

Figure S1

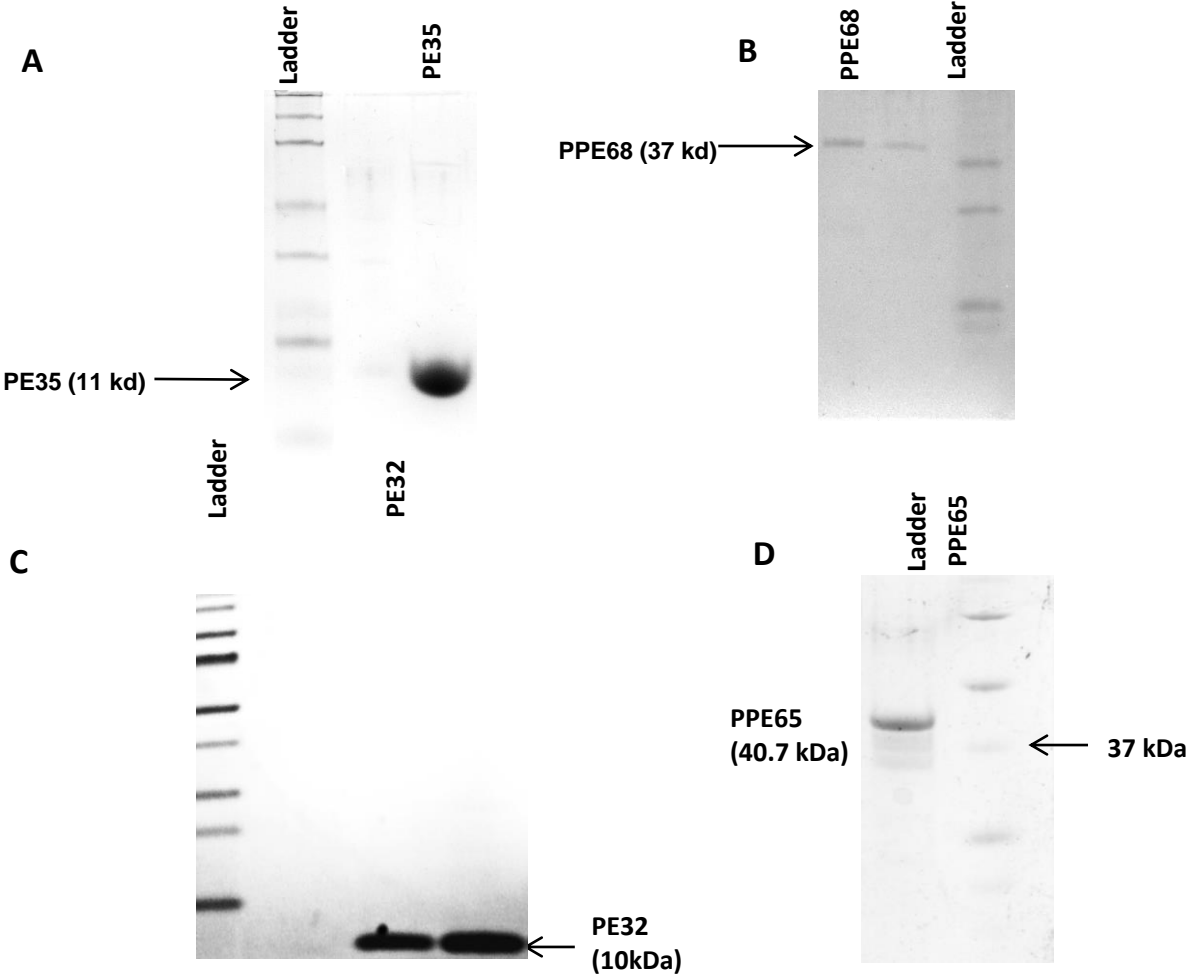


Figure S1: SDS-PAGE image of the purified PE32, PPE65, PE35 and PPE68 proteins.

Figure S2

	Disordered (80-100%)	Partially Disordered (20-80%)	Ordered (0-20%)
PPE(69)		65	4
PE(35)	1	32	2
PE_PGRS(64)	31	31	3

Figure S2: Structural analysis of the PE-PPE/PGRS protein family using RONN.

