Supplementary Materials

Comparative structure analysis of the multi-domain, cell envelope proteases of lactic acid bacteria

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Content of supplementary material

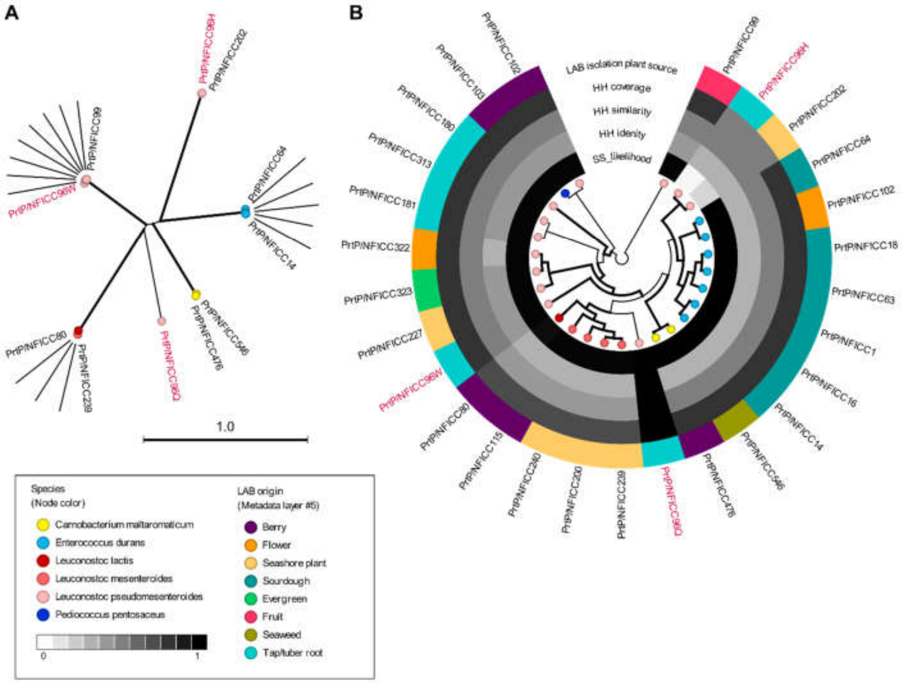

Fig. S1 PrtP protein homologs from plant derived lactic acid bacteria (LAB) strains
The entire PrtP homologous protein sequences were used to build a phylogenetic tree, with the tree topography determined by the neighbor-joining method. Bootstrapping with 100 replicates was used to assess tree reliability. Most branches were supported by bootstrapping above 70%, as shown by the thick lines. The phylogenetic tree is shown as a radial tree (A) and a circular cladogram with metadata layers (B). The PrtP homologs were discovered using genome sequences of LAB strains derived from various plant isolates. The LAB strains were from the National Food Institute Culture Collection (NFICC), and the proteases were named PrtP, followed by the name of the LAB strain. Three PrtP homologs (red) were found in a single LAB strain, with names including the last letter of the corresponding contig names. The LAB strains included different species (node color). The black branch lengths of the radial phylogenetic tree were proportional to the Jukes-Cantor distances, as indicated by the scale bar. The circular cladogram showed five metadata layers #1 – #5. The metadata layer #1 to #4 were colored according to the gray scale bar values. Metadata layer #1 displayed the predicted likelihood for a secretory signal (SS) peptide, whereas metadata layer #2, #3 and #4 displayed the protein sequence identity, similarity and coverage of pairwise HHpred comparisons between PrtP$_{Wg2}$ and each of listed PrtP homologs.

