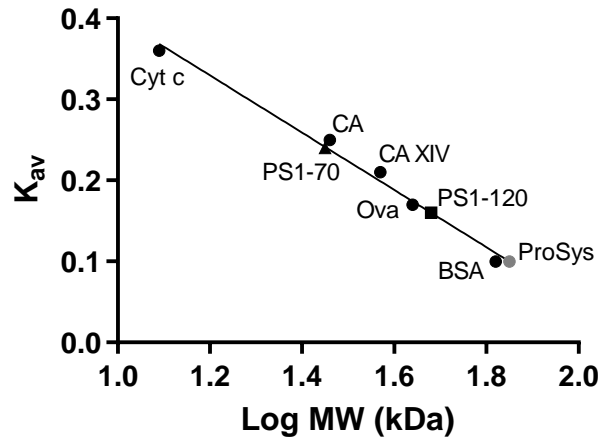
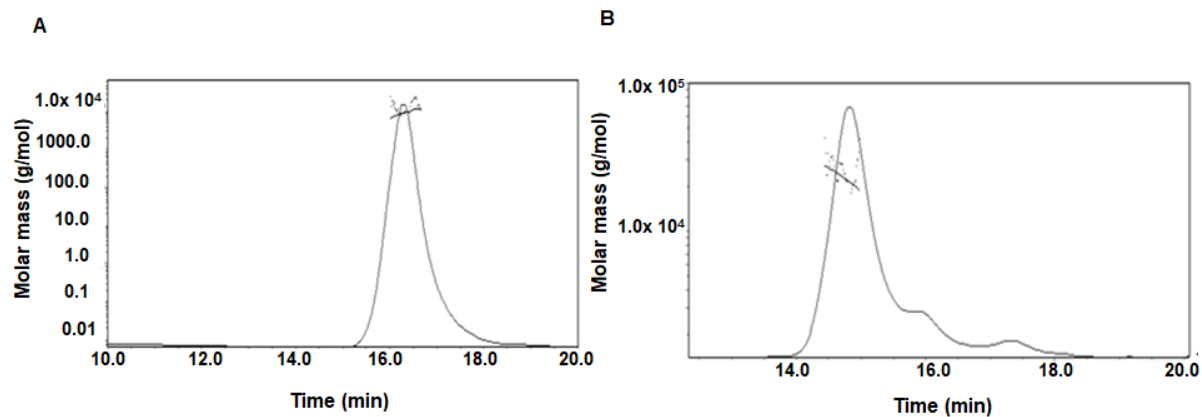


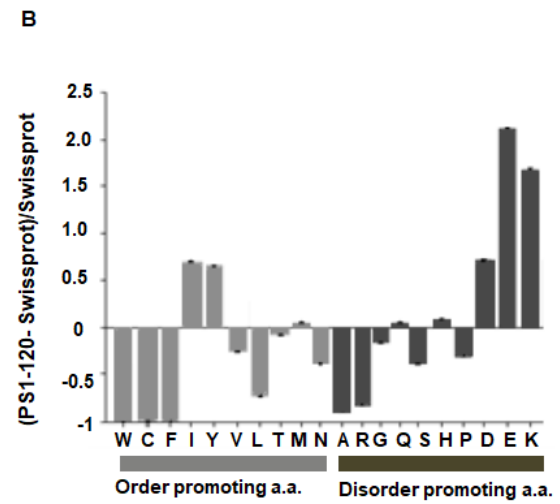
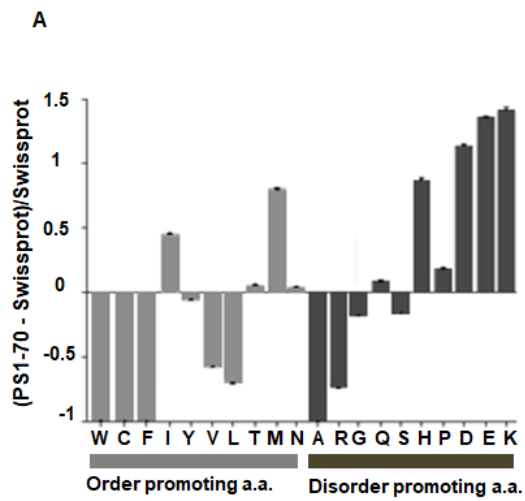
Supplementary Material



