCCAGCATGCATAATCTTCTTCGCT
S9Cfn(R603A)-F	GAAGATTATGCATGCTGGCTGGCAGAA
S9Cfn(R603A)-R	CAGCCAGCATGCATAATCTTCTTCGCT
S9Cfn(H633A)-F	GGTGAAAATGCTGAAGTGGCTGGCAG
S9Cfn(H633A)-R	CTCAGTTCAGCATTTCACCAACGAAAC
S9Cfn(R637A)-F	GAACTGAGTGCAAGCGGAAACCGAAA
S9Cfn(R637A)-R	CGGTTGCCGCTTGCACTCAGTTCATGATT



S9Cfn crystal structure are overlaid using *EScript* 3.0 (Pravda *et al.*, 2018). The sequences corresponding to the cavity loop of S9Cfn are marked. The residues of the catalytic triad are marked with asterisks (*), while the substrate-binding arginines (Arg543, Arg603 and Arg637) are marked with triangles (\blacktriangle).

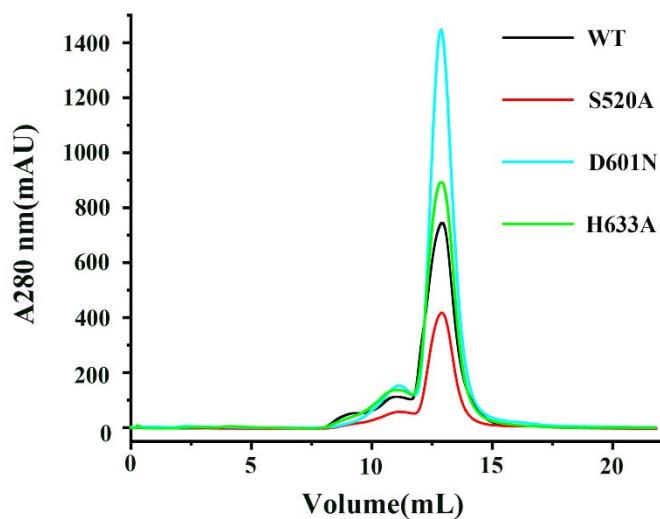


Figure S2 Size-exclusion chromatography profiles of the catalytic triads in the S9Cfn wild-type and mutant enzymes are shown using Superdex 200 Increase 10/300 (GE Healthcare).

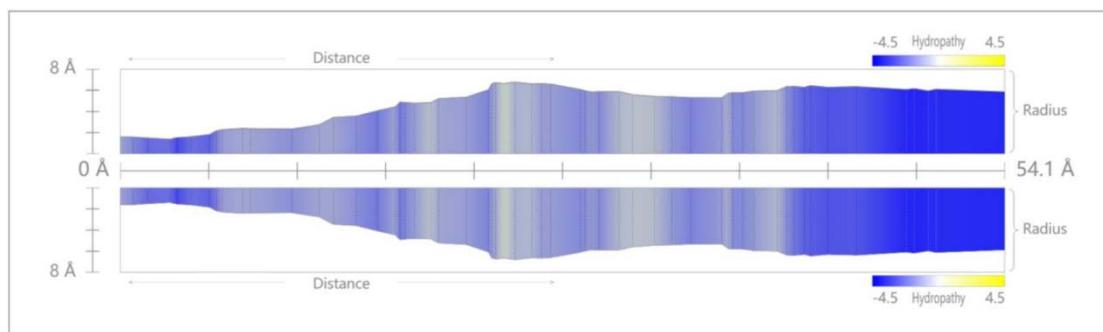


Figure S3 Parameters of the substrate channel in the S9Cfn monomer. Data were produced by *MOLEonline* (Pravda *et al.*, 2018).



Figure S4 Superposition of the S9Cfn tetramer with S9Cdr (PDB ID: 5YZN). Cartoons of the S9Cfn tetramer is colored green, and the S9Cdr tetramer is shown in orange.