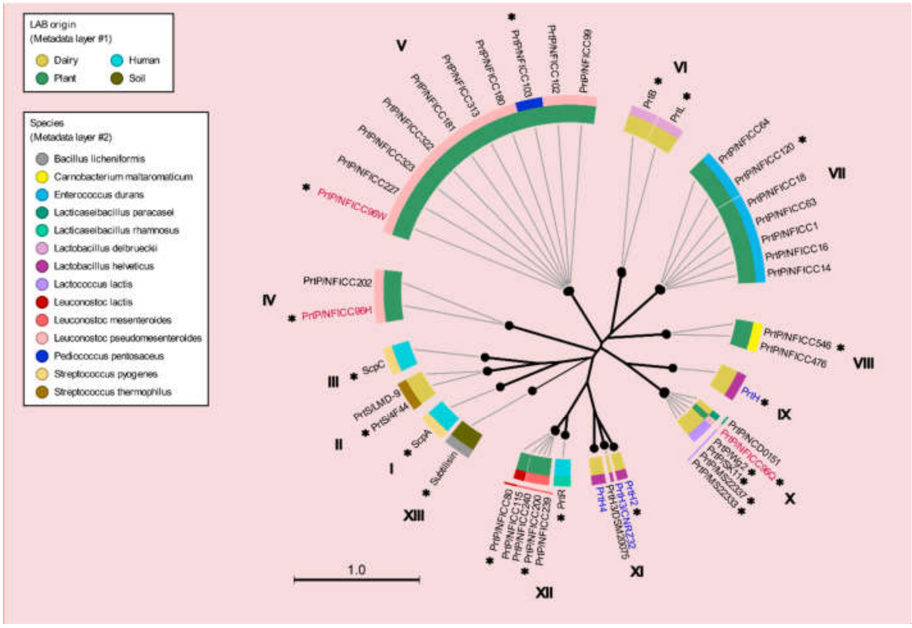

Fig. S2 Phylogenetic three of PrtP protein homologs based on their whole protein sequences
The entire protease sequences were used to construct the radial phylogenetic tree. The names of the LAB strains were provided as part of the protease name to distinguish between the proteases with the same name. The LAB strains derived from plant sources were from the National Food Institute Culture Collection (NFICC). The neighbor-joining method was used to generate the tree topography. Black branch lengths were proportional to the Jukes–Cantor distances, indicated by the scale bar. Bootstrapping with 100 replicates was used to assess the tree reliability. All branches were verified with bootstrapping above 70 %, as shown by the thick lines. PrtP homologs were found in different bacteria strains of different species (outer circle layer) that were derived from different origins (inner circle layer). Three PrtP homologs (red) were from *Leuconostoc pseudomesenteroides* NFICC96 like the four PrtP homologs (blue) were from *Lactobacillus helveticus* CNRZ32. The clusters of PrtP homologs were assigned I – XIII based on the phylogenetic tree in figure 1, but follow the same order in this tree, counting clockwise after subtilisin. PrtP homologs marked with asterisks were included for further structural analysis.

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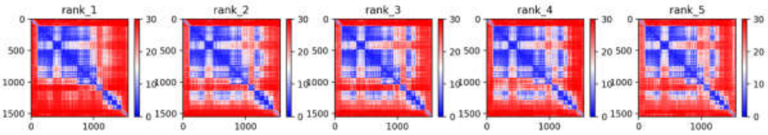
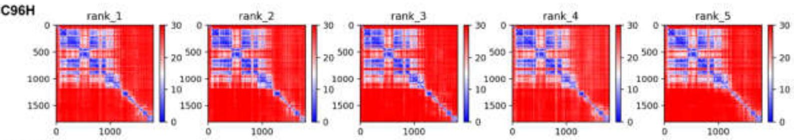
PrtS$_{4F44}$



PrtP$_{NFICC96H}$



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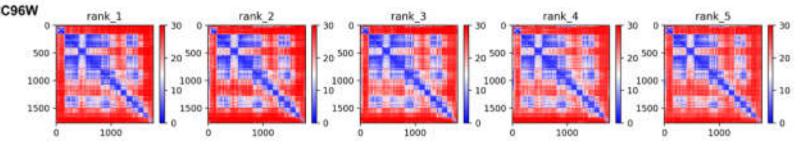

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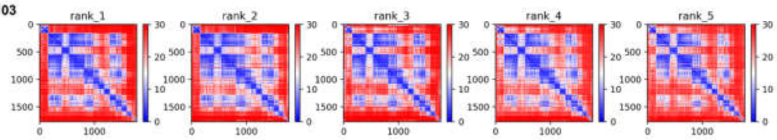
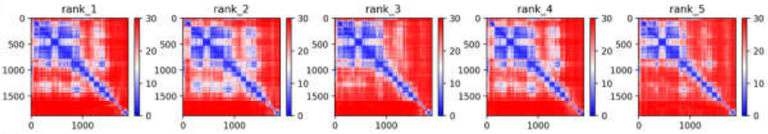
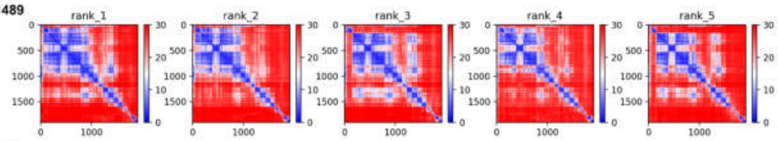


