

**Wheat powdery mildew resistance gene *Pm13* from *Aegilops longissima*
encodes a unique mixed lineage kinase domain-like protein**

Li et al.

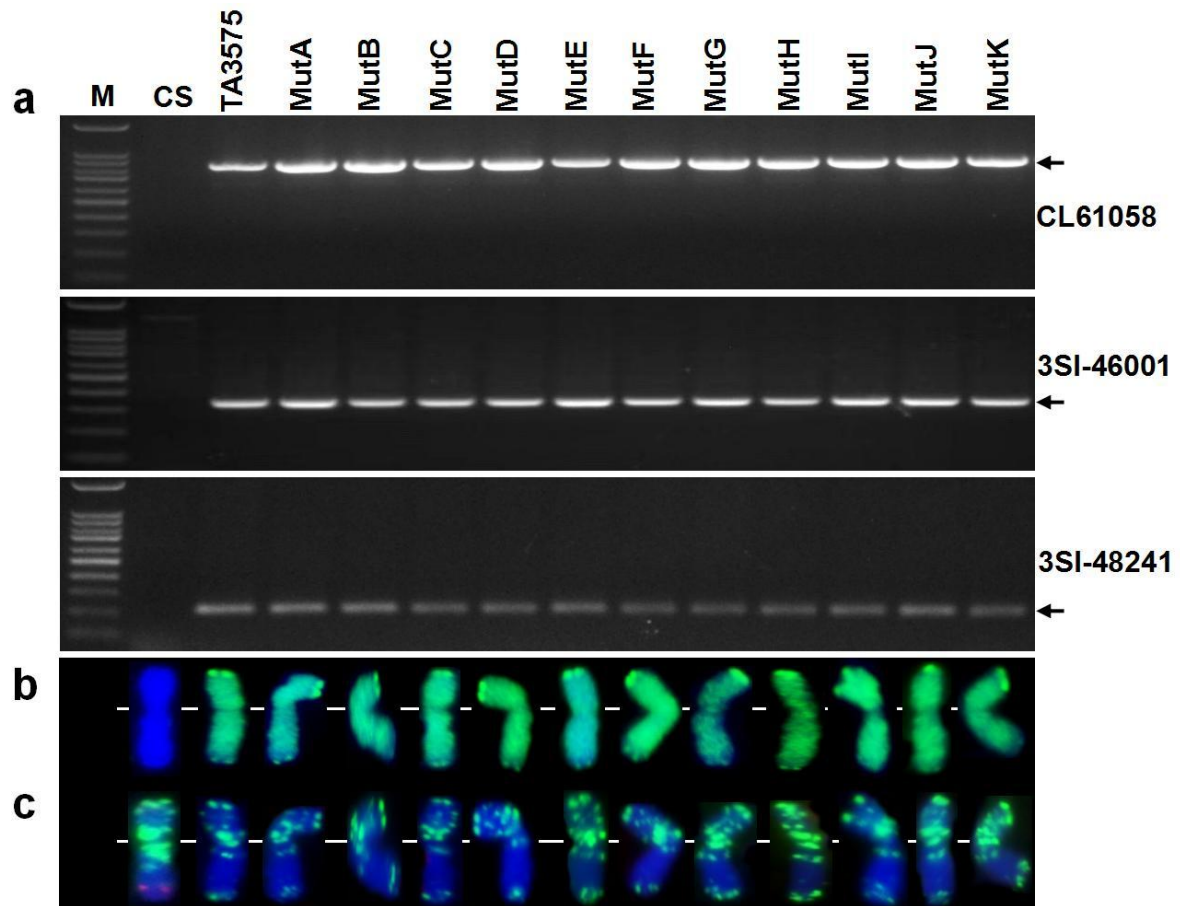
Supplementary Figs. 1-10



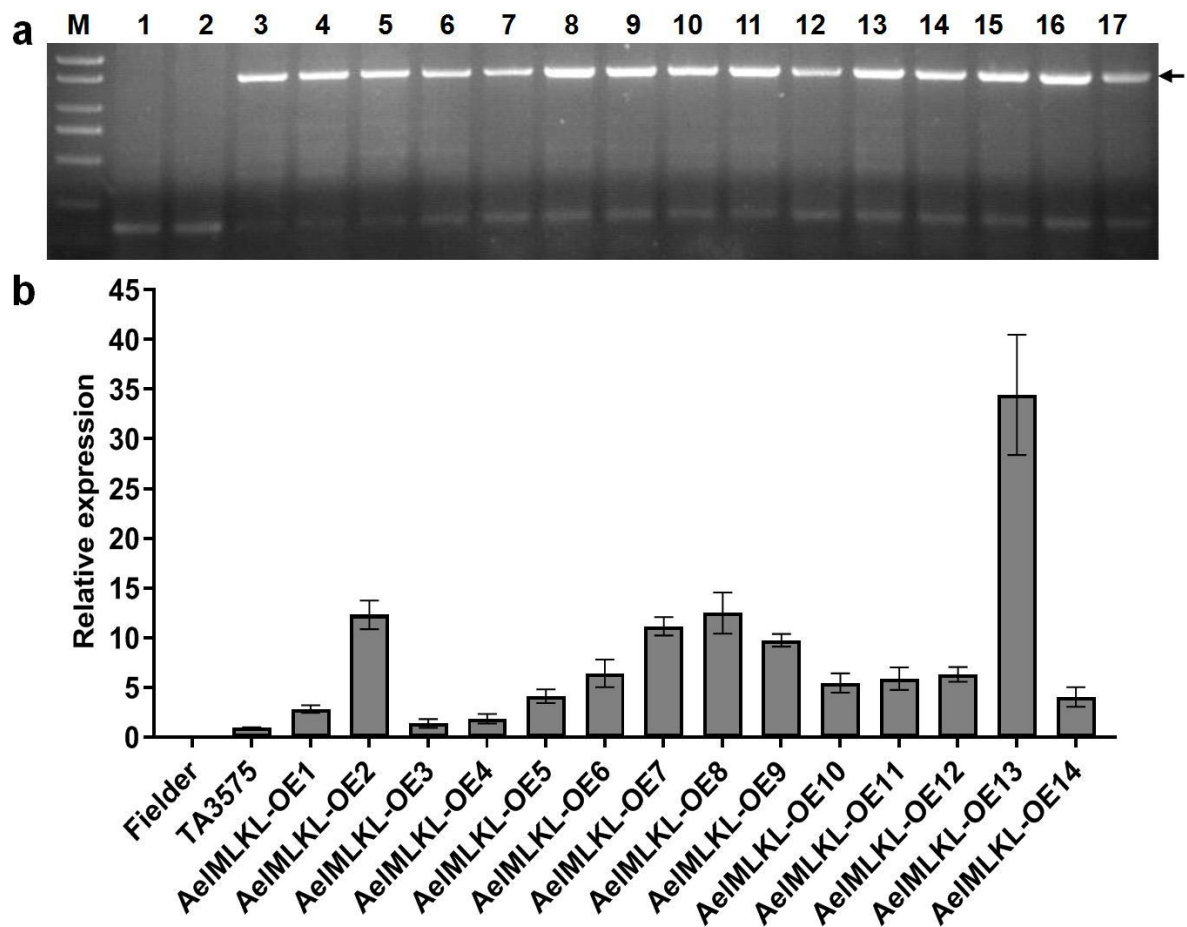
Supplementary Fig. 1 Initial mapping of *Pm13a*. A-V: Twenty-two different types of CS-*Ae. longissima* 3S^l#2 recombinants. R1B: CS-*Ae. longissima* T3S^l#1S-3BS.3BL recombinant. R2B: CS-*Ae. longissima* T3S^l#1S-3DS.3DL recombinant. R indicated resistance to powdery mildew, S indicated susceptible to powdery mildew.

