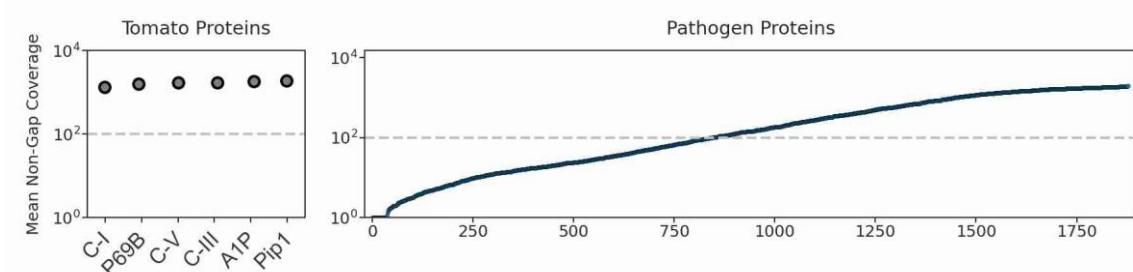


## Supplementary Figures and Tables

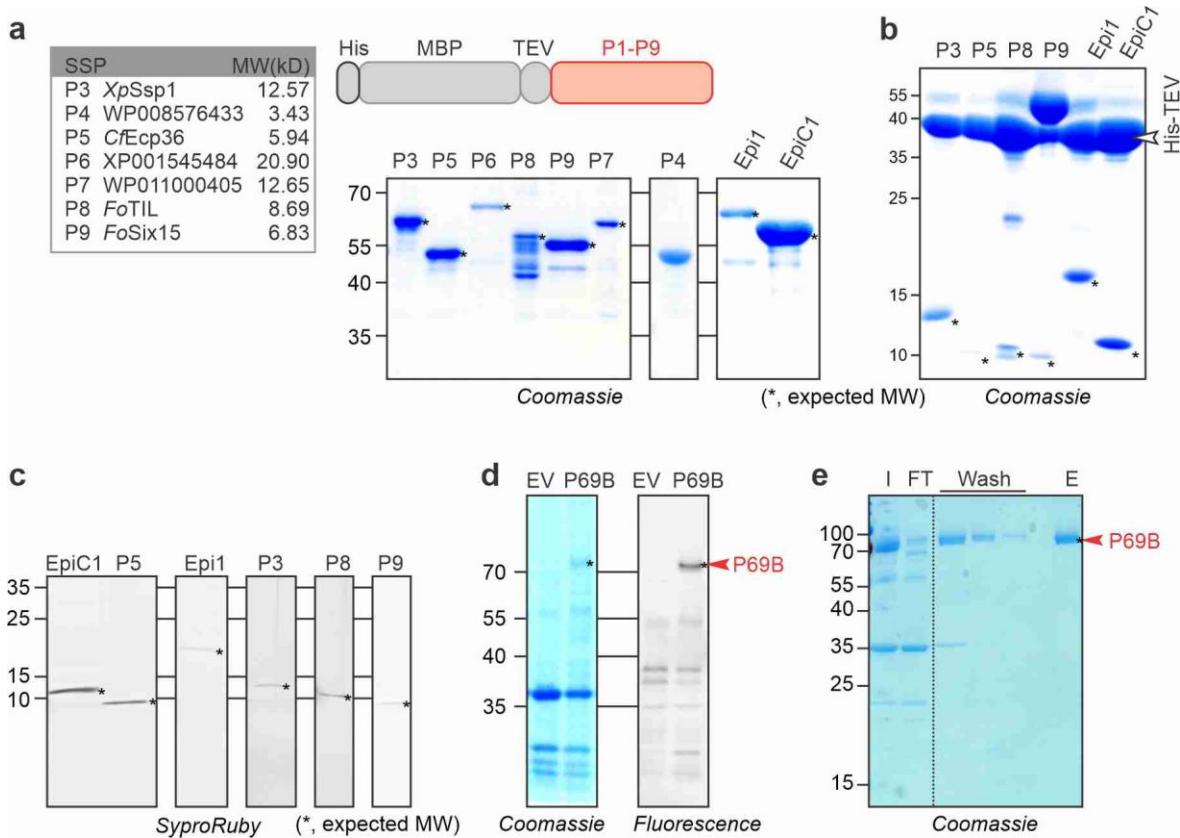
*AlphaFold-multimer predicts cross-kingdom interactions at the plant-pathogen interface*

Felix Homma, Jie Huang, Renier A. L. van der Hoorn



**Supplementary Figure 1** MSA depth of SSPs and hydrolases used in Fig. 2.

The mean non-gap multiple sequence alignment (MSA) depth is shown for the tomato hydrolases (left) and the 1,879 SSPs (right), ranked by MSA depth. The dash line indicates the desired minimum of 100 MSA depth.



