

# IUCrJ

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**Supporting information for article:**

**Structural insights into the molecular mechanism of phytoplasma immunodominant membrane protein**

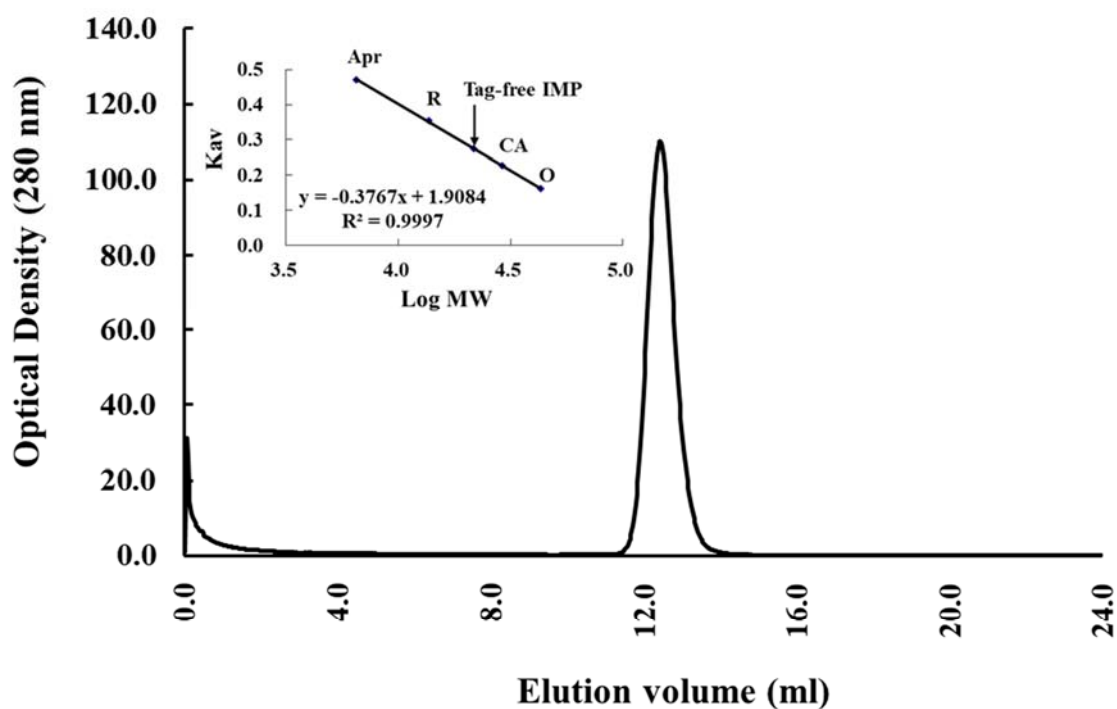
**Chang-Yi Liu, Han-Pin Cheng, Chan-Pin Lin, Yi-Ting Liao, Tzu-Ping Ko, Shin-Jen Lin, Shih-Shun Lin and Hao-Ching Wang**