PrtL$_{CRL581}$



PrtB$_{NCDO1489}$



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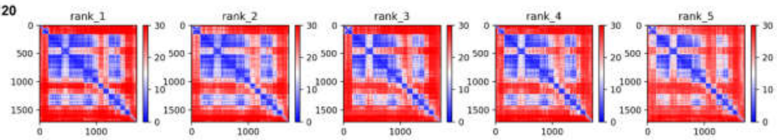
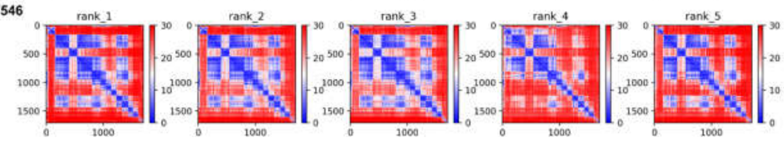
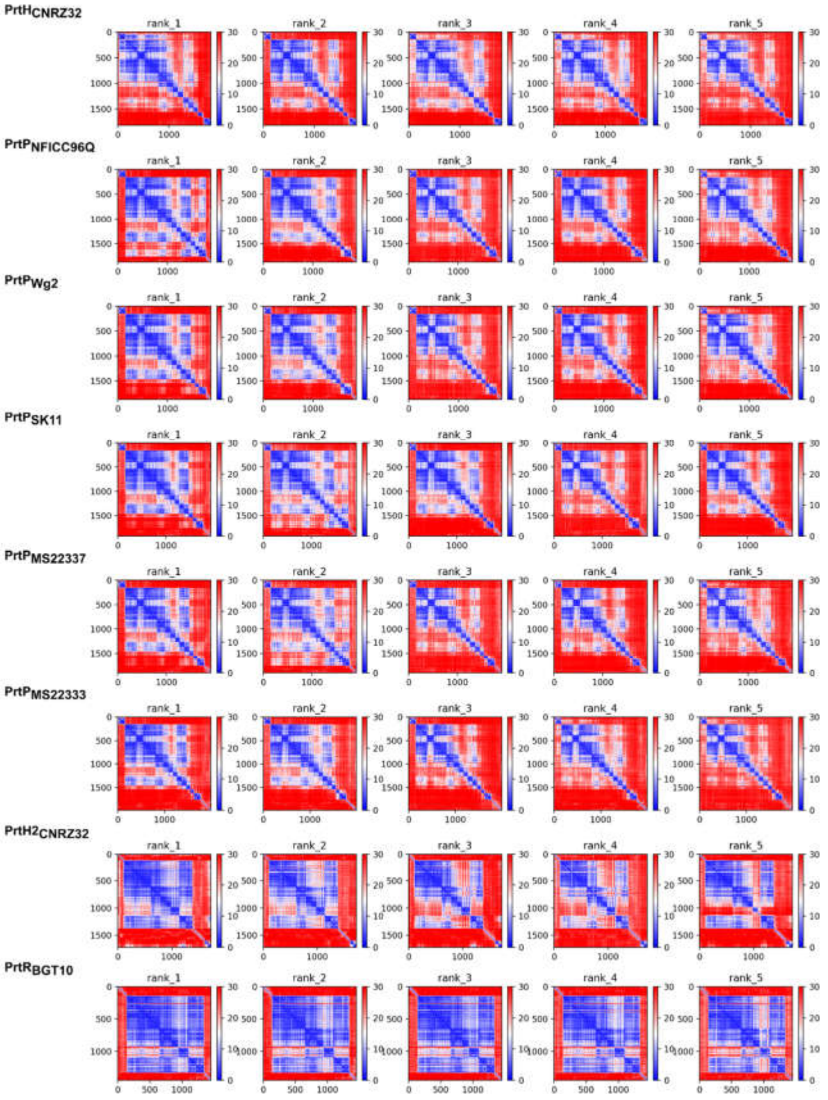