**Supplementary Figure 2** Production of candidate inhibitors and P69B for inhibition assays.

**a** Candidate inhibitors lacking their endogenous signal peptides were codon-optimized for expression in *E. coli* and cloned in-frame with a 6-His purification tag; the maltose binding protein (MBP) and the cleavage site of tobacco etch virus (TEV) protease. Fusion proteins were expressed in *E. coli* and purified on HisPur™ Ni-NTA resin and amylose resin, subsequently. Fusion proteins were detected at their predicted molecular weight (MW, \*) by SDS-PAGE and Coomassie staining. Purification of candidate inhibitors was repeated at least once for each candidate independently. **b** Putative inhibitors were released from their purification tags upon incubation with His-TEV protease. This experiment was repeated at least once for each inhibitor independently. **c** Purified inhibitor candidates after removal of their purification tags and TEV proteins, stained with Sypro Ruby. This experiment was repeated at least once for each inhibitor independently. **d** P69B-His is efficiently expressed by agroinfiltration. P69B with a C-terminal His tag and an empty vector (EV) control were transiently expressed by agroinfiltration. Apoplastic fluids were isolated, labeled with FP-TAMRA, separated on SDS-PAGE and scanned for fluorescence (right) and stained with Coomassie (left). Expression of P69B was repeated at least twice to similar effect. **e** Purification of P69B-His on Ni-NTA. I, input sample; FT, flow through; E, eluate. The MW marker is presented in kDa on the left of the gels. Purification of P69B was repeated twice with similar results.

1

lycop MGLLKILLVF IFCSFWPTI QSNLEYTIHV VESPESLVTQ QSLTDLGSY YLSFLPKTAT TISSSGNEEA ATMISYHNV MTGFAARLTA EQVKEMEKKH  
 pimpi MGLLKILLVF IFCSFWPTI QSNLEYTIHV VESPESLVTQ QSLTDLGSY YLSFLPKTAT TISSSGNEEA ATMISYHNV MTGFAARLITA EQVKEMEKKH  
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 habro MGLLKILLVF IFCSFWPTI QSNLEYTIHV VESPESLVTQ QSLTDLGSY YLSFLPKTAT TISSSGNEEA ATMISYHNV MTGFAARLITA EQVKEMEKKH  
 corn MGLLKILLVF IFCSFWPTI QSNLEYTIHV VESPESLVTQ QSLTDLGSY YLSFLPKTAT TISSSGNEEA ATMISYHNV MTGFAARLITA EQVKEMEKKH  
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 chile NGHGTHTAST AAGAFVKGAN VYGNANGTAV GVPLAHIAY KVVCNSVGCS ESDVLAAMDS AIDDGVILS MSLGGGPIPF HRDNIAIGAY SATERGILVS  
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 penne GLVYDTPFED YPVYLCGLKY TDQQVGNLQI RRVNCSEVK SILEAQLNPS FSIFGLGSTP QTYYTRTVNV GDATSSYKVE VASPEGAIE VEPSELNFSE  
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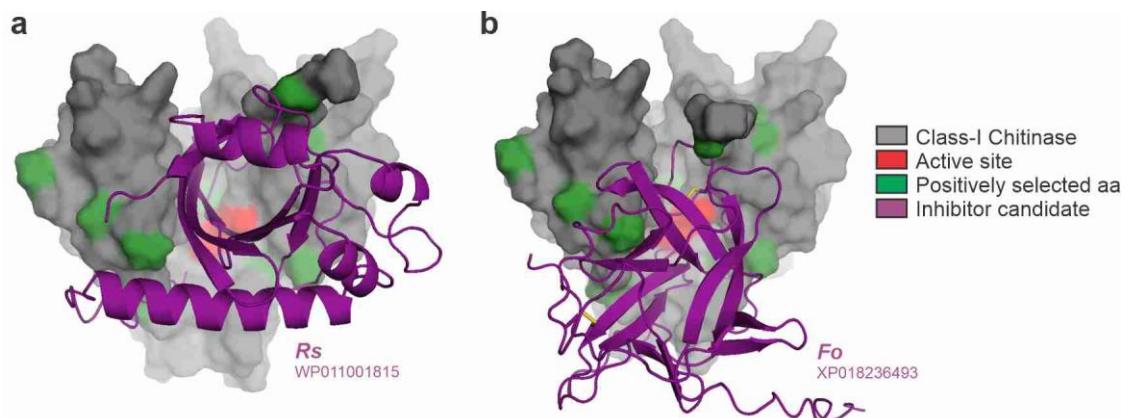
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 habro LNQKLTYQVT FSKTTNSNSP EVIEGFLKWT SNRHSVRSP1 AVVFA  
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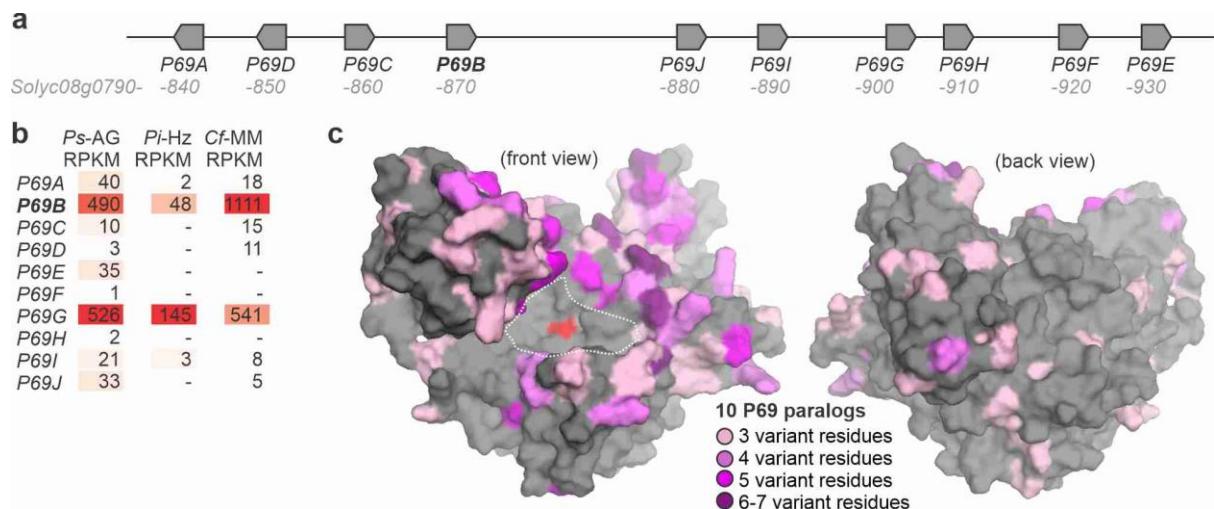
### Supplementary Figure 3 Amino acid alignment of P69B orthologs from wild tomato species.