**Table S1** The list for potential PHYL1-interacting proteins.

DDS ID	AGI	Protein name	Abbreviation	PubMed ID
<b>Photosynthesis</b>				
DDS57960	AT2G39730.1	Rubisco activase	RCA	25849391
DDS57462	ATCG00490.1	Ribulose-bisphosphate carboxylases	RBCL	26025532
DDS41686	ATCG00120.1	ATP synthase subunit alpha	ATPA	19697039
DDS44062	AT1G56190.1	Phosphoglycerate kinase family protein	cPGK2	24930633
DDS43812	ATMG01190.1	ATP synthase subunit 1	ATP1	25835989
DDS18960	AT1G12900.1	Glyceraldehyde 3-phosphate dehydrogenase subunit 2	A GAPA-2	17854848
DDS12824	AT5G14780.1	Formate dehydrogenase	FDH	28988354
DDS19554	AT5G13120.1	Cyclophilin 20-2	CYP20-2	22041789
DDS19944	AT5G66190.1	Ferredoxin-NADP(+)-oxidoreductase 1	FNR1	22300243
DDS59400	AT5G38410.3	Rubisco small subunit 3b	RBCS3B	22223809
DDS57901	AT1G06680.1	Photosystem II subunit P-1	PSBP-1	17604269
<b>Defense response</b>				
DDS18837	AT3G12500.1	Basic chitinase	B-CHI	DDS18837
DDS14248	AT1G20510.1	OPC-8:0 CoA ligase1	OPCL1	DDS14248
DDS23169	AT1G70840.1	MLP-like protein 31	MLP31	DDS23169
DDS58113	AT3G14420.1	Glycolate oxidase 1	GOX1	DDS58113
DDS20437	AT4G37990.1	Elicitor-activated gene 3	ELI3.1	DDS20437
DDS45044	AT4G37990.1	Elicitor-activated gene 3	ELI3.2	DDS45044
DDS57458	AT2G38870.1	PR-6 proteinase inhibitor family	PR-6 PI	DDS57458
DDS30631	AT1G70840.1	MLP-like protein 31	MLP31	DDS30631
DDS11033	AT2G23620.1	Methyl esterase 1	MES1	DDS11033
DDS47142	AT5G61410.1	D-ribulose-5-phosphate-3-epimerase	RPE	DDS47142
DDS59062	AT4G37980.1	Cinnamaldehyde and hexenal reductase	CHR	DDS59062
DDS58088	AT1G74020.1	Strictosidine synthase	STR	DDS58088
<b>Cytoskeleton</b>				
DDS53318	AT5G09810.1	Actin 7	ACT7	29531328
DDS19330	AT5G19780.1	Tubulin alpha-5	TUA5	1498608
DDS52148	AT1G50010.1	Tubulin alpha-2 chain	TUA2	30833711
DDS18929	AT4G38510.5	V-ATPase B subunit 2	VAB2	22371505
<b>Cellular respiration</b>				
DDS48404	AT1G59900.1	Pyruvate dehydrogenase complex E1 alpha	E1 alpha	7590338
DDS57166	AT2G36530.1	Enolase	ENO2	25620024
DDS50893	AT3G13930.1	Mitochondrial pyruvate dehydrogenase subunit 2-2	MTE2-2	26194327
DDS11268	AT2G44350.2	Citrate synthase 4	CS4	22115780
DDS52367	AT1G42970.1	Glyceraldehyde-3-phosphate dehydrogenase subunit	B GAPB	11442054