PrtP$_{NFICC546}$




PrtH$_{CNRZ32}$
PrtP$_{NFICC96Q}$
PrtP$_{Wg2}$
PrtP$_{SK11}$
PrtP$_{MS22337}$
PrtP$_{MS22333}$
PrtH2$_{CNRZ32}$
PrtR$_{BGT10}$
rank_1
rank_2
rank_3
rank_4
rank_5
0
500
1000
1500
0
10
20
30

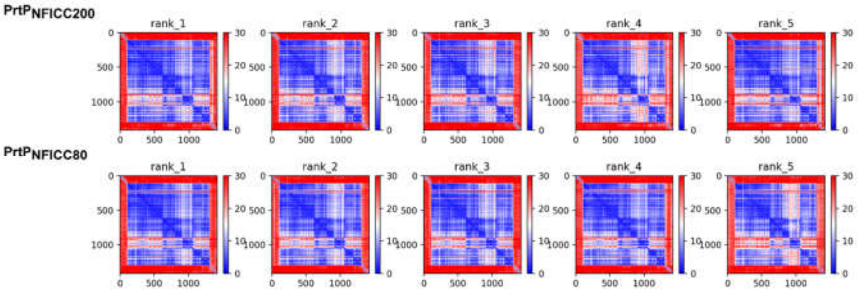
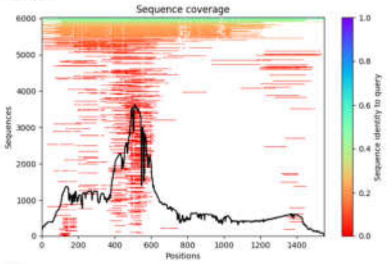
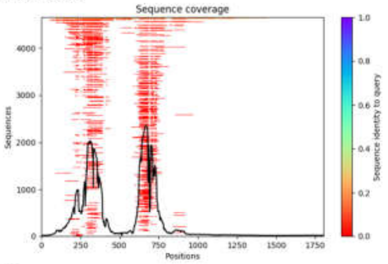


Fig. S3 Predicted aligned error (PAE) plots for AlphaFold 2 (AF) models
PAE provided a distance error for every pair of residues in each AF models of the PrtP homologs. AF had created five structural models for every PrtP homolog that were ranked from 1 to 5. The PAE values were shown as pixels in the heat maps and ranged from 0 to 35 Å. The residue numbers of the proteases were displayed along the horizontal and vertical axes, indicating PAE value for the corresponding residue pair. The prepeptides, with the exception of PrtP$_{NFICC96H}$, were not included in the structure models and thus not in the residue numbers. Low PAE values (< 5 Å) indicated that the relative position of two domains were confidently predicted.