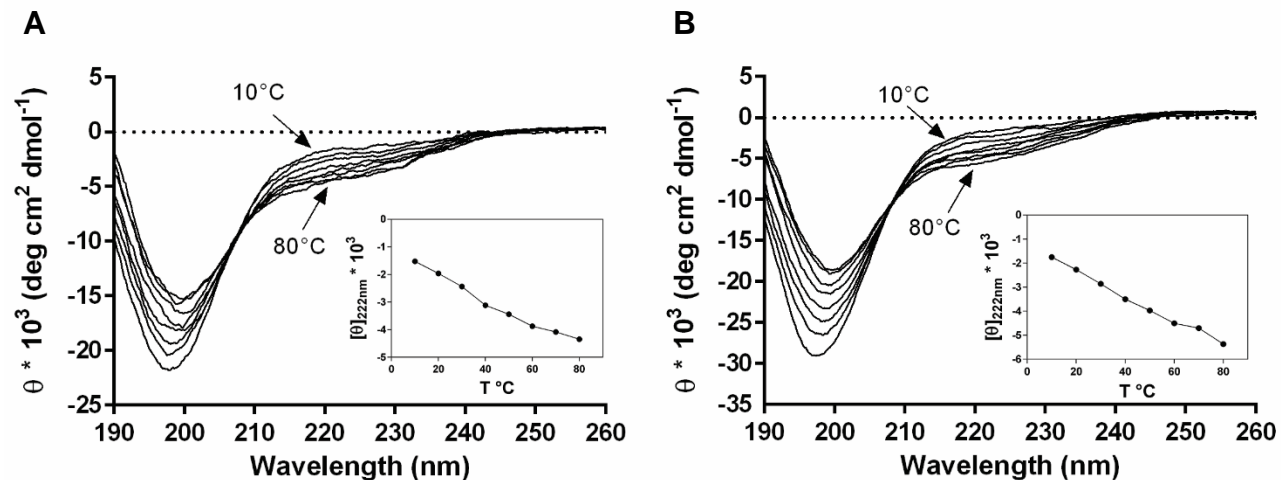
Supplementary Figure S1. Apparent molecular mass of PS1-70 and PS1-120. Apparent molecular mass of PS1-70 and PS1-120 as calculated by SEC. Calibration curve was built using five standard proteins (black circle). $K_{av} = (V_e - V_o) / (V_t - V_o)$, where V_e = protein elution volume, V_t = column volume, and V_o = void column volume. ProSys: grey circle; PS1-70: black triangle and PS1-120: black square.



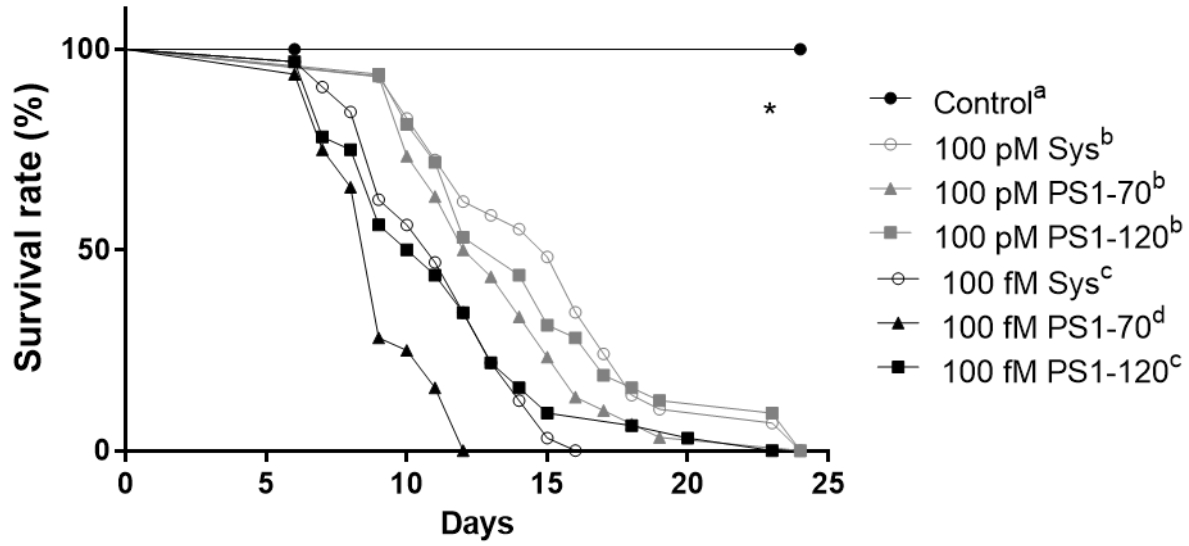
Supplementary Figure S2. Multi Angle Light Scattering of ProSys fragments. The peaks of (A) PS1-70 and (B) PS1-120 are representative of monomeric proteins.