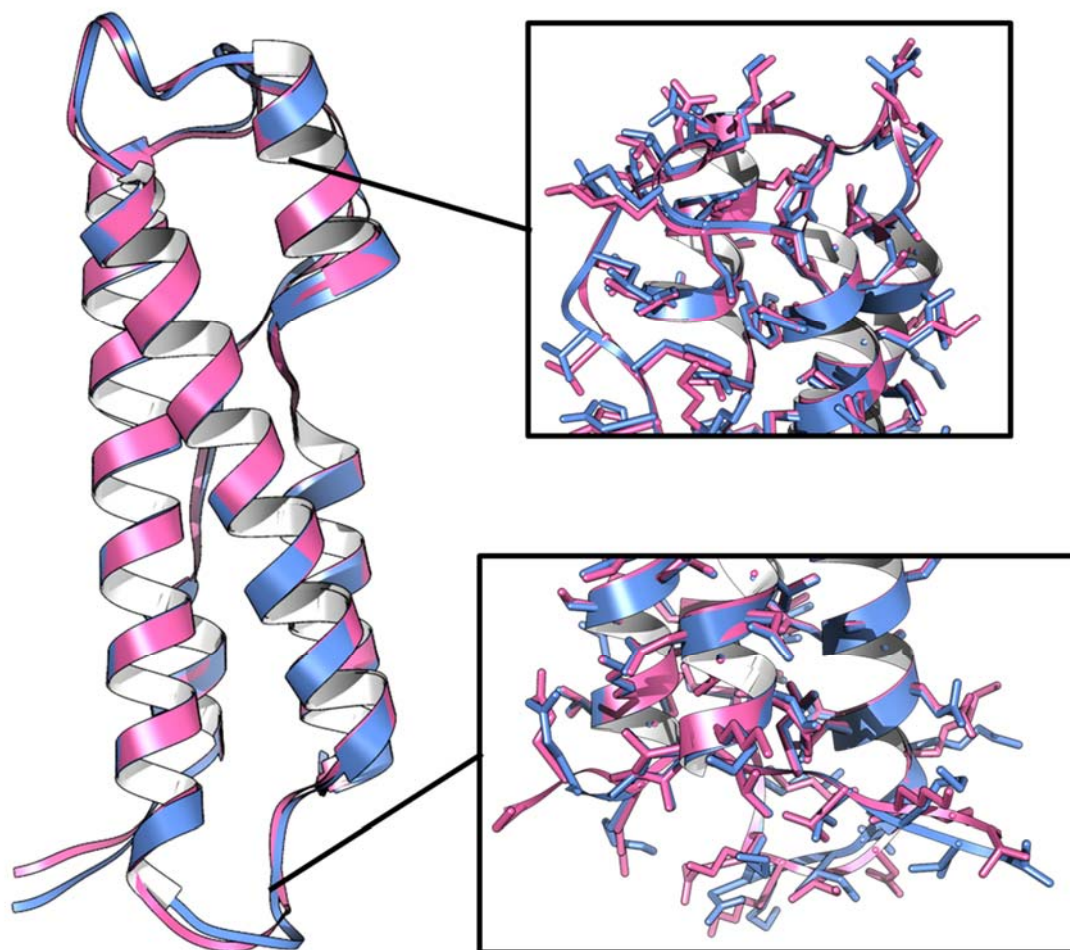
<b>Intracellular protein transport</b>				
DDS19123	AT4G02080.1	Secretion-associated RAS super family 2	SAR2	12242382
DDS51812	AT5G55190.1	Ran GTPase 3	RAN3	17530257
DDS19827	AT1G45000.1	AAA-type ATPase family protein	AAA-type ATPase	20118269
<b>Cell division</b>				
DDS51921	AT5G20720.1	Chaperonin 20	CPN20	21633907
DDS13025	AT5G03340.1	Cell division cycle 48C	CDC48C	12427991
<b>Amino acid metabolism</b>				
DDS11601	AT3G19480.1	Phosphoglycerate dehydrogenase 3	PGDH3	24058165
DDS11169	AT5G35630.1	Glutamine synthetase 2	GLN2	30368031
DDS44040	AT4G33010.1	Glycine decarboxylase P-protein 1	GLDP1	26603271
DDS19141	AT4G13930.1	Serine hydroxymethyltransferase 4	SHM4	16951055
DDS53279	AT4G14880.1	O-acetylserine (thiol) lyase isoform A1	OAS-TLA1	20429919
<b>Fatty acid oxidation</b>				
DDS19389	AT2G33150.1	Peroxisomal 3-ketoacyl-CoA thiolase 3	PKT3	20463027
<b>ROS-scavenging</b>				
DDS21519	AT4G09010.1	Ascorbate peroxidase 4	APX4	24407512
DDS58222	AT5G06290.1	2-cysteine peroxiredoxin B	2-Cys PrxB	29128357
DDS57429	AT2G28190.1	Copper/Zinc superoxide dismutase 2	CSD2	31093688
<b>Growth and development</b>				
DDS14940	AT1G47480.1	alpha/beta-Hydrolases superfamily protein		17597079
DDS18899	AT3G04400.1	Embryo defective 2171	EMB2171	27247031
DDS12009	AT4G26300.1	Embryo defective 1027	EMB1027	21139083
DDS53303	AT2G18420.1	Gibberellin-regulated GASA/GAST/Snakin family protein	Snakin/GASA	22836500
<b>Proton pump</b>				
DDS58014	AT3G01390.1	Vacuolar membrane ATPase 10	VMA10	11004467
DDS18932	AT1G78900.1	Vacuolar ATP synthase subunit A	VHA-A	22943363
<b>Protein quality control</b>				
DDS13016	AT1G02560.1	Nuclear encoded CLP protease 5	NCLPP5	26085677
DDS18881	AT1G09130.3	ATP-dependent caseinolytic protease/crotonase family protein	CLP	18230142
DDS51845	AT4G24280.1	Chloroplast heat shock protein 70-1	CPHSC70-1	20193958
DDS51861	AT5G56030.2	Heat shock protein 81-2	HSP81-2	30194279
DDS51072	AT3G23990.1	Heat shock protein 60 (HSP60)	HSP60	20018591
DDS18094	AT3G05530.1	Regulatory particle triple-A ATPase 5A	RPT5A	31156663
DDS42413	AT5G58290.1	Regulatory particle triple-A ATPase 3	RPT3	20516081
<b>Carbohydrate metabolism</b>				
DDS46369	AT5G51970.1	Sorbitol dehydrogenase	SDH	25755662
DDS57950	AT3G57520.1	Seed imbibition 2.1	SIP2.1	20739305
DDS53269	AT3G57520.1	Seed imbibition 2.2	SIP2.2	20739305
<b>DNA repair</b>				