P69B orthologs were extracted from genomes from wild tomato species and aligned. Residues that are different from P69B of cultivated tomato are highlighted in light grey, dark grey or red for n = 2, 3 and 4 variant residues, respectively. Note that only position 400 has 4 different residues.



**Supplementary Figure 4** Two candidate inhibitors interact with positively selected sites in Class-I chitinases.

Positively selected sites reported previously (Bishop et al., 2000) were plotted onto the AFM-predicted models of tomato Class-I chitinase in complex with inhibitors from *Ralstonia solanacearum* **(a)** and *Fusarium oxysporum f. sp. lycopersici* **(b)**.



**Supplementary Figure 5** Expression and variation of the ten P69 paralogs of tomato.

**a** Genomic P69 cluster contains ten paralogs. Sequence was extracted from the genome of cv. Heinz (Tomato Genome Consortium, 2012). **b** Expression levels in reads per kilobase per million (RPKM) of *P69* genes in three different cultivars infected with *Ps* (cv. Ailsa Craig, Rosli et al., 2013); *Pi* (cv. Heinz, Hwang et al., 2020) and *Cf* (cv. Money Maker, Ilyas et al., 2016). **c** Protein sequences of the ten P69 paralogs of tomato (P60A-I) were aligned and the number of variant residues at each position was plotted on the model of the P69B structure. The variation (purple) is surrounding the active site (red) in the substrate binding groove (dashed line) as a ‘ring-of-fire’.

**Supplementary Table 1** RMSD and TM scores for control complexes.

AFM model	Structure	Comparison of monomers	RMSD (Å)	TM
Pip1-EpiC2B (compatible)	3IMA	Pip1 - Papain	<b>0.94</b>	<b>0.9517</b>
		EpiC2B - Tarocystatin	<b>2.37</b>	<b>0.7827</b>
		Interface*	<b>0.85</b>	<b>0.89</b>
P69B-Epi1a (compatible)	1YU6	P69B - Subtilisin	<b>1.74</b>	<b>0.9217</b>
		Epi1a - OMTKY3	<b>1.44</b>	<b>0.5541</b>
		Interface*	<b>1.12</b>	<b>0.83</b>
Pip1-Epi1a (incompatible)	3IMA	Pip1 - Papain	<b>0.96</b>	<b>0.9509</b>
	1YU6	Epi1a - OMTKY3	<b>1.72</b>	<b>0.5252</b>
P69B-EpiC2B (incompatible)	1YU6	P69B - Subtilisin	<b>1.77</b>	<b>0.9220</b>
	3IMA	EpiC2B - Tarocystatin	<b>2.47</b>	<b>0.7687</b>

\*, Calculated from the PDB files by identifying residues that have differential solvent exposure when the complex partner is omitted.

DALI was used to identify experimentally-resolved structures with similarity to AFM-predicted control complexes: tarocystatin-papain (3IMA) for Pip1-EpiC2B and subtilisin-OMTKY3 (1YU6) for P69B-Epi1a. TMalign was used to calculate TM and RMSD values for the monomers in the AFM model compared to the resolved structures. The interface was determined by selecting the residues that show different solvent exposure when the complex partner is omitted. Scores that are above the set threshold are printed in green bold and below the threshold in red.

**Supplementary Table 2** Analysed RNAseq samples

SRA run ID	Pathogen	Tomato cultivar	Tissue/time
SRR5467166	<i>Rs</i> GMI1000 WT	'Bonny Best'	Petiole/3dpi
SRR5467167	<i>Rs</i> GMI1000 WT	'Bonny Best'	Petiole/3dpi
SRR5467168	<i>Rs</i> GMI1000 WT	'Bonny Best'	Petiole/3dpi
SRR6924534	<i>Bc</i> B05.10 WT	'Marmande'	Leaf/1dpi
SRR6924535	<i>Bc</i> B05.10 WT	'Marmande'	Leaf/1dpi
SRR6924536	<i>Bc</i> B05.10 WT	'Marmande'	Leaf/1dpi
SRR6050413	<i>Fol</i> 4287 WT	'Moneymaker' (S)	Root/1dpi
SRR6050414	<i>Fol</i> 4287 WT	'Motelle' (R)	Root/1dpi
SRR1171035	<i>Cf</i> race-0WU	'Heinz Cf-0' (S)	Leaf/4dpi
SRR1171040	<i>Cf</i> race-0WU	'Heinz Cf-0' (S)	Leaf/8dpi
SRR1171043	<i>Cf</i> race-0WU	'Heinz Cf-0' (S)	Leaf/12dpi
SRR1171047	<i>Cf</i> IPO1979	'Moneymaker'	Leaf/6dpi