Marker	Phys. pos.	Different types of 3S ^l #2 recombinants																		
		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s
CL10208	880550																			
3SI-32271	1936335																			
3SI-35091	2106061																			
3SI-39481	2369549																			
3SI-39601	2377548																			
CL22345/ <i>Msp</i> I	2444869																			
CL87265	2542972																			
3SI-44061	2644005																			
CL61058	2680166																			
3SI-44661	2680772																			
CL897	no exact hit																			
<i>Pm13a</i>	no exact hit	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S
3SI-46001	2760331																			
3SI-28A	2783963																			
3SI-29B	2795802																			
3SI-31B	2805206																			
3SI-39A	2826014																			
3SI-41A	2831767																			
3SI-48241	2894867																			
3SI-53031	3182533																			
CL51739	3739743																			
CL9359	4258118																			
CL3352	4483400																			
CL56281	4555120																			

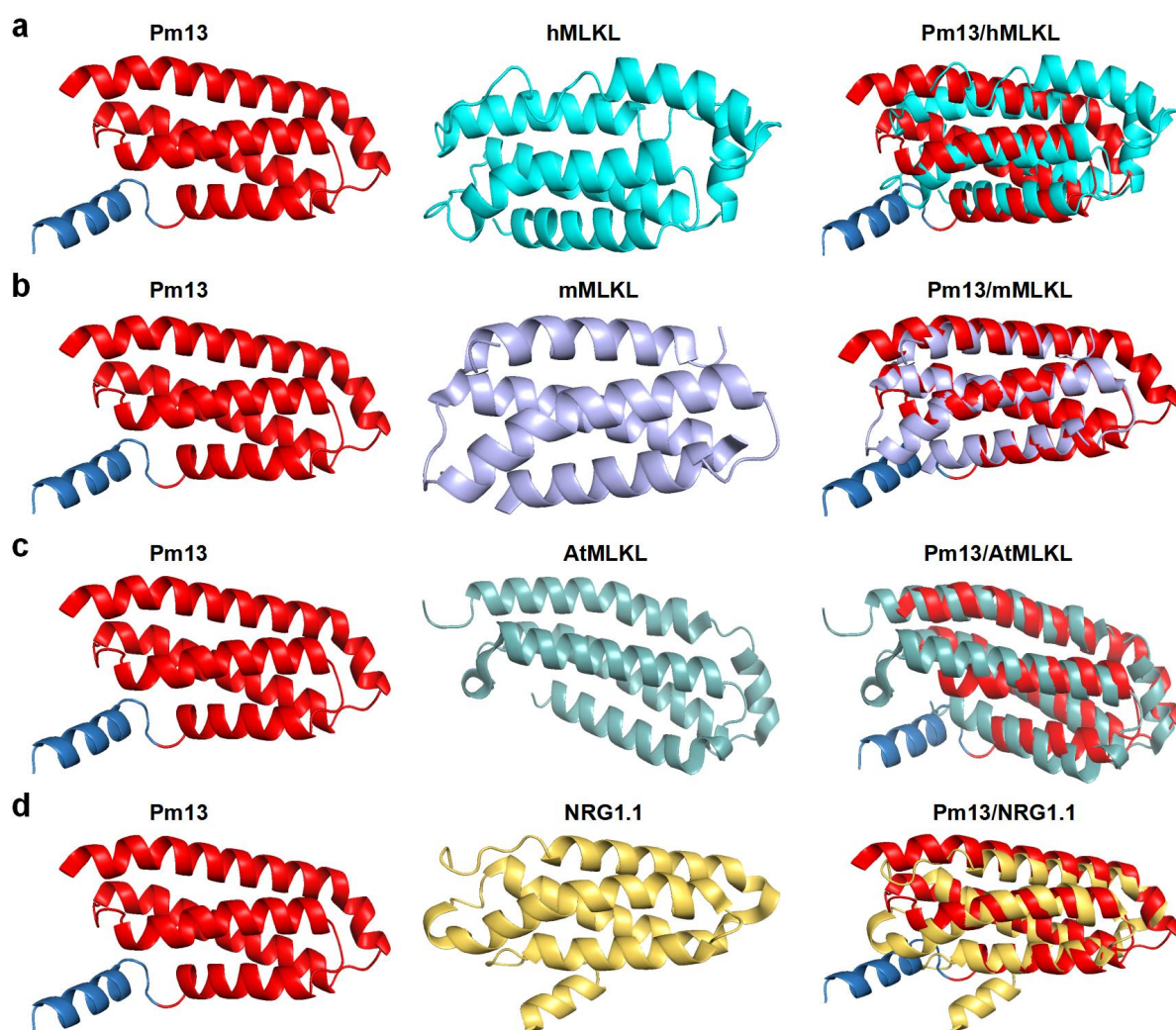
Supplementary Fig. 2 High-resolution mapping of *Pm13a*. a-s: Nineteen different types of CS-*Ae. longissima* 3S^l#2 recombinants. Blue box indicated the presence of *Ae. longissima* 3S^l#2-specific molecular markers, while gray box indicated the absence of *Ae. longissima* 3S^l#2-specific molecular marker. R: resistance to powdery mildew, S: susceptible to powdery mildew.