Each subfamily was divided into three groups, i.e. completely disordered members with more than 80% disorder, partially disordered members with disorder between 20-80% and ordered proteins having less than 20% structural disorder

Figure S3

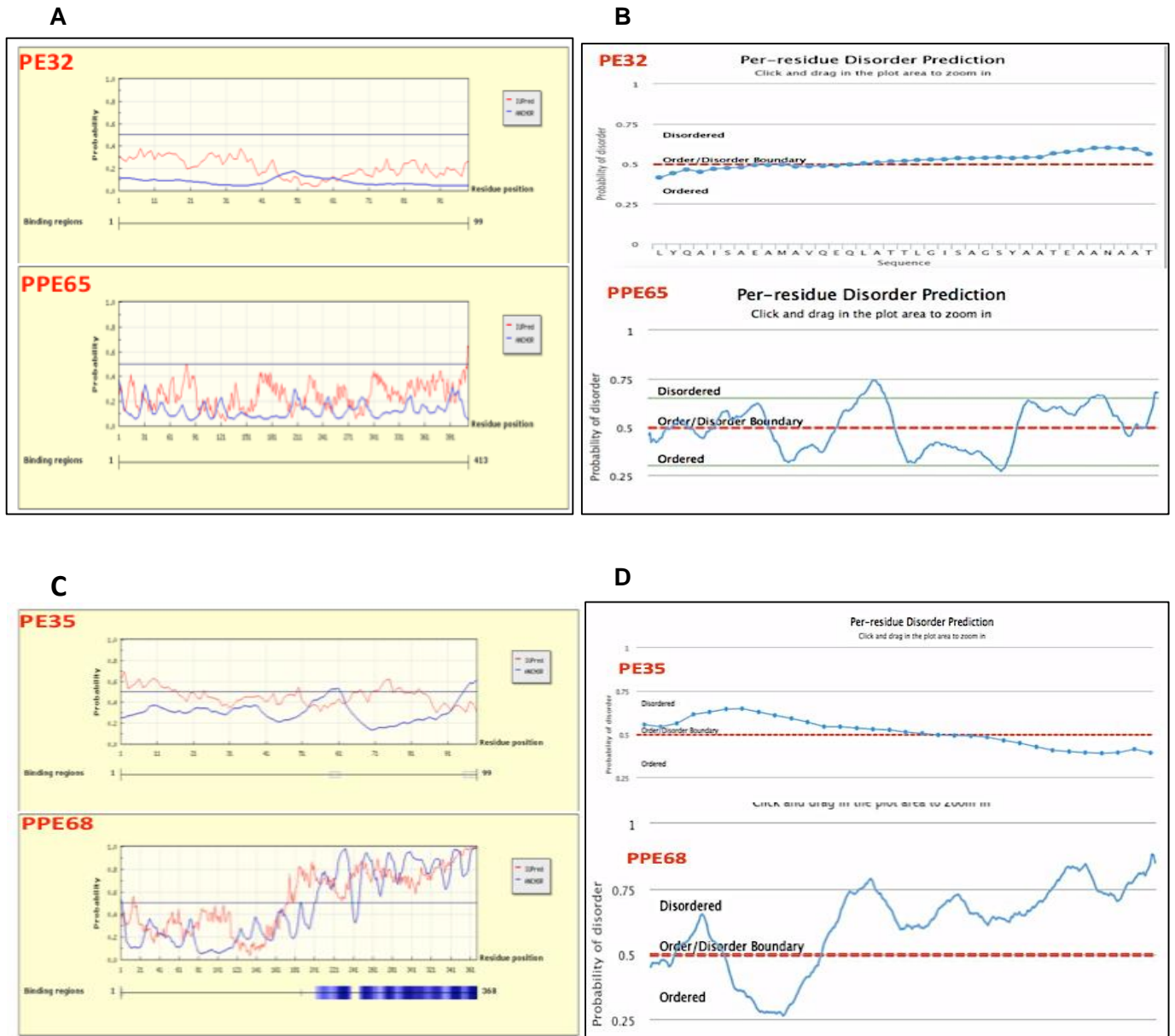


Figure S3: Structural disorder predictions for PE32, PE35, PPE65 and PPE68: (A) Predicted IUPred disordered regions and disordered binding sites using the ANCHOR tool in PE32 and PPE65. (B) Predicted disordered regions in PE32 and PPE65 using RONN. (C) Predicted IUPred disordered regions and disordered binding sites in PE35 and PPE58 using ANCHOR. (D) Disordered regions in PE35 and PPE68 as predicted by the RONN tool.