DDS52608	AT1G20340.1	DNA-damage-repair/toleration (DRT112)	protein	112	DRT112	19825610
<b>Phytoplasma protein</b>						
	AGT17678.1	immunodominant membrane protein			IMP	9611800
<b>Purine and pyrimidine metabolism</b>						
DDS59111	AT4G09320.1	Nucleoside diphosphate kinase 1			NDPK1	28586477
DDS57175	AT4G12440.2	Adenine phosphoribosyl transferase 4			APT4	12010467
<b>Detoxification</b>						
DDS19410	AT3G48000.1	Aldehyde dehydrogenase 2B4			ALDH2B4	19320993
<b>Lignin biosynthesis</b>						
DDS20250	AT4G39330.1	Cinnamyl alcohol dehydrogenase 9 (CAD9)			CAD9	20829305
<b>Energy</b>						
DDS42971	AT2G35660.1	FAD/NAD(P)-binding oxidoreductase family protein				14576160
DDS51803	AT3G53620.1	Pyrophosphorylase 4			PPA4	22566496

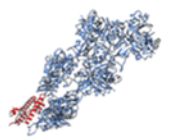
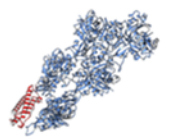
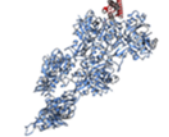
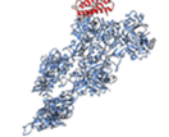
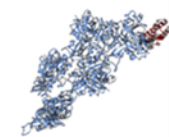

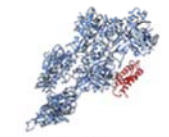

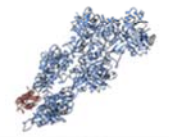
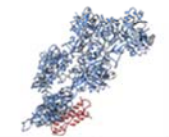


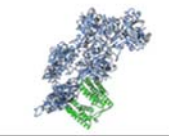
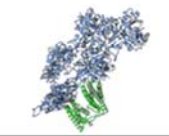
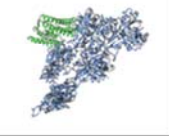

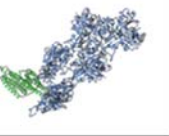
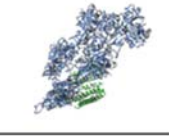
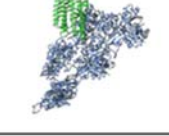
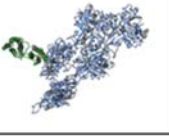
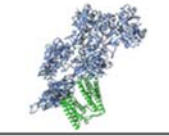
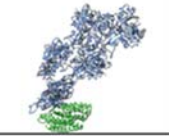
	Tag-free IMP
Theoretical MW	16.04 kDa
Calculated MW	21.66 kDa