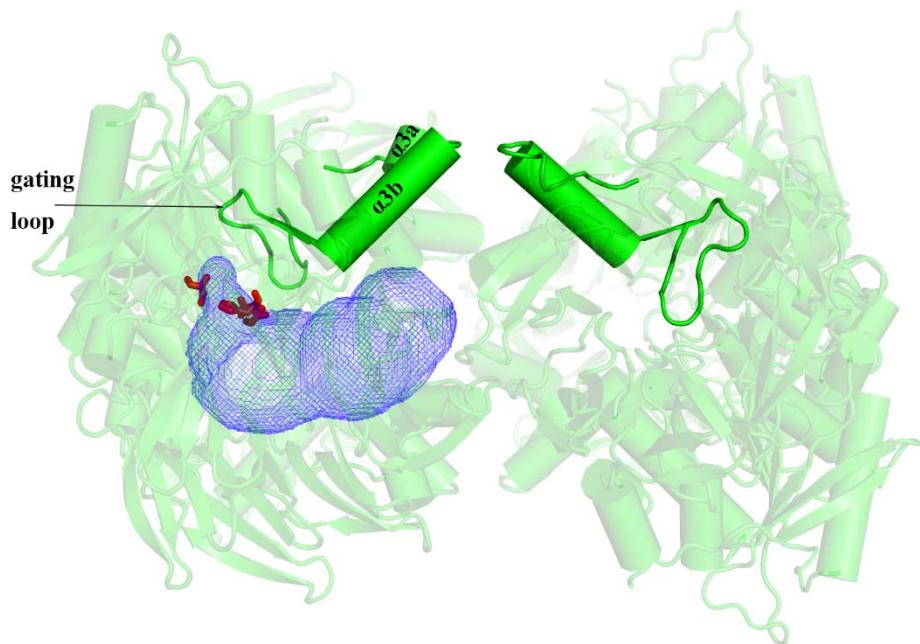


Figure S5 Side view of the S9Cfn tetramer and substrate tunnel. The catalytic triad is shown as red sticks, and the tunnel is shown as a blue mesh.