Supplementary Fig. 3 3S[#]2-specific molecular markers, GISH and FISH validation of 11 TA3575 EMS susceptible mutants. **a** Molecular markers analysis of 11 TA3575 EMS susceptible mutants. M, 100 bp Ladder DNA marker. Arrows pointed to the polymorphic bands of the 3S[#]2-specific molecular markers present in TA3575 and 11 mutants and absent in CS. **b** GISH patterns of 11 TA3575 EMS susceptible mutants. Total gDNA of *Ae. longissima* was labeled with fluorescein-12-dUTP and visualized with green fluorescence. Wheat chromatin was counterstained with 4',6-diamidino-2-phenylindole (DAPI) and visualized with blue fluorescence. **c** FISH patterns of 11 TA3575 EMS susceptible mutants. FAM-modified oligonucleotides (pSc119.2-1 and (GAA)₁₀) were in green color. TAMRA-modified oligonucleotides (pAs1-1, pAs1-3, pAs1-4, pAs1-6, AFA-3 and AFA-4) were in red color. Wheat chromatin was counterstained with DAPI and visualized with blue fluorescence.

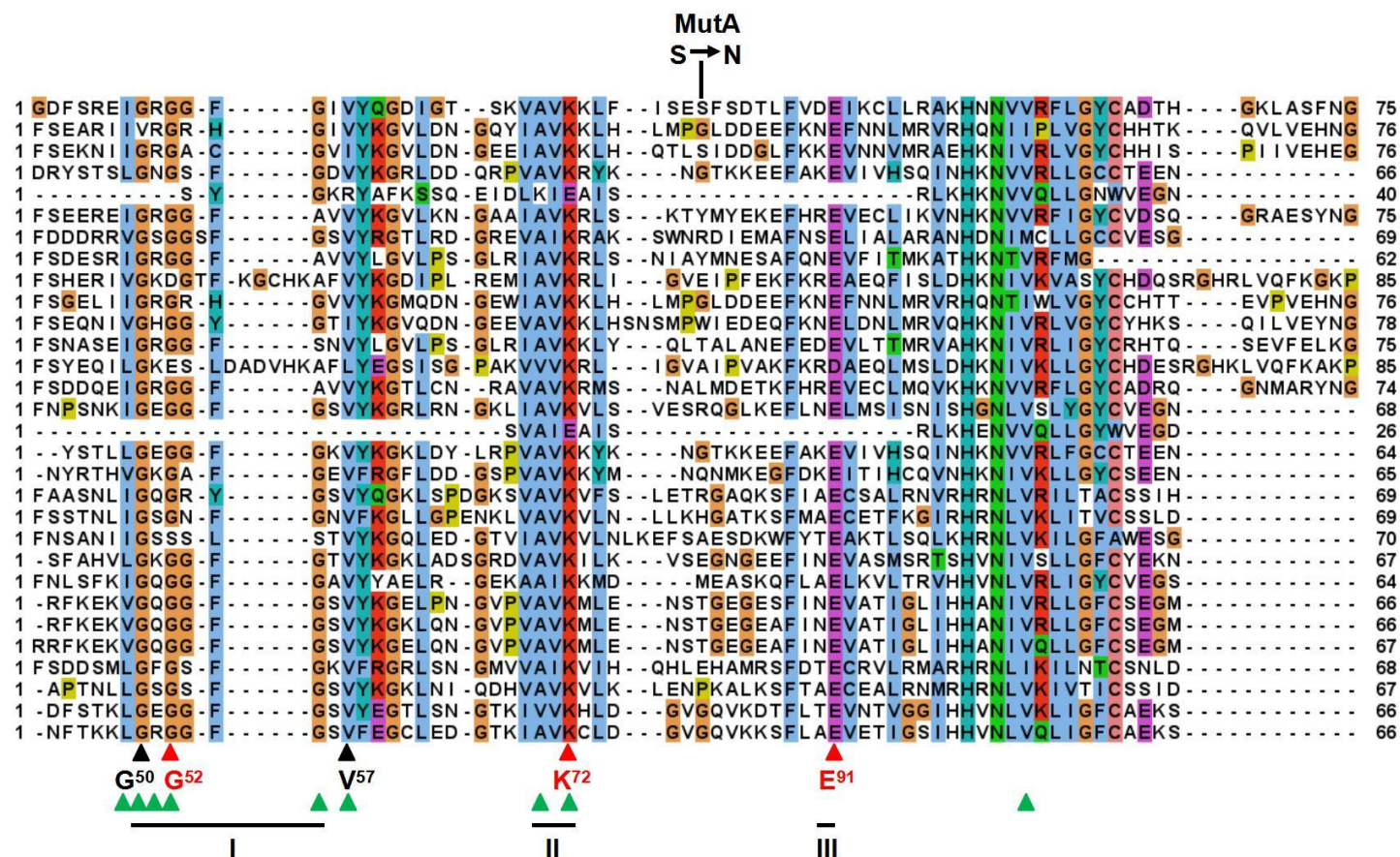


Supplementary Fig. 4 Molecular detection of T₀ and T₁ transgenic plants with *AelMLKL* gene mediated by *Agrobacterium tumefaciens*. **a** Detection of putative T₀ transgenic plants by PCR amplification of *AelMLKL* gene using primer *AelMLKL_OE*. M, DL2000 Plus DNA marker; 1, common wheat CS; 2, common wheat cultivar Fielder; 3, positive control of expression vector pWMB110 containing the full sequence of *AelMLKL*; 4-17, 14 individual T₀ transgenic plants. Arrows pointed to the polymorphic bands of molecular marker *AelMLKL_OE*. **b** The transcriptional levels of *AelMLKL* gene in T₁ transgenic plants derived from selfing of different T₀ individuals were examined before inoculation at the seedling stage. Fielder was used as negative control. TA3575 was used as positive control. Error bars represent the means $\pm$ SD from three biological replicates.