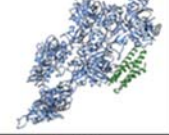


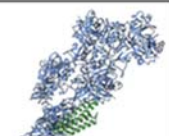
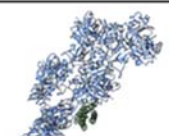

**Figure S1** Gel filtration analysis with Superdex 75 column. The standard curve for size exclusion chromatography was drawn by measuring standard proteins: Aprotinin (APR, 6.5 kDa), Ribonuclease A (R, 13.7 kDa), Carbonic anhydrase (CA, 29 kDa) and Ovalbumin (O, 43 kDa).



**Figure S2** Comparison of IMP structures from AlphaFold prediction (pink) and X-ray analysis (light blue).

IMP_A_with_Actin_Zea mays pollen_top10_models					
	Rank 1	Rank 2	Rank 3	Rank 4	Rank 5
					
Docking Score	-186.34	-170.51	-163.56	-160.14	-159.19
Confidence Score	0.6741	0.6011	0.5674	0.5505	0.5458
Ligand rmsd (Å)	63.83	62.54	87.75	80.60	103.87
	Rank 6	Rank 7	Rank 8	Rank 9	Rank 10
					
Docking Score	-157.98	-157.04	-155.95	-152.00	-151.87
Confidence Score	0.5398	0.5351	0.5297	0.5100	0.5093
Ligand rmsd (Å)	66.32	62.73	50.68	64.69	53.87

TLNRD1_A_with_Actin_Zea mays pollen_top10_models					
	Rank 1	Rank 2	Rank 3	Rank 4	Rank 5
					
Docking Score	-262.13	-247.16	-239.56	-237.05	-235.45
Confidence Score	0.9040	0.8747	0.8571	0.8508	0.8467
Ligand rmsd (Å)	101.12	102.03	44.82	117.01	53.42
	Rank 6	Rank 7	Rank 8	Rank 9	Rank 10
					
Docking Score	-233.88	-233.27	-233.07	-231.35	-230.57
Confidence Score	0.8426	0.8410	0.8404	0.8358	0.8336
Ligand rmsd (Å)	76.33	78.40	43.06	103.47	96.45

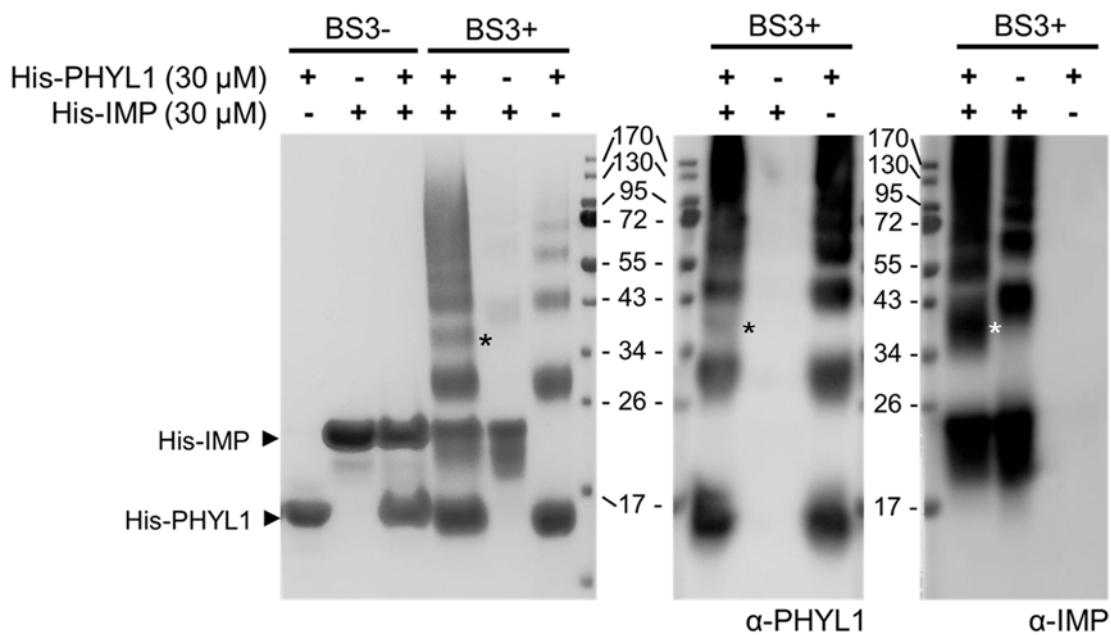
TLNRD1_4H_A_with_Actin_Zea mays pollen_top10_models					
	Rank 1	Rank 2	Rank 3	Rank 4	Rank 5
					