Figure S4

(A)

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CLUSTAL O(1.2.4) multiple sequence alignment
PE25_MYCTU MSFVITNPEALTVAATEVRRIRDRAIQSDAQVAPMTTAVRPPAADLVSEKAATFLVEYAR 60
PE32_MYCTU MSIMHAEPEMLAATAGELQSIGNAVARAGNAAVAGPTTGVVPAADLVSLLTASQFAAHAQ 60
PE35_MYCTU MEKMSHDPIAADIGT-QVSDNALHGVTAGSTALTSVTGLVPAGADEVSAQAATAFTSEGI 59
* . : :* : : . . . : . * . : * . * * * * : * : : . .
PE25_MYCTU KYRQTIAAAAAVLVEEFAHALTTGADKYATAEADNIKTFS- 99
PE32_MYCTU LYQAISAEAMAVQEQLATTLGISAGSYAATEANAATIA- 99
PE35_MYCTU QLLASNASAQDQLHRAGEAVQDVARTYSQIDDGAAGVFAE 99
* * . . . : : * . * : : : :
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(B)

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CLUSTAL O(1.2.4) multiple sequence alignment
PPE41_MYCTU -MHFEAYPPEVNSANIYAGPGPDSMLAAARAWRSLDVEMTAVQRSFNRTLLSLM-DAWAG 58
PPE65_MYCTU MLDFAQLPPEVNSALMYAGPGSGPMLAAAAWEALAELOTTASTYDALITGLADGPWQG 60
PPE68_MYCTU -MLWHAMPPELNTARLMAGAGPAPMLAAAAGWQTLAALDAQAVELTARLNSLG-EAWTG 58
: : * * * : * * : * * * * * * * . * : * . : : : . * * *
PPE41_MYCTU PVVMQLMEAAKPFVRWLTDLVQLSEVERQIHEIVRAYEWAHDMVPLAQIYNNRAERQI 118
PPE65_MYCTU SSAASMVAAATPQVAWLRSTAGQAEQAGSQAVAAASAYEAFFATVPPEIAANRALIMA 120
PPE68_MYCTU GGS DKALAAATPMVVWLQTASTQAKTRAMQATAQAAAYTQAMATTPSLPEIAANHITQAV 118
. : * * . * * * * . * . * . * * * : * * :
PPE41_MYCTU LIDNNALGQFTAQIADLDQEY-DDFWEDEGEVMDRDLRVSDALSCLTPWKAPPPIAHST 177
PPE65_MYCTU LLATNFLGQNTAAIAATEAQY-AEMWAQDAAMGYAGASA-AATQLSPFNPAAQITINPA 178
PPE68_MYCTU LTATNFFGINTIPIALTEMDYFIRMWNQAALAMEVYQAETAV-NTLFEKLEPMASILDPG 177
* . * : * * * * : : * : * : . . * * : : : : .
PPE41_MYCTU VLVAP---VSP---ST--ASSRTDT----- 194
PPE65_MYCTU GLASQAASVGVQAVSQAANAQALTDIPKALFGLSGIFT-----NEPPWLT-DLGKALGLTG 232
PPE68_MYCTU ASQS-----TTNPIFGMPSPGSSTPVQGQLPPAATQTLGQLGEMSG 217
:
PPE41_MYCTU -----
PPE65_MYCTU HTWSSDGSLIVGGVLDGDFVQGVGTGSAELDA-SVAMDTFGKWVSPARLMVTQFKDYFGLA 291
PPE68_MYCTU PMOQLTQPLQOVTSLF-SQVGGTGGGNPADEEAAQMGLLGTSP----LS-----N 262
PPE41_MYCTU -----
PPE65_MYCTU HDLPKWASEGAKAAGEAAKALPAAVPAIPSAAGLSGVAGAVGQAASVGGGLKVPVWTATTP 351
PPE68_MYCTU H--PLAGGSGPSA-G-----AGLLRAESLPGAGGSLTRTPMLMSQLI----EKPVAP 306
PPE41_MYCTU -----
PPE65_MYCTU AASPAVLAASNGLGAAAAAEGSTHAFGGMPLMGSGA-GRAFNNFAAPRYGFKPTVIAQPP 410