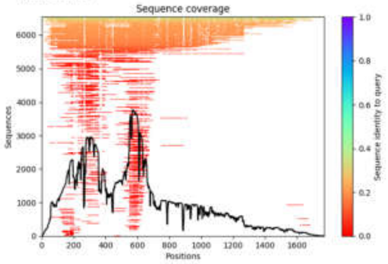
PrtS$_{4F44}$



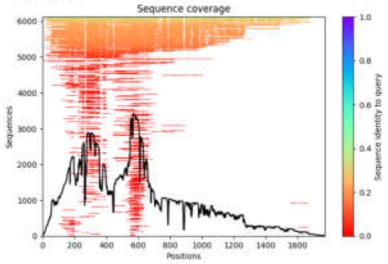
PrtP$_{NFICC96H}$



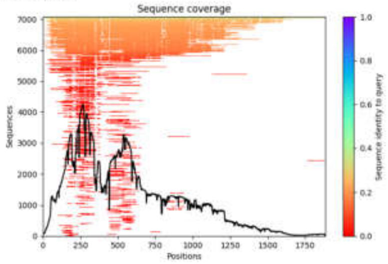
PrtP$_{NFICC96W}$



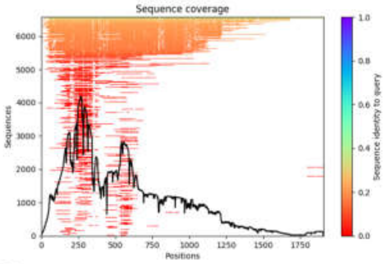
PrtP$_{NFICC103}$

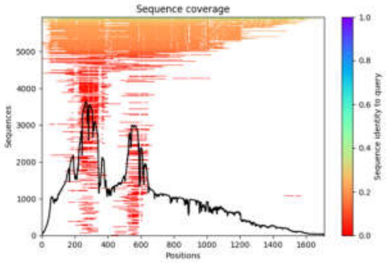


PrtL$_{CRL581}$

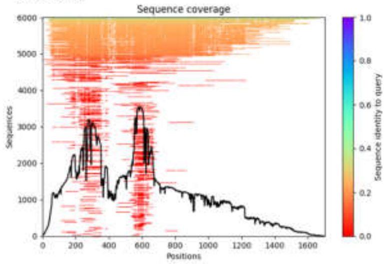


PrtB$_{NCDO1489}$

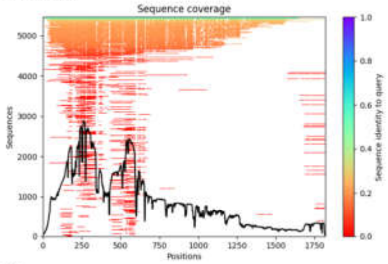




PrtP$_{NFICC546}$



PrtH$_{\text{CNRZ32}}$



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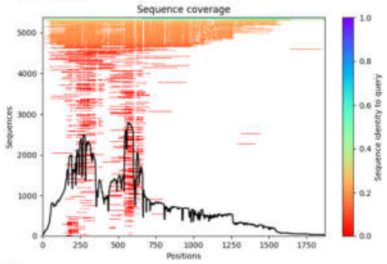
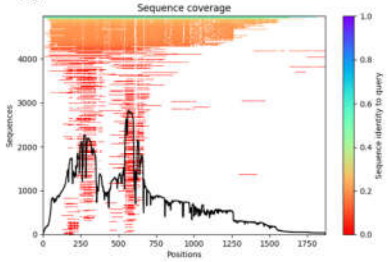
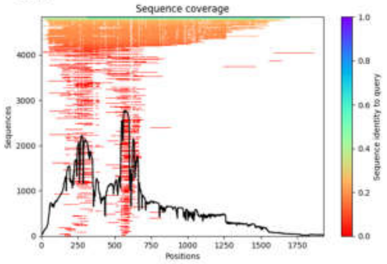