Docking Score	-168.27	-168.13	-166.98	-163.89	-163.54
Confidence Score	0.5903	0.5897	0.5841	0.5690	0.5673
Ligand rmsd (Å)	101.39	103.91	103.92	55.88	93.85
	Rank 6	Rank 7	Rank 8	Rank 9	Rank 10
					
Docking Score	-161.81	-159.52	-159.34	-157.16	-153.63
Confidence Score	0.5588	0.5475	0.5466	0.5357	0.5181
Ligand rmsd (Å)	58.69	104.87	129.43	101.75	75.35

\*The models are ranked according to the docking scores.

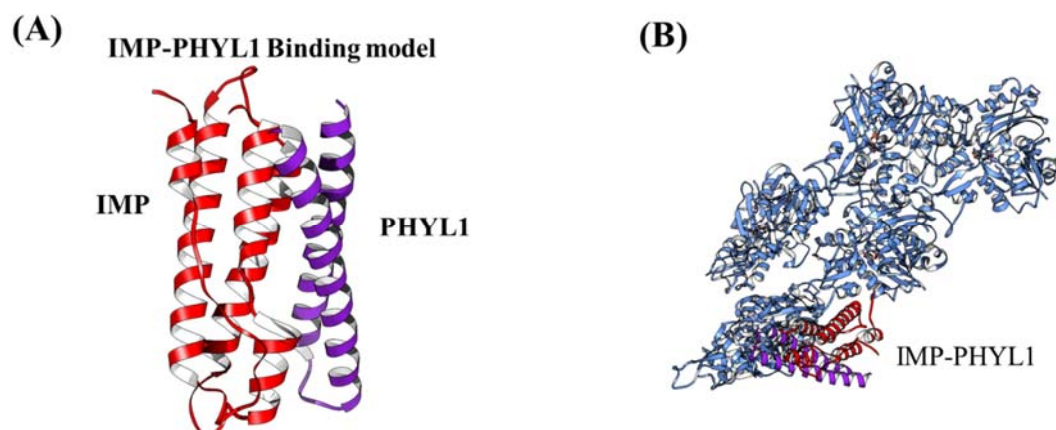
\*The ligand RMSDs from the input structures or modeled structures by homology modeling.

**Figure S3** Top 10 solutions of Hdock server docking results for F-Actin filaments and IMP/TLNRD1/TLNRD1\_4H. The top 10 binding models were proposed for IMP (red)/Actin, TLNRD1 (light green)/Actin and TLNRD1\_4H (dark green)/Actin by HDOCK server, each with Docking Score and Confidence Score indicated.





**Figure S4** Cross-linking analysis and western blot assay to confirm the binding of IMP and PHYL1. Cross-linking analysis of PHYL1 and IMP. Shifting band due to protein interaction was marked with an asterisk. Both anti-PHYL1 and anti-IMP antibodies were diluted 1:5000 for detection.



**Figure S5** Structural analysis modelling of IMP, PHYL1 and F-Actin. (A) The proposed IMP/PHYL1 binding model by HDOCK server. Docking Score, -199.51; Confidence Score, 0.729. (B) The proposed IMP/PHYL1-Actin binding model by HDOCK server with Docking Score, -198.65 and Confidence Score, 0.726. The F-Actin model is coloured in blue, the IMP model is coloured in red while the PHYL1 model is coloured in purple.