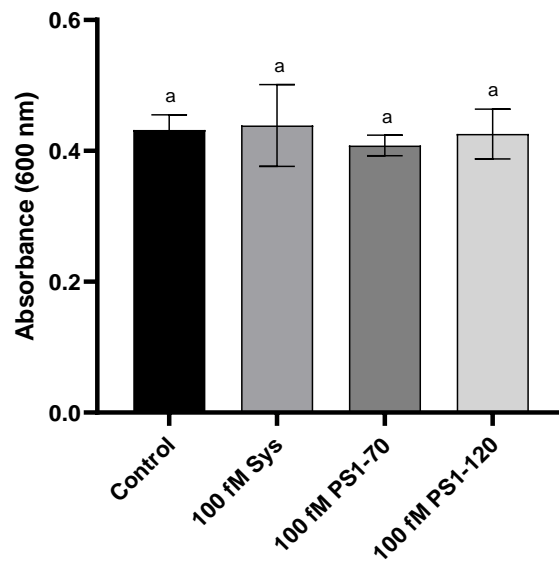
Supplementary Figure S3. Sequence properties of PS1-70 and PS1-120. Amino acid compositional analysis of (A) PS 1-70 and (B) PS 1-120 performed by Composition Profiler tool.



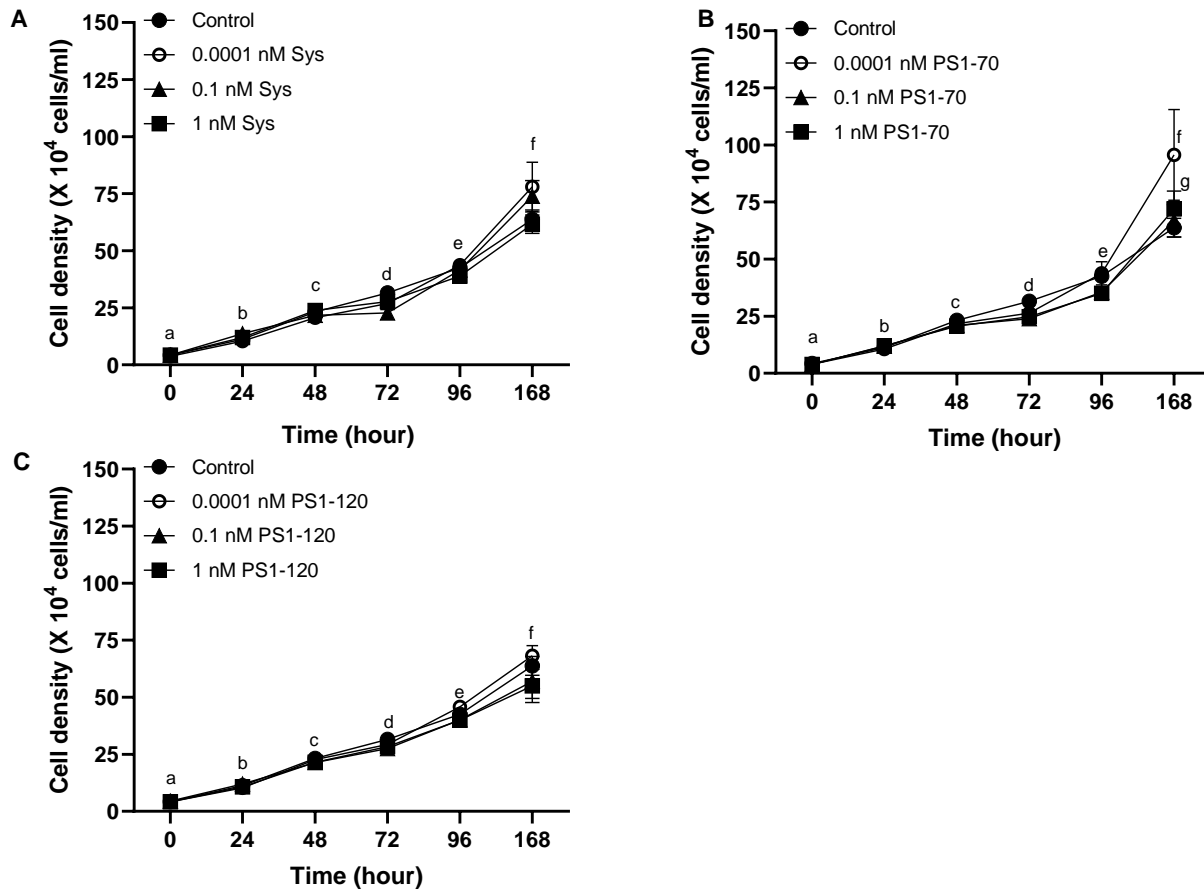
Supplementary Figure S4. CD spectra of PS1-70 and PS1-120 at different temperatures. Temperature effect on the secondary structure content of **(A)** PS1-70 and **(B)** PS1-120 at increasing temperature (10°C, 20°C, 30°C, 40°C, 50°C, 60°C, 70°C and 80°C). Changes in molar ellipticity at 222 nm as a function of the thermal treatment are shown in the inset for both fragments. Spectra were acquired in 10 mM phosphate buffer, pH 7.4.