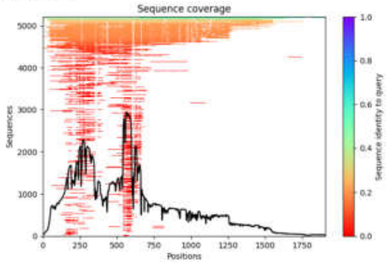
PrtP$_{\text{Wg2}}$



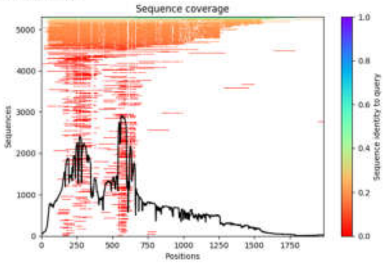
PrtP$_{\text{SK11}}$



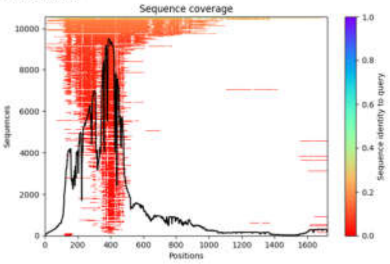
PrtP$_{\text{MS22337}}$



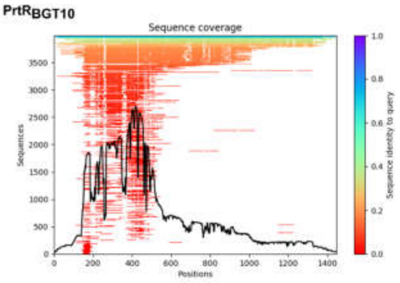
PrtP$_{\text{MS22333}}$



PrtH2$_{\text{CNRZ32}}$



PrtR$_{\text{BGT10}}$



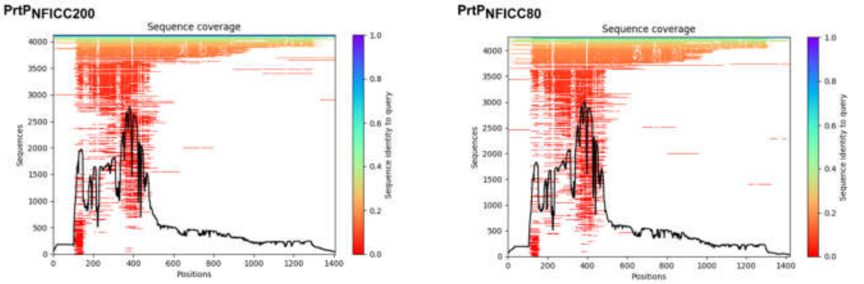
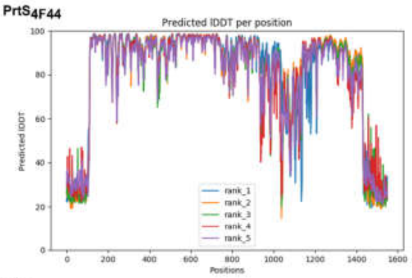
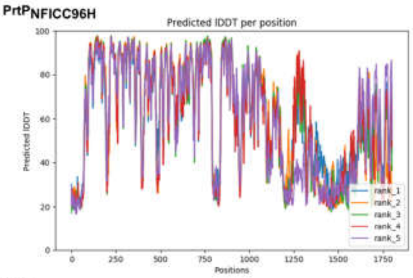

PrtP$_{NFICC200}$
PrtP$_{NFICC80}$

Fig. S4 Multiple sequence alignment (MSA) depth for AlphaFold 2 (AF) models
The plots showed the quality of the MSA used for AF structure modeling. The numbers of protein sequences were shown along the vertical axis, whereas the amino acid position of the query sequence was shown along the horizontal axis. The prepeptides, with the exception of PrtP$_{NFICC96H}$, were not included in the structure models and thus not in the residue numbers. The coverage and identity of the protein sequences to the query sequence was displayed. The plots were output from the ColabFold with the AF implementation, of which the PrtP homologs were used as query sequences.

PrtS$_{4F44}$



PrtP$_{NFICC96H}$



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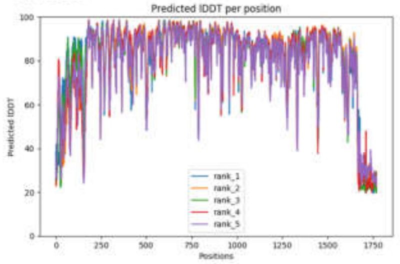
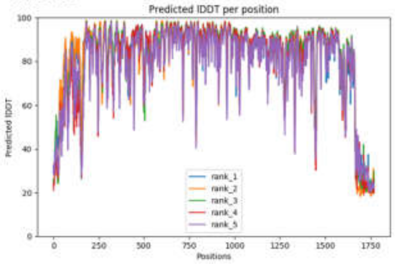
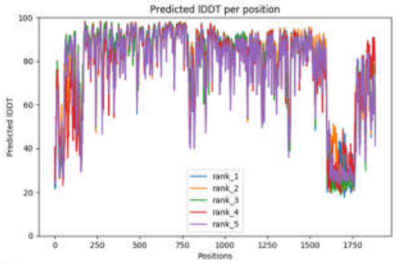


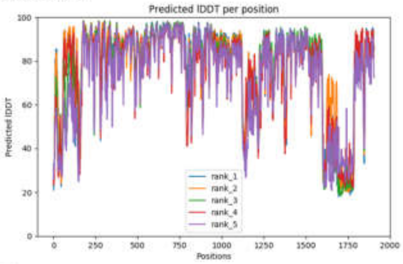
PrtP$_{NFICC103}$



PrtL$_{CRL581}$



PrtB$_{NCDO1489}$



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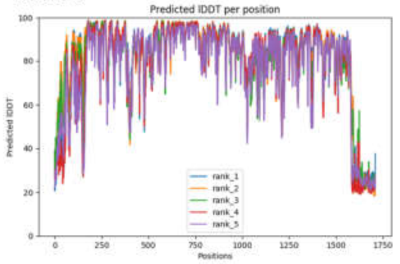