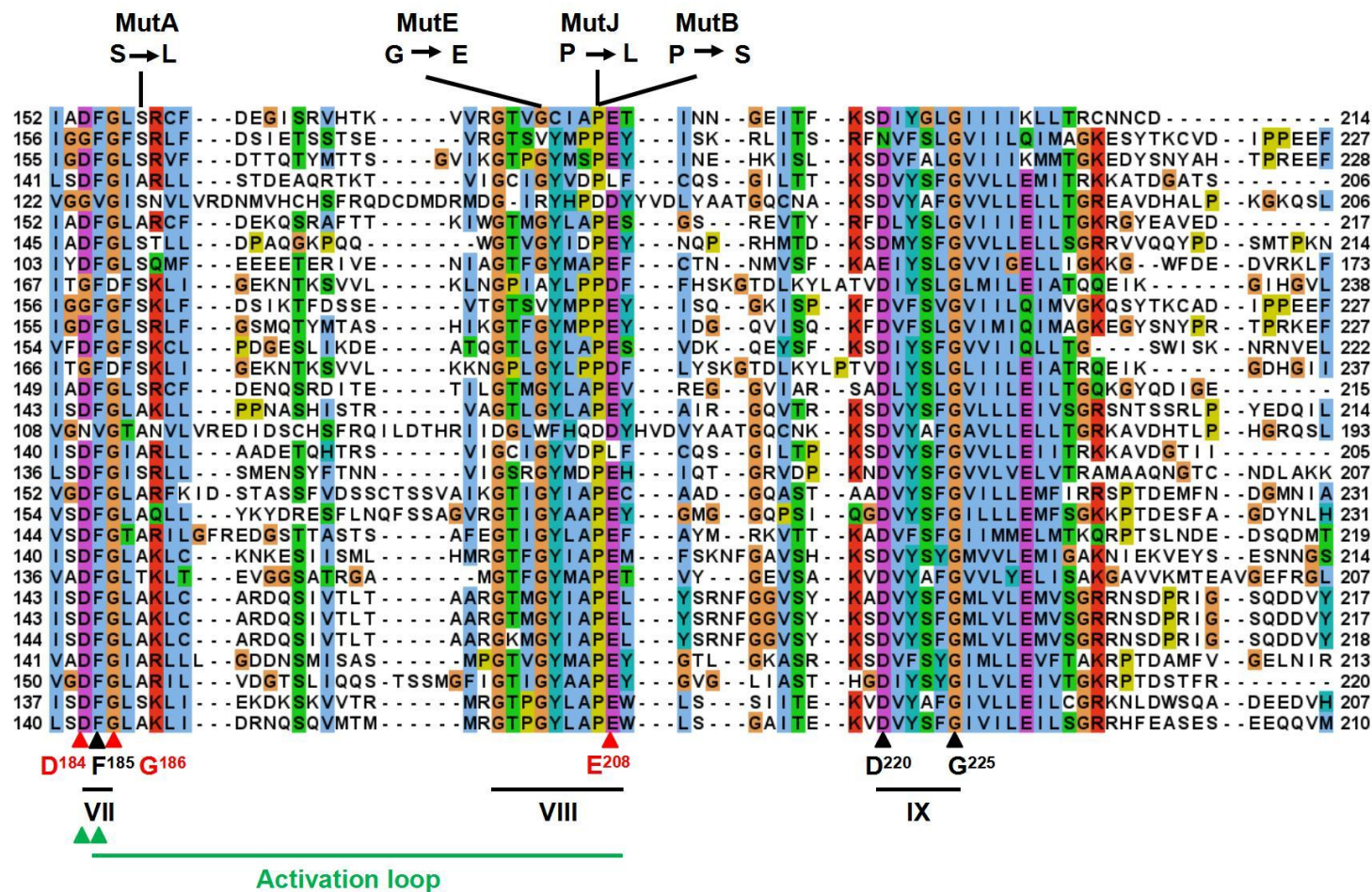


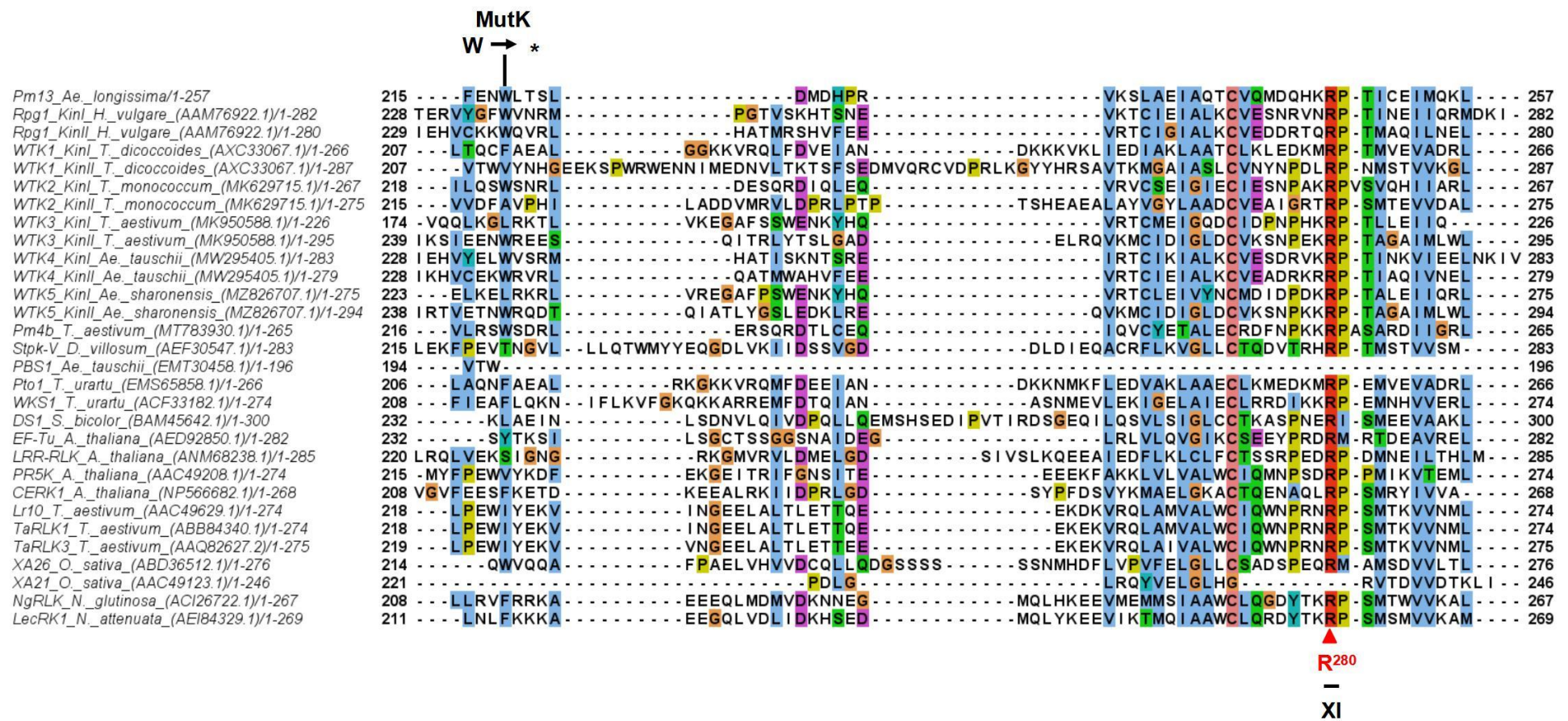
Supplementary Fig. 5 Pm13 HeLo domain superimposed well with HeLo domain of human MLKL (hMLKL), mouse MLKL (mMLKL) and *Arabidopsis thaliana* MLKL (AtMLKL), and CC_R domain of *Nicotiana benthamiana* NRG1.1. **a** Superimposition of the Pm13 HeLo domain (red) with hMLKL HeLo domain (cyan, PDB ID code 2MSV). **b** Structural overlay of the Pm13 HeLo domain (red) with mMLKL HeLo domain (lightblue, PDB ID code 4BTF). **c** Superposition of the Pm13 HeLo domain (red) and AtMLKL HeLo domain (lightteal, PDB ID code 6KA4). **d** Superposition of the Pm13 HeLo domain (red) and NRG1.1 CC_R domain (paleyellow, PDB ID code 7L7V).

Pm13_Ae_longissima/1-257
Rpg1_KinI_H_vulgare (AAM76922.1)/1-282
Rpg1_KinII_H_vulgare (AAM76922.1)/1-280
WTK1_KinI_T_dicoccoides (AXC33067.1)/1-266
WTK1_KinII_T_dicoccoides (AXC33067.1)/1-287