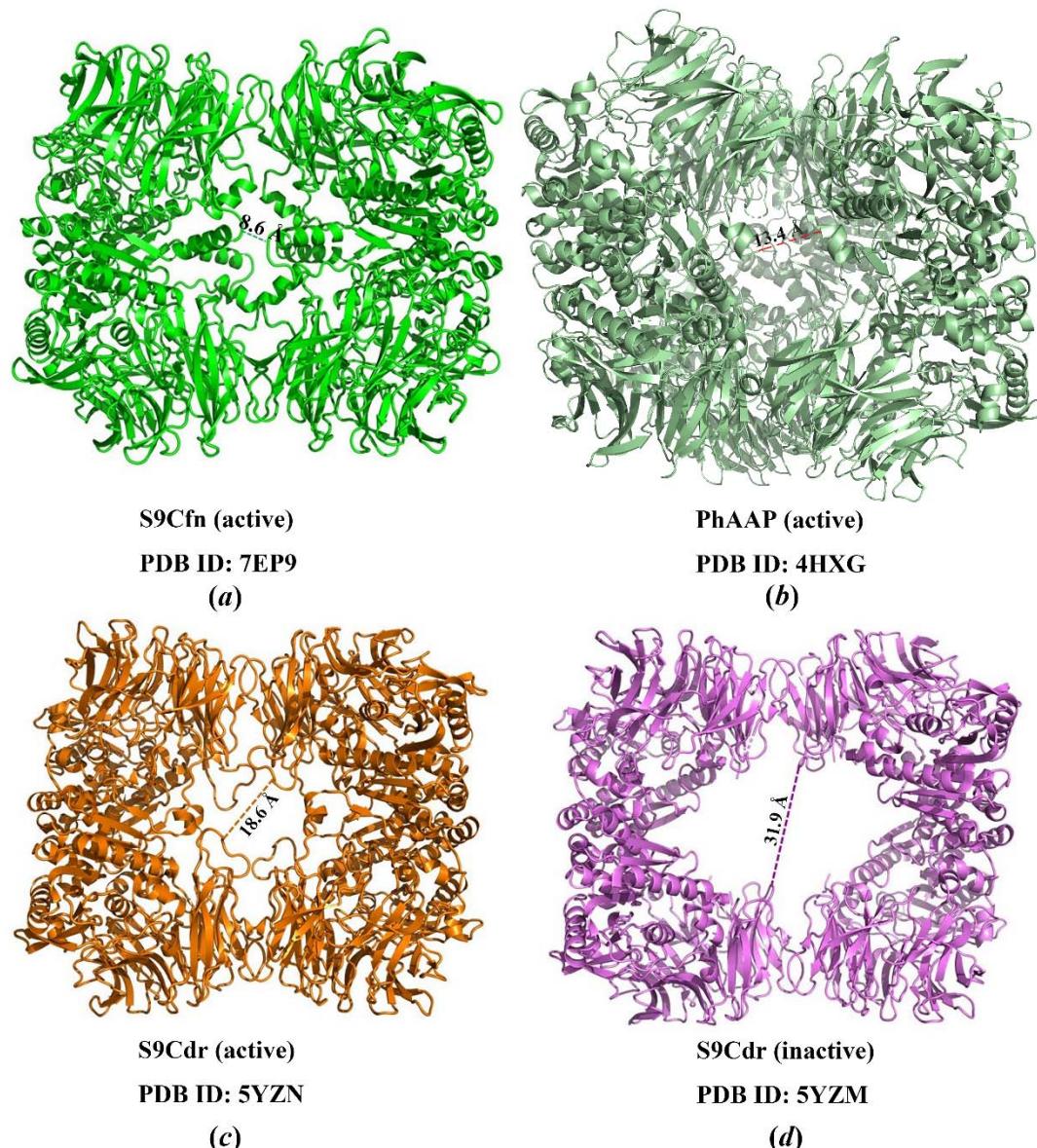


Figure S6 The narrowest distances of the oligomeric pores in the S9Cfn tetramer, PhAAP hexamer and S9Cdr tetramer. The cartoon of the S9Cfn (active) tetramer is colored green, that of the PhAAP hexamer (active) is colored pale green, that of the S9Cdr (active) tetramer is shown in orange and that of the S9Cdr (inactive) tetramer is colored violet.