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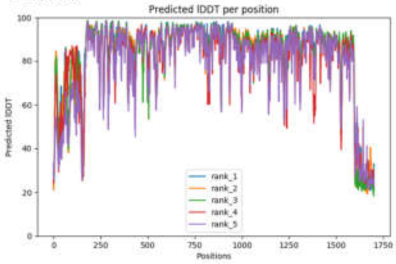

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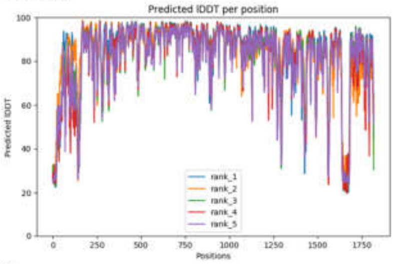

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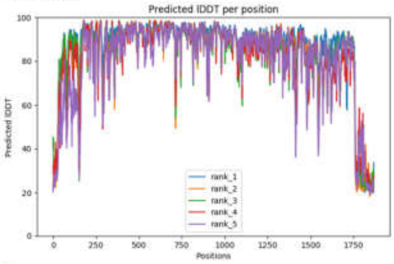

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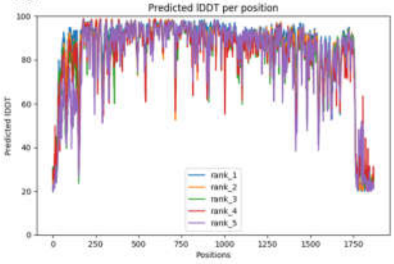

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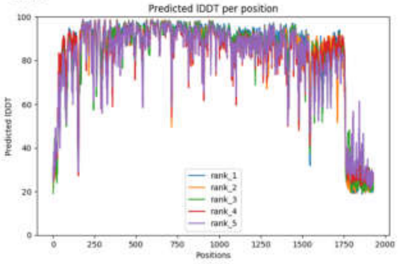

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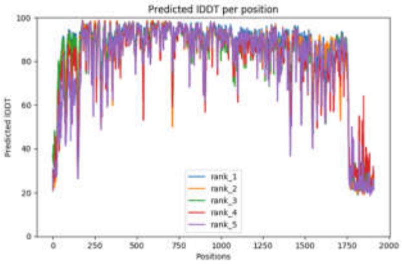

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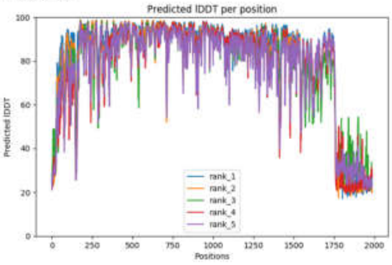

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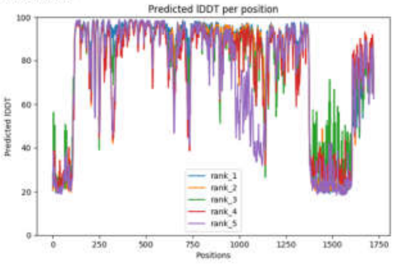

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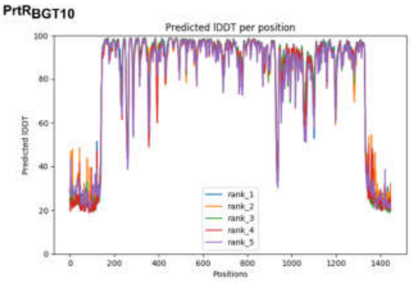