**Supplementary Table 3** Transcript levels of six tomato genes in (non)infected plants

<i>Cladosporium fulvum</i>		Day-6, susceptible tomato (n=3) Mean±SD		Day-6, resistant tomato (n=3) Mean±SD	
name	Accession	Mock	Cf	Mock	Cf
P69B	Solyc08g079870.3.1	352 ± 371	1111 ± 283	221 ± 56	2628 ± 117
Pip1	Solyc02g077040.4.1	223 ± 263	661±304	118 ± 34	1358 ± 166
A1P	Solyc08g067100.2.1	78 ± 8	88 ± 4	72 ± 9	116 ± 10
C-I	Solyc09g098540.3.1	234 ± 14	195 ± 7	224 ± 12	168 ± 13
C-III	Solyc05g050130.4.1	90 ± 103	232 ± 65	44 ± 18	377 ± 35
C-V	Solyc07g005090.4.1	28 ± 9	51 ± 9	24 ± 7	61 ± 6
<i>Fusarium oxysporum</i>		Day 1, susceptible tomato (n=1)		Day 1, resistant tomato (n=1)	
name	Accession	Mock	Fo	Mock	Fo
P69B	Solyc08g079870.1.1	3.69	17.09	1.49	5.00
Pip1	Solyc02g077040.2.1	2.14	6.49	3.21	3.62
A1P	Solyc08g067100.2.1	290.53	339.77	285.60	347.14
C-I	Solyc09g098540.2.1	446.14	316.62	363.06	311.31
C-III	Solyc05g050130.2.1	61.33	141.36	60.73	89.85
C-V	Solyc07g005090.2.1	172.49	210.57	168.12	172.49
<i>Phytophthora infestans</i>		Days 1/2/3, susceptible tomato, Pi (n=4) Mean±SD			
name	Accession	Mock	1dpi Pi	2dpi Pi	3dpi Pi
P69B	Solyc08g079870.1.1	37 ± 1	107 ± 3	48 ± 2	46 ± 1
Pip1	Solyc02g077040.2.1	39 ± 1	24 ± 1	19 ± 1	16 ± 1
A1P	Solyc08g067100.2.1	48 ± 1	60 ± 1	40 ± 1	28 ± 1
C-I	Solyc09g098540.2.1	140 ± 3	158 ± 5	76 ± 1	41 ± 1
C-III	Solyc05g050130.2.1	13 ± 1	131 ± 5	92 ± 2	43 ± 2
C-V	Solyc07g005090.2.1	10 ± 1	28 ± 1	32 ± 1	59 ± 1
<i>Botrytis cinerea</i>		Day 1, susceptible tomato, WT Bc (n=3)			
name	Accession	Rep1	Rep2	Rep3	Mean±SD
P69B	Solyc08g079870.1.1	5.77	2.74	10.18	6 ± 4
A1P	Solyc08g067100.2.1	19.80	11.35	20.89	17 ± 5
C-I	Solyc09g098540.2.1	10.95	4.75	17.27	11 ± 6
C-III	Solyc05g050130.2.1	0	0	0	0
C-V	Solyc07g005090.2.1	265.26	286.11	451.3	334 ± 102
<i>Ralstonia solanacearum</i>		Day-3, susceptible tomato, Rs (n=3)			
name	Accession	Rep1	Rep2	Rep3	Mean±SD
P69B	Solyc08g079870.1.1	503.40	954.43	734.40	731±226
A1P	Solyc08g067100.2.1	97.34	101.76	89.39	96±6
C-I	Solyc09g098540.2.1	905.04	867.27	871.69	881±21
C-III	Solyc05g050130.2.1	661.37	1111.08	865.27	879±225
C-V	Solyc07g005090.2.1	54.25	56.65	45.60	52±6

FPKM values of tomato genes, extracted from Ilyas et al., 2016 (Cf); Zhao et al., 2018 (Fo); Huang et al., 2020 (Pi); Müller et al., 2018 (Bc); Khokhani et al., 2017 (Rs).