WTK2_KinI_T_monococcum (MK629715.1)/1-267
WTK2_KinII_T_monococcum (MK629715.1)/1-275
WTK3_KinI_T_aestivum (MK950588.1)/1-226
WTK3_KinII_T_aestivum (MK950588.1)/1-295
WTK4_KinI_Ae_tauschii (MW295405.1)/1-283
WTK4_KinII_Ae_tauschii (MW295405.1)/1-279
WTK5_KinI_Ae_sharonensis (MZ826707.1)/1-275
WTK5_KinII_Ae_sharonensis (MZ826707.1)/1-294
Pm4b_T_aestivum (MT783930.1)/1-265
Stpk-V_D_villosum (AEF30547.1)/1-283
PBS1_Ae_tauschii (EMT30458.1)/1-196
Pto1_T_urartu (EMS65858.1)/1-266
WKS1_T_urartu (ACF33182.1)/1-274
DS1_S_bicolor (BAM45642.1)/1-300
EF-Tu_A_thaliana (AED92850.1)/1-282
LRR-RLK_A_thaliana (ANM68238.1)/1-285
PR5K_A_thaliana (AAC49208.1)/1-274
CERK1_A_thaliana (NP566682.1)/1-268
Lr10_T_aestivum (AAC49629.1)/1-274
TaRLK1_T_aestivum (ABB84340.1)/1-274
TaRLK3_T_aestivum (AAQ82627.2)/1-275
XA26_O_sativa (ABD36512.1)/1-276
XA21_O_sativa (AAC49123.1)/1-246
NgRLK_N_glutinosa (ACI26722.1)/1-267
LecRK1_N_attenuata (AEI84329.1)/1-269