Supplementary Figure S5. Effect of 100 pM and 100 fM Sys, PS1-70 and PS1-120 on *S. littoralis* larvae. Survival rate of experimental *S. littoralis* larvae. Different letters denote significant differences in the survival curve (Log-Rank test, $P < 0.0001$).



Supplementary Figure S6. *In vitro* antifungal vitality assay in presence of ProSys fragments. Spores of *B. cinerea* were added to each well filled with 1 ml of PDB1/2 medium containing the peptides at the final concentration of 100 fM, except for the broth sterility control wells. Fungal growth was assessed 24 hours after pathogen inoculation by evaluating the OD₆₀₀ of the medium. Different letters indicate statistically significant differences (One-Way ANOVA, $P < 0.05$). Error bars indicate standard error.



Supplementary Figure S7. Growth curves of *Chlamydomonas reinhardtii* treated with different concentrations of peptides. (A) Sys, (B) PS1-70, and (C) PS1-120, used at different concentrations. (One way ANOVA, $P < 0.05$, not significant). Error bars indicate standard deviation.

Supplementary Table S1. List of primer sequences, expected amplicon size and number of PCR cycles used.

Primer name	Sequence (5'-3')*	Fragment name	Amplified length (bp)	PCR cycle	Number of PCR cycle
P11F1	CGCGCGCCATGGGAACCTTCAT ATGATATCA	PS1-70	236 bp	98°C for 10 seconds	30
	CGCGCGCTCGAGTTACTACTCCTC ATGTTCCATCTTTGGTA			66°C for 15 seconds	
P11R1				72°C for 30 seconds	
P11F1	CGCGCGCCATGGGAACCTTCAT ATGATATCA	PS1-120	386bp	98°C for 10 seconds	30
	CGCGCGCTCGAGTTACTAAGTCTC TTTTTCAACTATTTTCTCTTTTC			66°C for 15 seconds	
P11R3				72°C for 30 seconds	

*NcoI restriction site is underlined; XhoI restriction site is italicized and underlined.

Supplementary Table S2. List of primers used for Real-time qPCR analysis.

Primer	Sequence (5'-3')	Amplicon length (bp)	Gene name	Accession number
ACO2 Fw	AACTCCTCAAAGACGGTGA			
ACO2 Rv	TCCCGTTCGTGATTACCTCG	95	<i>ACO2</i>	Solyc12g005940
AOS Fw	GATCGGTTTCGTCCGAGAAGAA			
AOS Rv	GCGCACTGTTTATTCCCCACT	101	<i>AOS</i>	Solyc11g069800
EF Fw	CTCCATTGGGTCGTTTTGCT			
EF Rv	GGTCACCTTGGCACCAGTTG	101	<i>EF-1a</i>	Solyc06g005060
Lox C Fw	TTGCCTATGGTGCTGAATGGA			
Lox C Rv	CAAGCCATGTGGTTCATTTGG	101	<i>Lox C</i>	Solyc01g006540
Lox D Fw	TTCATGGCCGTGGTTGACA			
Lox D Rv	AACAATCTCTGCATCTCCGG	102	<i>Lox D</i>	Solyc03g122340
Pal Fw	CCACATTCAGCAACAAGGGC			
Pal Rv	ACACGGGGTAATGTTGCTGT	126	<i>PAL</i>	Solyc09g007910
Pin I Fw	GAAACTCTCATGGCACGAAAAG			
Pin I Rv	CACCAATAAGTTCTGGCCACAT	114	<i>Pin I</i>	Solyc09g084470
Pin II Fw	CCAAAAAGCCAAATGCTTG			
Pin II Rv	TGTGCAACACGTGGTACATCC	116	<i>Pin II</i>	Solyc03g020060

Tm: melting temperature; LA: length amplicon; NC: number of cycles;¹ calculated according to the rule of Wallace: 4°C for G and C, 2°C for the A and T (Wallace, 1999).