**Supplementary Table 4** RNA-seq values of SSPs in infected plants\*

<i>Cf</i>	4dpi SRR1171035	6dpi SRR1171047	8dpi SRR1171040	12dpi SRR1171043	Mean(±SD)
KAH3648627 ( <i>Cf</i> Ecp36)	212.485	436.287	578.257	691.188	479.55 ± 206.34
<i>Fo</i>		MoneyMaker (S) SRR6050413	Motelle (R) SRR6050414	Mean(±SD)	
XP018243121 ( <i>Fo</i> TIL)		180	461	340.50 ± 198.70	
APP91304 ( <i>Fo</i> Six15)		270.034	144.81	207.42 ± 88.55	
XP018236493		204.966	227.051	216.01 ± 15.62	
XP018248187		5.39765	5.71688	5.56 ± 0.23	
XP018241286		1.79816	5.8976	3.85 ± 2.90	
<i>Rs</i>	SRR5467166	SRR5467167	SRR5467168	Mean(±SD)	
WP011000405	0	6.374	13.972	6.78 ± 6.99	
WP011001815	528.431	656.721	612.85	599.33 ± 65.20	
WP011002292	2.7102	2.8454	3.11861	2.89 ± 0.21	
<i>Bc</i>	SRR6924534	SRR6924535	SRR6924536	Mean(±SD)	
XP001545484	2.47535	2.34842	2.50272	2.44 ± 0.82	
XP001560184	71.8476	79.0248	27.7998	59.56 ± 27.7	

\*, all values are in transcripts per million (TPM) reads of the pathogen during infection of tomato.

Infection of *Rs* and *Bc* was at 72 and 24 hpi, respectively.

**Supplementary Table 5** RMSD and TM scores for the hydrolases in 15 SPP-hydrolase complexes

Hydrolase	SSP	PDB	Protein(s) in PDB	RMSD (Å)	TM
P69B	WP046932418- <i>XpSsp</i>	1YU6	Subtilisin-OMTKY3	<b>1.7</b>	<b>0.92</b>
P69B	KAH3648627- <i>C/Ecp36</i>	1YU6	Subtilisin-OMTKY3	<b>1.7</b>	<b>0.92</b>
P69B	XP018243121- <i>FoTIL</i>	1YU6	Subtilisin-OMTKY3	<b>1.7</b>	<b>0.92</b>
P69B	APP91304- <i>FoSix15</i>	1YU6	Subtilisin-OMTKY3	<b>1.7</b>	<b>0.92</b>
P69B	XP001545484	1YU6	Subtilisin-OMTKY3	<b>1.7</b>	<b>0.92</b>
P69B	WP011000405	1YU6	Subtilisin-OMTKY3	<b>1.8</b>	<b>0.92</b>
P69B	WP008576433	1YU6	Subtilisin-OMTKY3	<b>1.8</b>	<b>0.92</b>
C-I	WP011001815	3IWR	Rice class-I chitinase	<b>1.4</b>	<b>0.95</b>
C-I	XP018236493	3IWR	Rice class-I chitinase	<b>1.4</b>	<b>0.92</b>
C-III	WP046931881	4TOQ	Class-III chitinase	<b>0.75</b>	<b>0.97</b>
C-III	XP001560184	4TOQ	Class-III chitinase	<b>0.76</b>	<b>0.97</b>
C-III	XP018248187	4TOQ	Class-III chitinase	<b>0.79</b>	<b>0.97</b>
C-III	XP018241286	4TOQ	Class-III chitinase	<b>0.86</b>	<b>0.97</b>
C-V	WP008572913	3ALG	Class-V chitinase	<b>1.4</b>	<b>0.94</b>
A1P	WP011002292	4ZL4	Plasmepsin	3.0	0.73

DALI was used to identify the experimentally resolved structure that is closest to that of the hydrolase in the SPP-hydrolase complex and the RMSD and TM scores were calculated for each comparison with TMalign. Scores that are above the set threshold are printed in green bold and below the threshold in red.

**Supplementary Table 6** RMSD and TM scores for the SSPs in 15 SPP-hydrolase complexes

SSP	PDB	RMSD (Å)	TM
WP046932418-XpSsp	x	x	x
KAH3648627-CfEcp36	6NK9	<b>1.9</b>	<b>0.63</b>
XP018243121-FoTIL	x	x	x
APP91304-FoSix15	x	x	x
XP001545484	1WPX	<b>2.6</b>	<b>0.68</b>
WP011000405	x	x	x
WP008576433	x	x	x
WP011001815	5UC0	<b>4.7</b>	<b>0.53</b>
XP018236493	4I1B	<b>3.6</b>	<b>0.62</b>
WP046931881	4MIS	<b>2.2</b>	<b>0.70</b>
XP001560184	3TC2	<b>3.7</b>	<b>0.61</b>
XP018248187	5FJQ	<b>2.7</b>	<b>0.46</b>
XP018241286	4OWL	<b>3.0</b>	<b>0.70</b>
WP008572913	1Y34	<b>2.8</b>	<b>0.66</b>
WP011002292	2HOD	<b>3.0</b>	<b>0.61</b>

DALI was used to identify the resolved structure that is closest to that of the SSP in the SPP-hydrolase complex and the RMSD and TM scores were calculated for each comparison with TMalign. Scores that are above the set threshold are printed in green bold and below the threshold in red. No good structures were identified for several complexes (x).