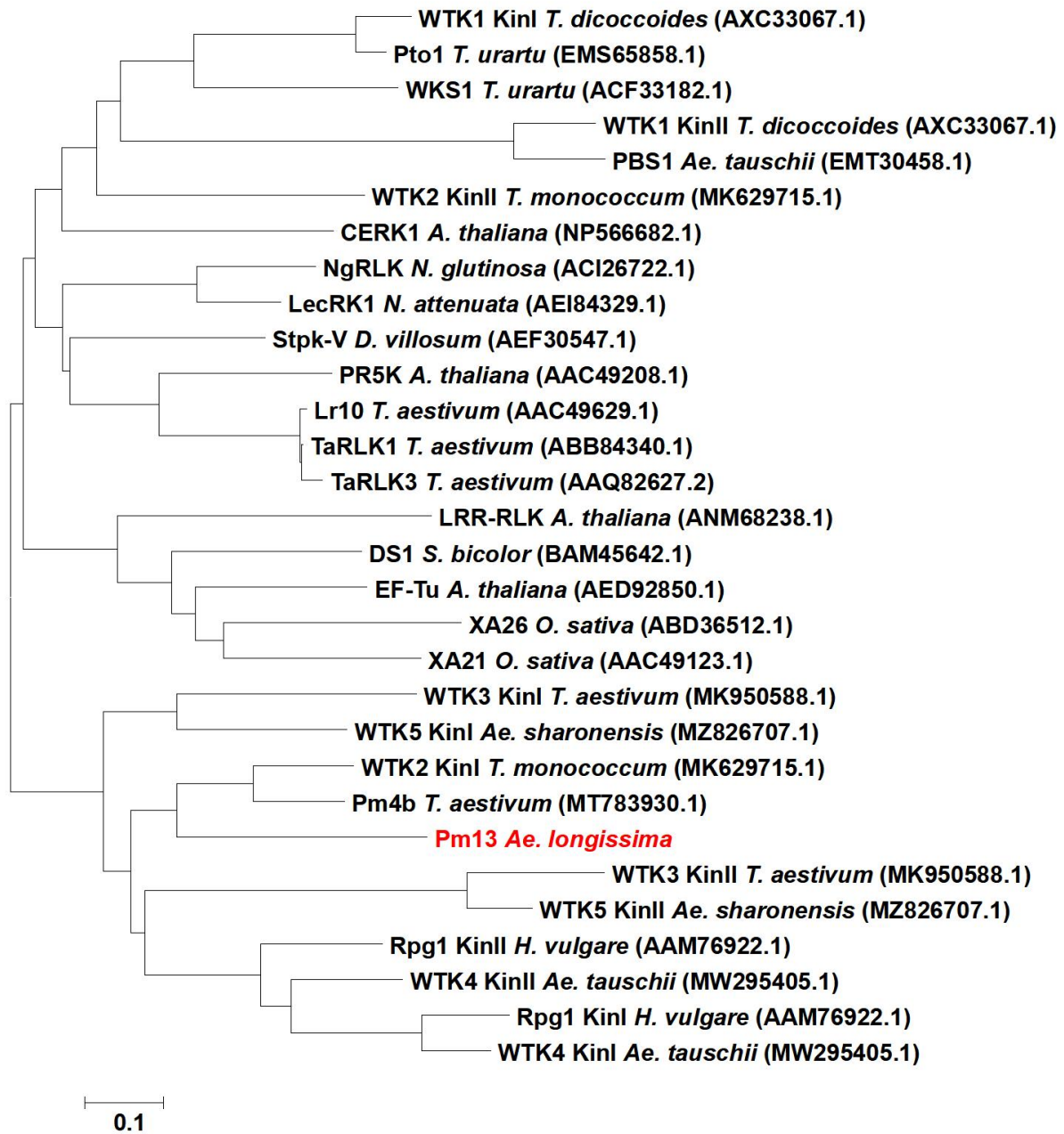
Pm13_Ae_longissima/1-257
Rpg1_KinI_H_vulgare (AAM76922.1)/1-282
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WTK1_KinI_T_dicoccoides (AXC33067.1)/1-287
WTK2_KinI_T_monococcum (MK629715.1)/1-267
WTK2_KinI_T_monococcum (MK629715.1)/1-275
WTK3_KinI_T_aestivum (MK950588.1)/1-226
WTK3_KinI_T_aestivum (MK950588.1)/1-295
WTK4_KinI_Ae_tauschii (MW295405.1)/1-283
WTK4_KinI_Ae_tauschii (MW295405.1)/1-279
WTK5_KinI_Ae_sharonensis (MZ826707.1)/1-275
WTK5_KinI_Ae_sharonensis (MZ826707.1)/1-294
Pm4b_T_aestivum (MT783930.1)/1-265
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XA26_O_sativa (ABD36512.1)/1-276
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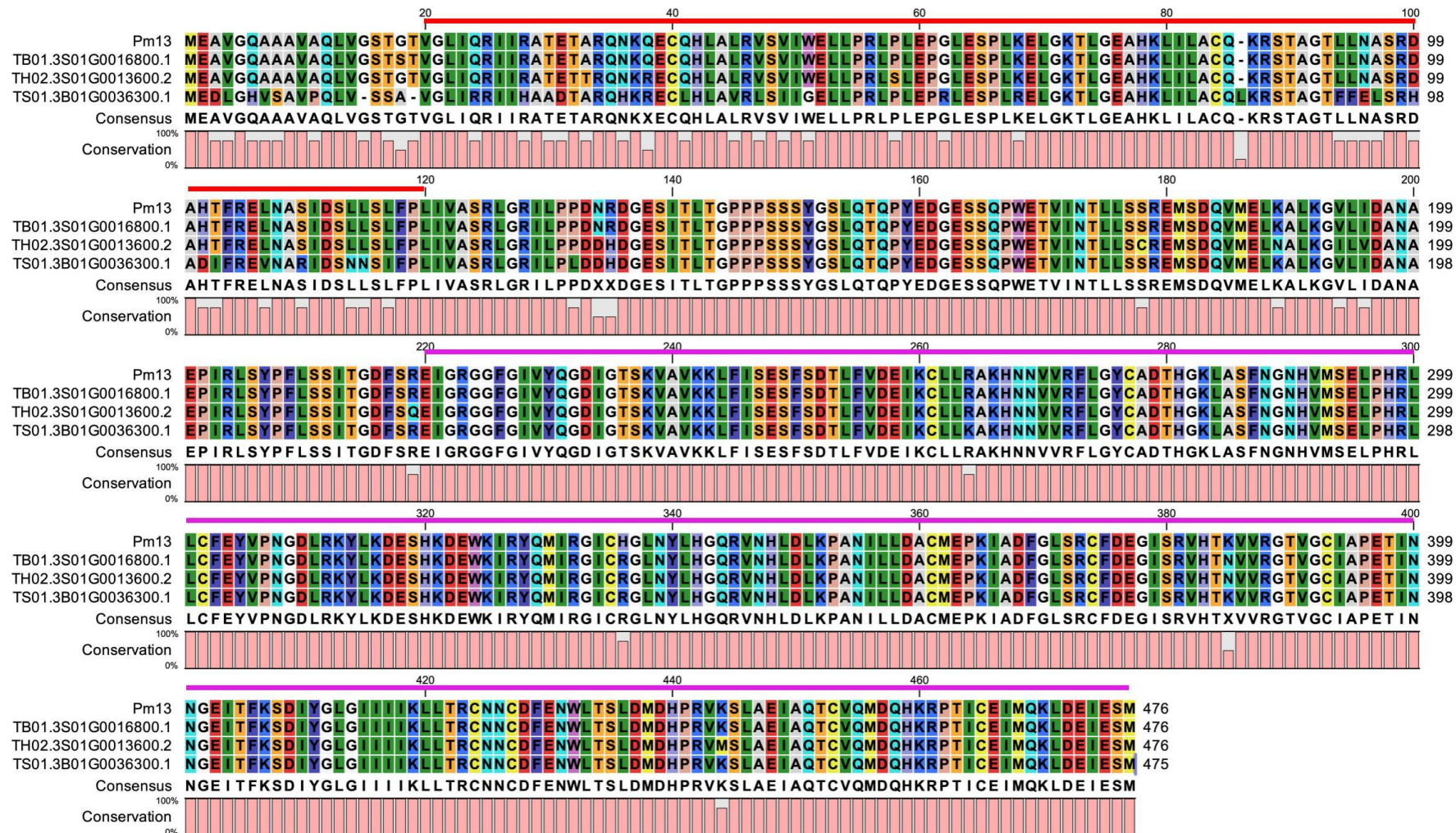