Supplementary Table S3. Weight increase of *S. littoralis* larvae fed on leaves of tomato plants treated with Sys peptide, PS1-70 and PS1-120 fragments. Averages \pm standard deviations are reported for Sys, PS1-70, PS1-120 and control (C) samples for all experimental time points. For each time point, mean values denoted with different letters are significantly different (One-way ANOVA: * $P < 0.05$; ** $P < 0.0001$ followed the Tukey-Kramer Honestly Significant Difference (HSD) multiple range test).

Days of feeding	C Larval weight (g)	Systemin Larval weight (g)	PS1-70 Larval weight (g)	PS1-120 Larval weight (g)	F	P value
1	0.002 \pm 3.33E-05 ^a	0.0023 \pm 2.94E-05 ^a	0.0024 \pm 3.41E-05 ^a	0.0023 \pm 3.24E-05 ^a	F (3, 124) = 0.5599	0.6425
3	0.004 \pm 7.98E-05 ^a	0.0032 \pm 7.22E-05 ^b	0.0033 \pm 9.64E-05 ^b	0.0035 \pm 0.0001 ^b	F (3, 123) = 15.27	< 0.0001**
5	0.0159 \pm 0.0036 ^a	0.0099 \pm 0.0026 ^{ab}	0.0068 \pm 0.0004 ^b	0.0070 \pm 0.0004 ^b	F (3, 124) = 3.619	0.0151*
7	0.0153 \pm 0.0006 ^a	0.0092 \pm 0.0007 ^b	0.0079 \pm 0.0008 ^b	0.0087 \pm 0.0006 ^b	F (3, 107) = 27.86	< 0.0001**
9	0.0240 \pm 0.0006 ^a	0.0082 \pm 0.0007 ^b	0.0084 \pm 0.0007 ^b	0.0093 \pm 0.0006 ^b	F (3, 75) = 147.4	< 0.0001**
11	0.0346 \pm 0.0007 ^a	0.0118 \pm 0.0010 ^b	0.0117 \pm 0.0011 ^b	0.0135 \pm 0.0013 ^b	F (3, 64) = 176.2	< 0.0001**
13	0.0439 \pm 0.0012 ^a	0.0157 \pm 0.0020 ^b	0.0109 \pm 0.0029 ^b	0.0115 \pm 0.0020 ^b	F (3, 44) = 92.95	< 0.0001**

Supplementary Table S4. Evaluation of larvicidal effect of the direct delivery of ProSys fragments at different concentrations on *Spodoptera littoralis* larvae.

Oral ingestion	Control	100 fM Sys	100 pM Sys	100 nM Sys	100 fM PS1-70	100 fM PS1-70	100 nM PS1-70	100 fM PS1-120	100 pM PS1-120	100 nM PS1-120
4th instar	16	16	16	16	16	16	16	16	16	16
pupal stage	15	16	15	16	16	15	15	16	16	15
% survival	93.75	100	93.75	100	100	93.75	93.75	100	100	93.75
Topical application	Control	100 fM Sys	100 pM Sys	100 nM Sys	100 fM PS1-70	100 fM PS1-70	100 nM PS1-70	100 fM PS1-120	100 pM PS1-120	100 nM PS1-120
4th instar	16	16	16	16	16	16	16	16	16	16
pupal stage	15	16	16	16	15	16	15	15	16	16
% survival	93.75	100	100	100	93.75	100	93.75	93.75	100	100

Supplementary Table S5. *C. reinhardtii* 96 hours growth rate inhibition using different concentration of Sys, PS1-70 and PS1-120.

Sample	Concentration (nM)	Growth rate inhibition (% of control)
Sys	0.0001	-7.9 ± 1.8
	0.1	1.2 ± 1.7
	1	3.4 ± 2.9
PS1-70	0.0001	-4.7 ± 3.9
	0.1	3 ± 2.6
	1	4.2 ± 2
PS1-120	0.0001	-4.7 ± 0.9
	0.1	3.8 ± 1.3
	1	1.3 ± 1.3