**Supplementary Table 7** Synthesised nucleotide sequences

**P69B Fragment-1**

GAAGACATAATGGGATTCTTCTTTACAATTGCCTCATTCTTGTCTACACTCTCTTATTCC  
AGTAATATCCCCTCACTCTTGACAGATTGGTAGTTACTATCTTCCTTCTGCCAAAACGGCTAC  
CTTGGTCAACCCTCAATCACTCTTGACAGATTGGTAGTTACTATCTTCCTTCTGCCAAAACGGCTAC  
TACGATCTCTCAAGTGGCAACGAGGAAGCCGCGACAATGATTTACTCATATCATAACGTAATGACTGGTT  
TCGCAGCTAGATTAACGTGCTGAGCAGGTAAAGAAATGGAAAAGAACATGGATTGTGTCGCACAGAA  
GCAAAGAATCTTGAGCCTACATACTACACACACTCTAGTTCCCTGGTTACAGCAAATATGGCGTTG  
GAAGGACTCAAATTATGGTAAGGGAGTGATCATTGGTGAATTGATACAGGTATCATTCCGACCATCC  
GCTTAGTGTGATGGCATGCCCGCACCTGCAAAATGGAAAGGTGTTGCGAGTCAATTACTAATA  
AGTGTAAATAACAAGCTTATAGGTGCCGATCTTACCAACTAGGTAATGGTCCCCAATTGATTCTATAGGGC  
ACGGAACGCACACAGCCAGTACTGTCGGCGTTCTGAAAGGAGCCAACGTGATGGTAATGCCGAC  
GGAACAGCGTAGGGTGGCACCATAGCTCATATTGCTATTATAAGGTGTCGAAATTCAAGTGGTTGTC  
GAGTCAGACGTACTTGCAGCCATGGACTCCGCAATAGACGATGGTGTGATATACTTCTATGCTTTGCT  
GGAGGTCCGATTCTTTACCCGGACAATATCGCTATAGGGCCTATACTGCTACTGAAACGTGGAATCCTC  
GTTCTGTTGCTAGCTGTAATAGTGGACCTTCTTATTACAGCTGCAATACCGCTCCGGATTCTAAGT  
TCGGAGCTTCACTTGGACCGTAAGATAAGGCAACTGTGAAACTGGCAACGGTAGGAATTGAAGGA  
GAATCAGCATATCGCTTAAGATAAGCAATGCTACATTCTTACACTCTTGATGCGGCAAAGAACGCTAA  
AGACCCCTAGCGAACACCCCTATTGCGCAGGGAACTCAACAGACCCGCTATCAGGGTAAAGTAGTTC  
TCTGAGTGTCTGGACATGTTGCAAACGTTGATAAAGGACAGCGGTAAAGGACGCTGGTAGTTGGA  
ATGATAATCATAAATCCAAGTCAAGTATGGAGTAACCAAGTCTGCTATGCTGTCATGTGTCCTGCTCGTA  
GTGAGCGCCGCTGATGGTATGTC

**P69B Fragment-2**

GAAGACATTGGTACTAAGATATTGGCATATATGAACACTACTAGCTCTCAGTAGGCCACAATTGCAATTCA  
GGAACGATCATTGGTACAAGAACGACCGATGGTGCAGCATTAGTCTCCGAGGCCCTCACGTGCATC  
CCCTGGTATCCTCAAGCCTGATATCATTGGACCTGGCGAAACATACTTGCCTGCGATGGCCTACTTCAGTGG  
TGACAATAAGAACACAAAGTCTACTTTAATATAATTAGTGGTACAAGCATGTCCTGCCCCACCTGTC  
AGTAGCTGCACTGCTTAATGTACGCATCCAGATTGGTCAACCAGCTGTTAAAGAGCGCTATGATGACGA  
CCGCTGACACCTGAAACCTCGCAAACCTCCTATTCTGACGAGCGTTGCTCCAGCTGACATCTGCAA  
TAGGCAGCCGGACATGTGAATCAGTGGCTAATGACCCAGGGTTGGTACGATACTCCTTCAAGAT  
TACGTCCCATACTCTGTGGACTTAAATACGGATCAACAGGTTGGTAAATTAAATCAGTAAAGACGTTGAAAC  
TGTTCGAAGTTAACAGCATCTTGGAAAGCTCAACTGAATTATCCAGTTCTATTGGACTAGGAAGT  
ACTCCTCAGACTTATAACAAGAACTGTGACTAATGTCGGAGATGCAACATCTTCTACAAAGTTGAGGTTG  
CAGCCCTGAAGGGTGCAGTGAAGTTAACCTAGCGAGCTTAACCTCCAGCTAAATCAGAAACTTA  
CGTACCAAGTAACATTCAAGACAACAACTCTAGTAATCCAGAAGTAATCGAGGGTTCTTAAATGG  
ACTAGTAATCGTCATAGTGTGCGATCTCCATGCCGTTGTAAGCGCAGGTGGCACCATCACCA  
CTGAGCTTATGTC