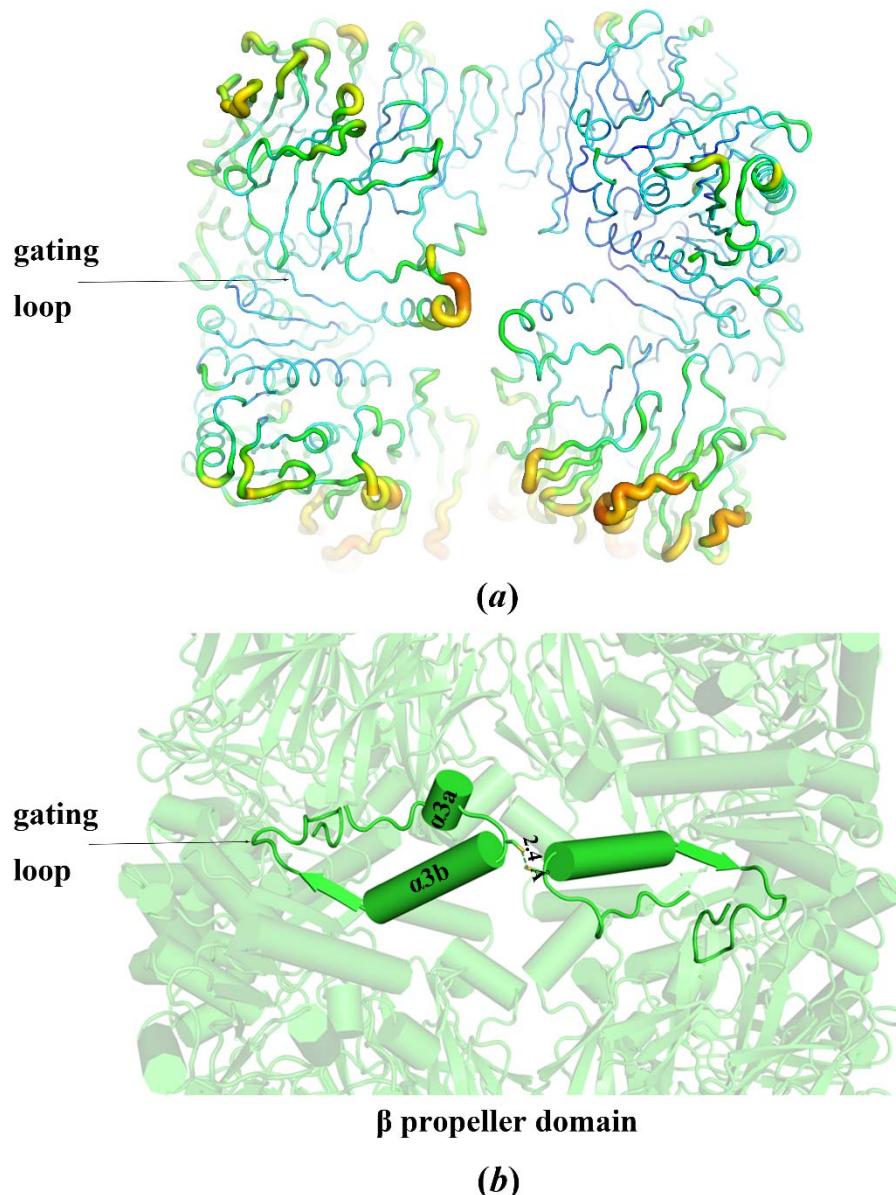


Figure S7 (a) S9Cfn colored by B-factor. (b) The narrowest distance of the oligomeric pore in the G148C S9Cfn variant produced by *PyMOL*. The distance between the two Cys148 residues from a pair of contralateral monomers was measured.

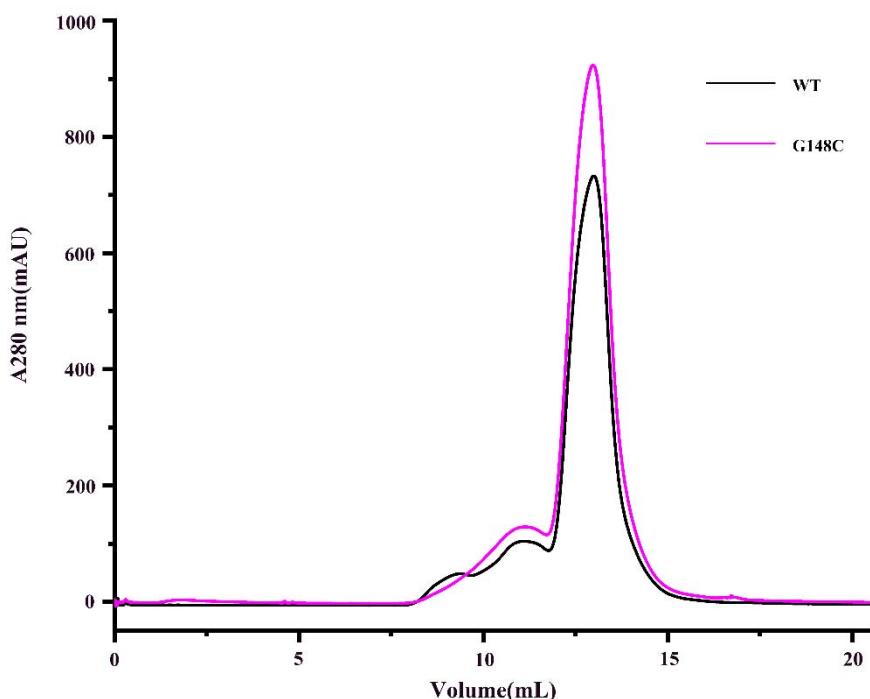


Figure S8 Size-exclusion chromatography profiles of the S9Cfn wild-type and G148C mutant enzymes are shown using Superdex 200 Increase 10/300 (GE Healthcare).

References

- Pravda, L., Sehnal, D., Toušek, D., Navrátilová, V., Bazgier, V., Berka, K., Svobodová Vařeková, R., Koča, J. & Otyepka, M. (2018). *Nucleic acids research*, **46**, 368-373.
Robert, X. and Gouet, P. (2014). *Nucleic acids research*, **42**, 320-324.