Supplementary Fig. 6 Kinase domain comparison analysis of Pm13. A multiple amino acid sequence alignment of 29 protein kinase catalytic domains involved in disease resistance was used to infer the Pm13 kinase domain architecture. In Pm13 (row 1) all the 14 key conserved residues of a protein kinase are present. In the alignment, red triangles indicated invariant residues (G⁵², K⁷², E⁹¹, D¹⁶⁶, N¹⁷¹, D¹⁸⁴, G¹⁸⁶, E²⁰⁸, R²⁸⁰), which were numbered with upper case numbers representing their position in the α form of the

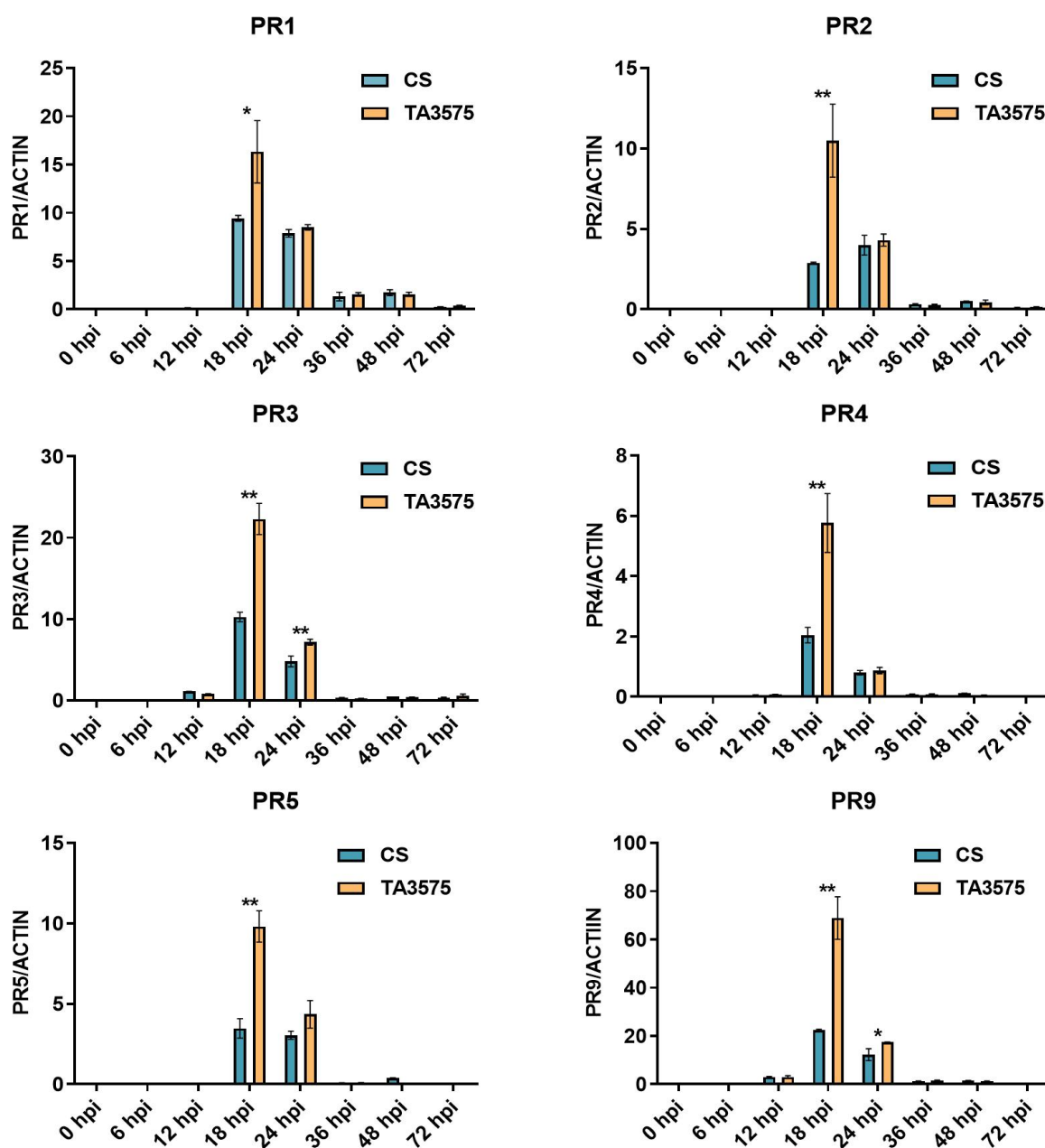
cAMP-dependent protein kinase catalytic subunit (cAPK). Likewise, black triangles indicate the nearly invariant residues (G⁵⁰, V⁵⁷, F¹⁸⁵, D²²⁰, G²²⁵). Based on the presence of a L residue at position R¹⁶⁵ of cAPK in subdomain VI, Pm13 Kinase was classified as a non-RD kinase. Moreover, conserved residues in subdomain VIb (D166 -> N171, DLKPAN in Pm13 vs. DLPKPEN in cAPK) and VIII (GTVGCIAP in Pm13 vs. GT/SXXY/FXAPE in cAPK) indicate that the Pm13 kinase domain is a serine/threonine protein kinase. Labels: red and black triangles, key invariant and mostly invariant residues in the protein kinase catalytic domains, respectively. Blue diamond pointed to the residue determining RD or non-RD kinases. Black asterisks, putative substrate binding site. Green triangles, ATP binding site. Conserved subdomains I, II, III, VIb, VII, VIII, IX and XI are highlighted by black bars labeled with Roman numerals. The catalytic loop and the activation loop are highlighted by purple and green bars. On top of the wrapped alignment, EMS missense and nonsense mutations resulted in loss of Pm13 function and corresponding amino acid changes are indicated by black thin line.