PPE68_MYCTU SVMPAAGSS-----ATGGAAPVGAGAMGQAQSGG----STRPGLVAPAP 349
PPE41_MYCTU -----
PPE65_MYCTU AGG----- 413
PPE68_MYCTU LAQEREDEDDWDEEDDW 368
```

Figure S4: Alignments of our investigated PE and PPE proteins to those with available complex structure. The (A) PPE41, PPE65 and PPE68 proteins as well as the (B) PE25, PE32 and PE35 proteins were aligned using Clustal Omega 1.2.4. The regions of the PPE and PE domains are marked by light grey and yellow backgrounds, respectively.

Figure S5

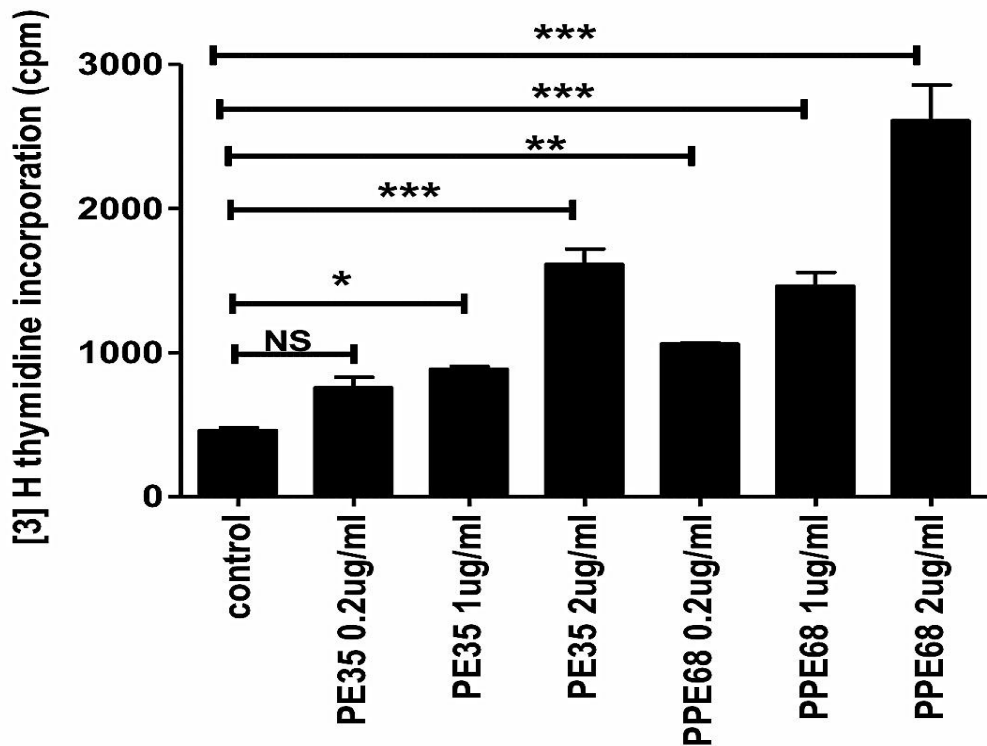
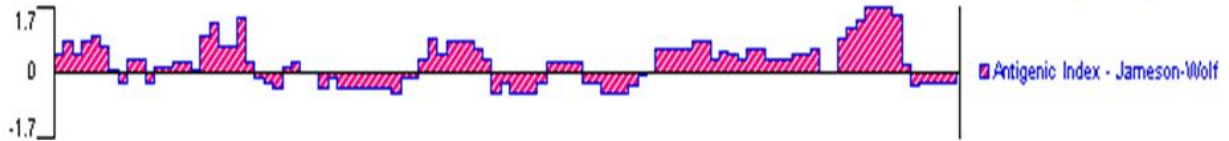


Figure S5: Individual recombinant PE35 and PPE68 proteins induce cell proliferation. Different concentrations of individual recombinant proteins were used for re-stimulating the splenocytes in culture for 72 hrs. 3[H] thymidine (0.5mci/ml) was added to each well and incubated at 37o C for 24 hours. Cells were harvested and β scintillation counter was employed for counting Beta activity. Beta activity was significant for the cells re-stimulated with 1 μ g/ml or higher dose of PE35 while it was significant for 0.2 μ g/ml or higher dose of PPE68, suggesting PPE68 to be more antigenic. Data represents experiments with three mice in each group . Statistical significance was determined by one-way ANOVA and data represented as mean \pm SD . p value <0.05 was considered statistically significant.

Figure S6

PE35



PPE68

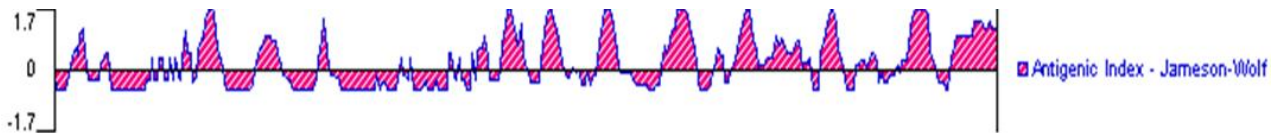


Figure S6: Antigenic index of PE35 and PPE68. Antigenicity plots depicting the antigenic regions of PE35 and PPE68.

Supplementary Table S1: Identifiers of the protein family members and secretome proteins used in the analyses.

PE subfamily

Gene Id	Protein name	Gene Id	Protein name	Gene Id	Protein name
YP_177690.1	PE1	YP_177794.1	PE13	YP_177882.1	PE25
YP_177696.1	PE2	YP_177797.1	PE14	YP_177907.1	PE27
YP_177697.1	PE3	YP_177805.1	PE15	YP_177685.1	PE29
YP_177710.1	PE5	YP_177810.1	PE16	YP_177975.1	PE31
YP_177717.1	PE6	YP_177825.1	PE17	YP_177999.1	PE32
YP_177766.1	PE7	YP_177834.1	PE18	YP_178000.1	PE33
YP_177779.1	PE8	YP_177837.1	PE19		
YP_177784.1	PE9	YP_177843.1	PE20		
CCP43841.1	PE10	YP_177858.1	PE22		
YP_177792.1	PE11/LIPX	YP_177867.1	PE23		
