

Supplemental Figure S1. Analysis of *Ps*LYR4-ECD binding to different chitosan oligomers using microscale thermophoresis (MST), (A) CO6-DA, (B) CO7-DA, (C) CO8-DA.



Supplemental Figure S2. Searching of binding sites in the PsLYK9-ECD revealed five potential places for binding. The binding sites are situated between LysM domains. Site 3 and site 4 are placed at the central groove, the 5 site occupies the area near the hypothetical transmembrane alpha helixes. Site 1 occupies the groove between LysM3 and LysM1 domains and site 2 is in the groove between LysM1 and LysM2.



Supplemental Figure S3. Diagram of PsLYK9-ECD binding with CO5 (binding site 1). This binding is characterized by many non-covalent bonds and good affinity. The complex with best GScore and model energy for the site 1 has 8 H-bonds, 3 salt bridges and 3 "bad" bonds. "Good" bonds are marked at the diagram: Ser186, Asn74, Glu178, Gly85, Gly177. "Bad" bonds are not marked at the figure: Asn89, Arg87, Met153. It is well seen that CO5 interacts with polar and negative charged aminoasids and glycine. The outer part of the ligand surface is available to the solvent and is able to make bonds with water or hydrophilic liquids. The CO5 surface is charged in according to inner PsLYK9-ECD cavity surface.



Supplemental Figure S4. Diagram of PsLYK9-ECD binding with CO5 (binding site 2). The ligand binds the protein near the groove but not inside it and characterized by 6 H-bonds, 1 salt bridge, 3 "bad" bonds. "Good" bonds are marked at the diagram: Gly55, Asp109, Gln111. "Bad" bonds are not marked at the figure: Thr149. The ligand interacts with polar, negatively and positively charged amino acids as well as with glycine and hydrophobic Val110. The CO5 surface is partly polar, negatively charged at the area of Thr149 and hydrophobic near the hydrophobic Pro60. Almost one-half of the ligand surface is available to the solvent.



Supplemental Figure S5. Localization of P145 in relation to binding area in PsLYK9-ECD. P145 is situated at the LysM2 lower lateral loop under the binding site 2. However, it does not influence to the binding as it does not bind with CO5 or CO8-DA.



Supplemental Figure S6. Diagram of PsLYK9-ECD binding with CO8-DA. The binding is characterized by 8 H-bonds, 1 aromatic bond, 10 bad bonds. "Good" bonds are marked at the diagram: Ser46, Thr112, Asp114, Gly121, Ser122, Ala125, Asn126, Gly206. "Bad" bonds are not marked at the figure: Thr112, Asp114, Ser122, Asn123, Arg175. The complex is primarily formed with polar amino acids, with one negative charged amino acid and with glycines. The surface of the CO8-DA interacts with PsLYK9-ECD surface primarily in hydrophobic way. However, the ends of the molecule and some atoms along its "body" are available to the solvent.

Supplemental Table S1. Mutant lines found in the TILLING collection and carrying a mutation in the *PsLyk9* gene.

#	Mutation	Families	Nature	AA Change	gDNA position	SIFT prediction	SIFT score	SIFT median sequence conservation	Sequence represented at this position
1	C→T	3693	MISSENSE	S122F	443	AFFECTED	0,03	3,06	57
2	C→T	4249	MISSENSE	P145L	512	AFFECTED	0,01	3,02	60
3	C→T	4542	MISSENSE	A184V	629	AFFECTED	0	3,02	60
4	G→A	3562	MISSENSE	A184T	628	AFFECTED	0	3,02	60
5	C→T	3079	MISSENSE	L470F	4340	AFFECTED	0,02	3,01	64
6	G→A	3631	MISSENSE	G485R	4533	AFFECTED	0	3,01	63