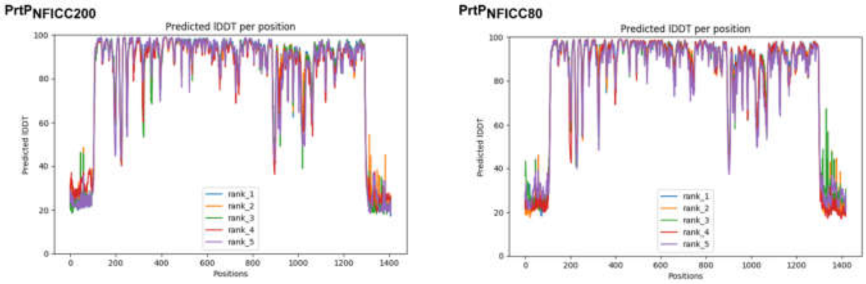
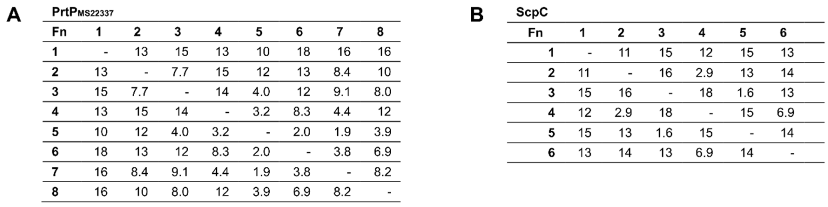


Fig. S5 The predicted local distance difference test (pLDDT) for AlphaFold 2 (AF) models
The plots showed pLDDT (0 – 100) along the vertical axes and the residues numbers of the proteases along the horizontal axes, illustrating the confidence of each amino acid position in each structure model (rank_1 – rank_5). The prepeptides, with the exception of PrtP$_{NFICC96H}$, were not included in the structure models and thus not in the residue numbers. The plots were output from ColabFold with the AF implementation, of which the PrtP homologs were used as query sequences.



A

$PrtP_{MS22337}$

Fn	1	2	3	4	5	6	7	8
1	-	13	15	13	10	18	16	16
2	13	-	7.7	15	12	13	8.4	10
3	15	7.7	-	14	4.0	12	9.1	8.0
4	13	15	14	-	3.2	8.3	4.4	12
5	10	12	4.0	3.2	-	2.0	1.9	3.9
6	18	13	12	8.3	2.0	-	3.8	6.9
7	16	8.4	9.1	4.4	1.9	3.8	-	8.2
8	16	10	8.0	12	3.9	6.9	8.2	-

B

ScpC

Fn	1	2	3	4	5	6
1	-	11	15	12	15	13
2	11	-	16	2.9	13	14
3	15	16	-	18	1.6	13
4	12	2.9	18	-	15	6.9
5	15	13	1.6	15	-	14
6	13	14	13	6.9	14	-

Fig. S6 Intramolecular comparison of fibronectin type-III-like (Fn) domains
The structure of the (Fn) domains were superimposed in pairs within $PrtP_{MS22337}$ (A) and ScpC (B). The RMSD values were given for each pair of Fn domains.

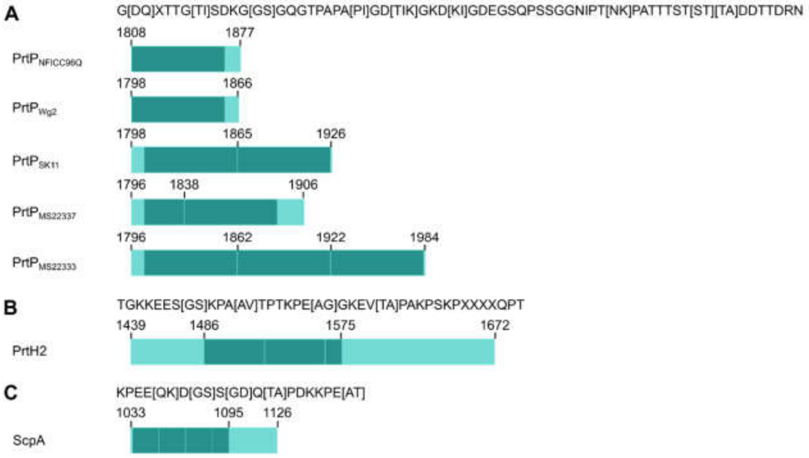


Fig. S7. Long tandem repeats in the cell wall spacing (W) domain
The W-domains of the PrtP homologs were shown as turquoise bar plots, in which darker colored boxes indicated the relative positions of long tandem repeats. The sequence pattern of the tandem repeats were given in top of figure A, B and C.