**His-MBP-TEV**

ATGGGCAGCAGCCATCATCATCATCATCATATGAAAATGAAAGAAGGTAACCTGGAATCTGGATTAACGG  
CGATAAAGGCTATAACGGTCTGCTGAAGTCGGTAAGAAATTGAGAAAGATACCGGAATTAAAGTCACC  
GTTGAGCATCCGGATAAACTGGAAGAGAAAATTCCCACAGGTTGCGGCAACTGGCGATGCCCTGACATTAT  
CTTCTGGGACACGACCGCTTGGCTACGCTCAATCTGGCTTGGCTACGTTACAACGGCAAGCTGATTGCTTACCCGA  
CGTTCCAGGACAAGCTGTATCCGTTACCTGGATGCCGACGTTACAACGGCAAGCTGATTGCTTACCCGA  
TCGCTGTTGAAGCTTATGCTGATTATAACAAAGATCTGCTGCCAACCCGCCAAAAACCTGGAGAG  
ATCCCCGGCGCTGGATAAAAGAACTGAAAGCAGGTAAGAGCGCCTGATGTTCAACCTGCAAGAACCGT  
ACTTCACCTGGCGCTGATTGCTGCTGACGGGGTTATGCGTTCAAGTATGAAAACGGCAAGTACGACATT  
AAAGACGTGGCGTGGATAACGCTGGCGAAAGCGGGTCTGACCTCTGGTTGACCTGATTTAAAGGCGAAACAGCGATGACC  
ACACATGAATGCAACACCGATTACTCCATCGCAGAAGCTGCCCTTAATAAAGGCGAAACAGCGATGACC  
ATCAACGGCCCGTGGCATGGTCAACATCGACACCAGCAAAGTGAATTATGGTGAACGGTACTGCCGAC  
CTTCAAGGGTCAACCCTAACCGTCTGGCGTCTGAGCGCAGGTATTAACGCCAGTCCGAACA  
AAGAGCTGGCAAAGAGTTCTCGAAAACATCTGCTGACTGATGAAAGTCTGGAAGCGGTTAATAAAGA  
CAAACCGCTGGTGGCGTAGCGCTGAAGTCTACGAGGAAGAGTTGGCGAAAGATCCACGTATTGCCGCA  
CTATGGAAAACGCCAGAAAGGTGAAATCATGCCAACATCCGCAAGATGTCGCTTCTGGTATGCCG  
CGTACTGCCGTGATCAACGCCGCCAGCGCTGTCAGACTGTCGATGAAAGCCCTGAAAGACGCGCAGACTA  
ATTGAGCTCGAACACAACAAACAATAACAATAACAACACCTCGGGATCGAGGAAAACCTGTATTTCA  
GGCGAACATCGGATCTCTAGAGTCGACCTGCAAGCAAGCT

**XpSsp1-WP046932418.1-P3**

GCTCCACCTACAGATACGGCAACTCCCCCCCCAGCGACGGGTTCCACCGTGGTCCGGCGCTGGCAAGGC  
GGTCTGGTGACCACCGACGTGCCGTACCGACGCCACTGTACCGTGAAGAACGTAAGGTAATTGTTGTC  
CATTCTGGCGTGCCTGAACGCTAACAGCGCAGCCGACCGAACCGAATGGTGTGTCAGTGCCTGCAAGCGGGT  
GGCATGATGAGCGTTGCCGCTCCGAGATCTCTGCCGACTGCCGACGGAAACCGTTCACTAG  
AGATGCCAACCGGATACCTCGCTGCCGACTCCGCCGACGGAAACCGTTCACTAG

**CfEcp36-KAH3648627.1-P5**

AAAAGGGGGTCAGGTGGAGAATGTAGAACACAATCCGACTGTTGTTACTGCGACCCGGGTTCCGGCTGCAT  
CCTGAGCTGTCAGGCAAGATCGTCTAGCACCAACGGCTATCGCTGCATTACCGCCGTCAGTCGGCTTGCA  
ACGTTGCCGTTGCAAGCTG

**FoTIL-XP\_018243121.1-P8**

GCTCCCAAGTGTAAAGCAGGAGAACAAATTTCGAGTGCAGGCCGCTTGCCCCTGACGTGCGACAAACC  
GGAACCGCGTCCGTACCAAACAATGTGTTCCGGTTGCTTGCAGGAGGTGCGCATTCTCGGTCAAA  
GCCAGCAGGTTCTGCGCCCTCAAGGCCTGTTGCTGCCGGTAGCATCCACAACCGCCCAATCTCGGT  
ATTTCGGATAGCCGTACCTGTAG

**FoSix15-APP91304.1-P9**

ACAATATAATTGTAGGGATGTATCACCAACCCCGCGATACCCGTAGCTGGTGTAAAACGAACACCCGGCGTG  
GCAAGGTTGCCAGCGTTTGCGAGCAGACTGCCGCTCACCCCGCGTAGACTACCCGACGGCTGCATGT  
ATCATCTGCAGGTTGGCGGTGATTACGACTGTTCTGCAAGTAG

**XP001545484.1-P6**

TTCACACCACCCGGATTGAACCTAGTTCAACGAACGATCTGACCGTCGCATACGGCTCGAAACTTGCCAC  
CAACGGTATTCAAATGCTGCGGACACCGCGTATGCTCCGATTCTGGGTACAGCGACCAAAATGTCCG  
GCACCTATGCGGTTATGATGGTTGATCCGGACATCCCGCCGCAAAGGTGGGTGGTAGACACCAGCCAATT  
CTGCATTGGATGCAGGCAGACTTGACCAACTAACTACTACGACCATGGTGGCCAGAAAATCTACGA  
GCTGATTAACGTGAAGAATACCTCTGCCTTGCGACGTACCTGCAGCCGAATCCGCCAGATATCGCTCCGA  
CCACCCACCGTTATACCCAGTTGCTCTTAACACCAACTGGCATGAATATGAGCTTGCCAGCCTGCAAACG  
GCGGGTAAAACCGCGGTAAATTCAACCGACGAACGTTGTTAAGTCTGCTAAGTTAACGGTGCTGATGGG  
TAACTCCTCGACGTGAGCTCGCGATAAAGCGATCAACAAACACCGCCGCTCACCGGCAGCAGCACCT  
AG