RT-PCR primers:					
PE_For	AGCTAGATCTTCTCGCCGTG				
PE_Rev	Rev CCACTCCCATCTTTAGCCACTAC				
MCA2_For	TATTATCCGAGTGATGGCAA				
MCA2_Rev	CTGCAACATTGACTGTTAGT				
PLC_For	TCAACCTGCCATCAATGTTCTC				
PLC_Rev	TTCTTTTGGACCATATCATCTACTGTC				
DELLA3_For	GCAATATAGAATTAACCGCCACAAC				
DELLA3_Rev	CGGATGAGCGGGACAACC				
WRKY33_For	ACCCGAATTGTCCAACGAAGAA				
WRKY33_Rev	TTAGCAGGAACAATCACAAGAGCA				
WRKY35_For	GGGTTGTGAGTGTGAGGATTGG				
WRKY35_Rev	TGGATGGTTGTGCGTTGAAGTA				
PUB22_For	TGTCCGATCACAAAACAACAGC				
PUB22_Rev	GAAGCTTCTTTGATGAGTTTCGC				
Ub_For	ATGCAGATYTTTGTGAAGAC				
Ub_Rev	ACCACCACGRAGACGGAG				
Actin_For	CTCAGCACCTTCCAGCAGATGTG				
Actin_Rev	CTTCTTATCCATGGCAACATAGTTC				
Primers for protein exp	pression in insect cells				
PsLYK9-ECD_For	GGCTCGAGATGTGCACAAAAGGTTGTTCATTAGCC (XhoI)				
PsLYK9-ECD_Rev	GGAAGCTTCGCTAATGATGATGATGATGATGAGACCCCACCCGC				
	AAGACC (HindIII, stop and 6His tag)				
PsLYR4-ECD_For	GGGGATCCACACTACTCATCCTCATAATCTCCA (BamH1)				
PsLYR4-ECD_Rev	GGGAATTCTCAATGATGATGATGATGATGACCACCACCGCGG				
	GAGGAGTTTGC (EcoRI, stop and 6His tag)				
PsLYR3-ECD_For	GGGGATCCCTAGGACAACAACCTTACATT (BamH1)				
PsLYR3-ECD_Rev	GGGAATTCTCAATGATGATGATGATGATGATGTTGAATTTGAGAAC				
	TTTTAGGCTTATC (EcoRI, stop and 6His tag)				
Primers for protein exp	pression in <i>E. coli</i> cells				
PsLYK9-ECD_For	CCCTCGAGTGCACAAAAGGTTGTTCATTAGCC (Xhol)				
PsLYK9-ECD_Rev	CCAAGCTTCGCTAAGCCCCACCCGCAAGAC (HindIII)				
PsLYR4-ECD_For GGGGATCCGACACTAACCATGTTTTCTCTCATCT (BamHI)					
PsLYR4-ECD_Rev GGGAATTCTCAACCACCACCGCGGGAGGAGTTTGC (Ecc					
PsLYR3-ECD_For GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG					
<i>Ps</i> LYR3-ECD_Rev	GGGGGAATTCTCATTGAATTTGAGAACTTTTAGGCTTATC				
	(EcoRI)				

Supplemental Table S2. List of primers used in this study.

The sites' metrics						
Site	SiteScore	Volume	Dscore	Size		
1	1.088	288.806	1.009	115		
2	0.605	60.025	0.590	33		
3	0.631	63.112	0.511	30		
4	0.615	75.117	0.516	36		
5	0.588	135.485	0.527	23		

The best CO5 – PsLYK9-ECD poses according to XP GScore for each site					
Ligand	XP GScore	docking score	glide emodel		
Ligand at site 1	-10.214	-9.082	-57.424		
Ligand at site 2	-9.795	-8.663	-75.486		
Ligand at site 3	-9.802	-8.670	-59.802		
Ligand at site 4	-9.151	-8.019	-59.665		
Ligand at site 5	-11.274	-10.142	-77.548		

The best CO8-DA – PsLYK9-ECD poses according to XP GS core for each site

Ligand	XP GScore	docking score	glide emodel
Ligand at site 2	-12.611	-12.232	-90.371
Ligand at site 3	-11.060	-10.681	-44.636