YP_177793.1	PE12	YP_177880.2	PE24		

PPE subfamily

Gene Id	Protein name	Gene Id	Protein name	Gene Id	Protein name
YP_177690.1	PPE1	YP_177830.1	PPE24	YP_177918.1	PPE46
YP_177704.1	PPE2	YP_177833.1	PPE25	YP_177932.1	PPE49
YP_177709.1	PPE3	YP_177835.1	PPE26	YP_177934.1	PPE50
YP_177711.1	PPE4	YP_177836.1	PPE27	YP_177935.1	PPE51
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YP_177715.1	PPE6	YP_177840_1	PPE29	YP_177937.1	PPE53
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YP_177726.2	PPE10	YP_177844.1	PPE32	YP_177964.1	PPE56
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YP_177790.1	PPE16	YP_177870.1	PPE38	YP_177985.1	PPE62
YP_177791.1	PPE17	YP_177871.1	PPE39	YP_177987.1	PPE63
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YP_177828.1	PPE23	YP_177913.1	PPE45	YP_178024.1	PPE69

PE_PGRS subfamily

Gene Id	Protein Name	Gene Id	Protein Name	Gene Id	Protein Name
YP_177692.1	PE_PGRS1	YP_177799.1	PE_PGRS24	YP_177909.1	PE_PGRS48
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YP_177750.1	PE_PGRS9	YP_177842.1	PE_PGRS32	YP_177981.2	PE_PGRS56
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YP_177798.1	PE_PGRS23	YP_177902.1	PE_PGRS47		

Mce family

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NP_218014.1	Rv3497c	NP_216485.1	Rv1969		
NP_214682.1	Rv0168	NP_218018.1	Rv3501c		

MmpL family

NP_214716.1	Rv0202c	NP_216073.1	Rv1557)	NP_215699.1	Rv1183
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NP_217458.1	RV2942C	NP_215661.1	Rv1145		
NP_214720.1	Rv0206c	NP_216855.1	Rv2339		
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Secretome

Gene Id	Gene id	Gene id	Gene id	Gene id
NP_214684.1	NP_215391.1	NP_217510.1	NP_215954.1	NP_215868.1
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Supplementary Table S2: Secondary structure content in the investigated recombinant proteins and their complexes in percentage

Recombinant protein	1615-1625 (unstructured /AAS)	1630-1640 (beta sheets)	1640-1650 & 1660-1680 (random coil/loop)	1650-1660 (alpha helices)	1680-1705 (turn/beta sheets)
PE32	16	10	25	32	17
PPE65	18		48	28	16
PE32&PPE65 complex	16	12	16	36	20
PE35	19	15	25	23	18
PPE68	24		33	29	14
PE35&PPE68 complex	10	18	20	32	20