**WP\_011000405.1-P7**

GCTGATGATGTTCCCGTAGCGGACGCATCACTCCAACCGAGCGTTGAGGCGGCCACCCGGTTGCTGC  
GGCGACCGCTCGTCAAGGTTTGCCAGGCAGTTGAGGCAAGCGCACTGGAAGGTATCGTGGTGGTACGC  
AGCCGCTGTACCAAGACCGTCAACGACGCCGCTTGCGACGGTACCGATAATGCCGCGATTAACGTT  
GCTACTGGCGCTAATATTGTACGTGACGGCAGCTCGCAACGCCCTGTCATCCCGACCGTTATTCAAAC  
ACCGGTGCGAACGTGCTGATCCAGAATGCGACCATCGTGAACGTGCAGTTCCGCCGTAG

**WP008576433.1-P4**

CTGTATTTCAAGGGCGAATTGGTTGTAATACAGTAGCTGGAGCAGGGAAAGACATGCAGGGTGGCGGTGA  
TAAAGTTGAGAAGACCGCTGAAAAGTGCAGCGACGGCAAATGTTAGGAATTGGATCCTCTAGAGTC

**Supplementary Table 8** Used plasmids

Plasmid	Description	Reference
pJK187	Binary vector (pL0V2-2x35S::SC)	This work
pET-32/28	Bacterial expression vector	Novinec et al., 2012
P19	Binary vector carrying p19 silencing inhibitor	Van der Hoorn et al., 2003
pFH20	Binary vector for P69B-His expression	This work
pHJ000	Bacterial expression vector pET-32/28-His-MBP-TEV	This work
pHJ028	Bacterial expression P3(WP046932418.1, <i>XpSsp1</i> )	This work
pHJ033	Bacterial expression P5(KAH3648627.1, <i>CfEcp36</i> )	This work
pHJ029	Bacterial expression P6(XP001545484.1,)	This work
pHJ032	Bacterial expression P7(WP011000405.1)	This work
pHJ030	Bacterial expression P8(XP_018243121.1, <i>FoTIL</i> )	This work
pHJ031	Bacterial expression P9(APP91304.1, <i>FoSix15</i> )	This work
pHJ043	Bacterial expression P4(WP008576433.1)	This work
pHJ046	Bacterial expression <i>PiEpi1</i>	This work
pHJ047	Bacterial expression <i>PiEpiC1</i>	This work

**Supplementary Table 9** Primer sequences

Primer	Use	Nucleotide sequence (5'-3')
His-MBP-F	Cloning	AAGAAGGAGATATAACCATGGGCAGCAGCCATCATCATCAT
His-MBP-R	Cloning	TGGTGGTGGTGGTGGCTCGAGAACGCTGCCTGCAG
Epi1-F	Cloning	CTGTATTTCAGGGCGAATTCCAAGGCCGAAGTCATCAG
Epi1-R	Cloning	GACTCTAGAGGATCCGAATTCTTATCCCTCCTGCGGTGTC
EpiC1-F	Cloning	CTGTATTTCAGGGCGAATTCCAAGTGGACGGCGGATACTC
EpiC1-R	Cloning	GACTCTAGAGGATCCGAATTCTACTTAACGGGTAATCG
P3-F	Cloning	CTGTATTTCAGGGCGAATTGCTCCACCTACAGATAACGG
P3-R	Cloning	GACTCTAGAGGATCCGAATTCTAGTGAACGGTTCCGTCGG
P4-F	Cloning	CTGTATTTCAGGGCGAATT
P4-R	Cloning	GACTCTAGAGGATCCGAATT
P5-F	Cloning	CTGTATTTCAGGGCGAATTCAAAAGGGGGTCAGGTGGAGA
P5-R	Cloning	GACTCTAGAGGATCCGAATTCTAGCAGCTGCAACCCGCAA
P6-F	Cloning	CTGTATTTCAGGGCGAATTCTCACACCACCCGGATTGA
P6-R	Cloning	GACTCTAGAGGATCCGAATTCTTAGGTGCTGCTGCCGGTGG
P7-F	Cloning	CTGTATTTCAGGGCGAATTGCTGATGATGTTCCCGTAGC
P7-R	Cloning	GACTCTAGAGGATCCGAATTCTACGGCGGAACTGCACGT
P8-F	Cloning	CTGTATTTCAGGGCGAATTGCTCCCAAGTGTAAAGCAGG
P8-R	Cloning	GACTCTAGAGGATCCGAATTCTACAGGTGACGGCTATGGG
P9-F	Cloning	CTGTATTTCAGGGCGAATTACAATATATTGTAGGGATGT
P9-R	Cloning	GACTCTAGAGGATCCGAATTCTACTTGAGAACAGTCGT
PJK187-F	Sequencing	CTATCCTTCGCAAGACCCCTC
PJK187-R	Sequencing	CTCAACACATGAGCGAAACC
pet32a-F	Sequencing	TAATACGACTCACTATAGGG
pet32a-R	Sequencing	GCTAGTTATTGCTCAGCGG