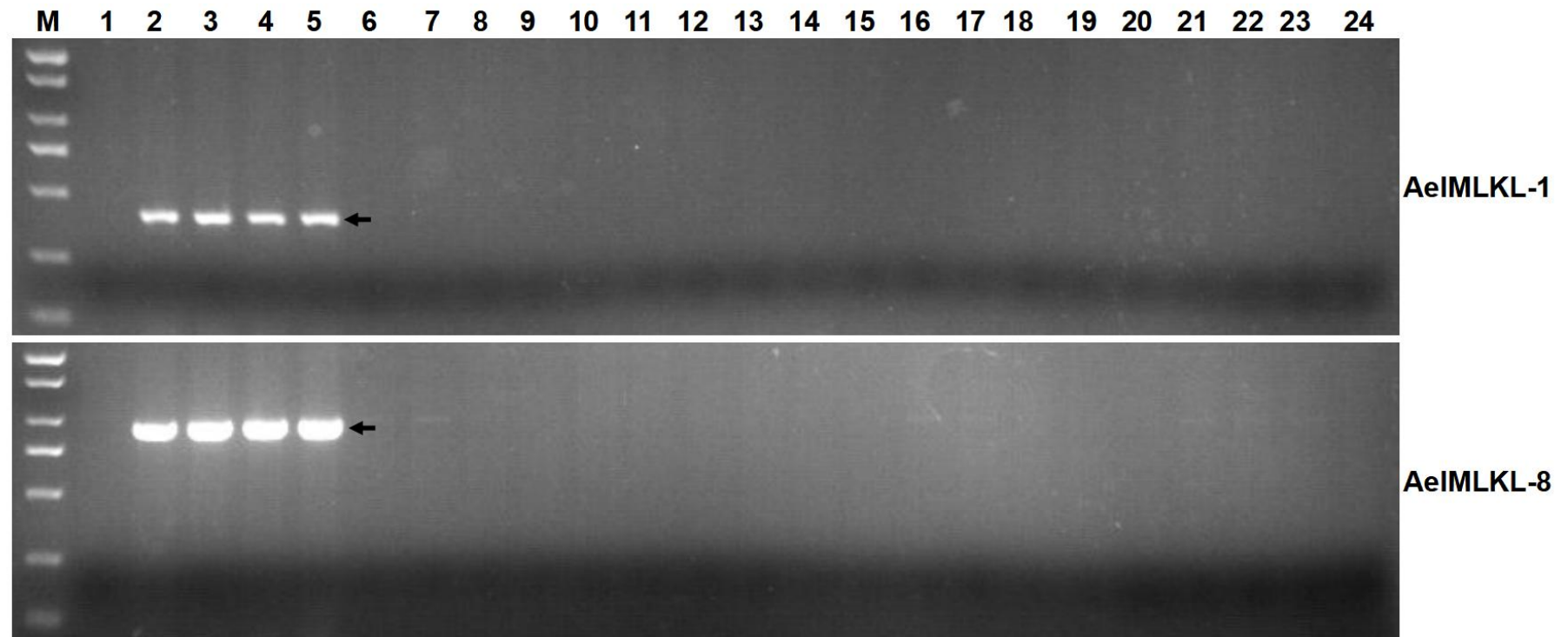
Supplementary Fig. 7 Phylogenetic analysis of kinase domains of Pm13 and described resistance proteins.



Supplementary Fig. 8 Protein sequence alignment of Pm13 and Pm13 homologues. Predicted HeLo (red) and STK (magenta) domains are indicated above the sequences.



Supplementary Fig. 9 Relative expression level of PR genes from one-week-old TA3575 and CS seedling plants at 0, 6, 12, 18, 24, 36, 48 and 72 hpi with *Bgt* isolate E09. Expression of the *TaActin* gene was used for normalization. Error bars indicate means $\pm$ SD (n = 3 biological replicates). Two-tailed Student's t-tests; *, $P < 0.05$, **, $P < 0.01$.



Supplementary Fig. 10 Validation of the usefulness of functional molecular markers AelMLKL-1 and AelMLKL-8 of *Pm13* in partial wheat lines. M, DL2000 Plus DNA marker; 1, CS; 2, CS-*Ae. longissima* 3S^l#2(3B) disomic substitution line TA3575; 3, CS-*Ae. longissima* T3S^l#1S-3BS.3BL recombinant R1B; 4, CS-*Ae. longissima* T3S^l#1S-3DS.3DL recombinant R2B; 5, the newly developed CS-*Ae. longissima* T3S^l#2S-3BS.3BL recombinant W12-3 with tiny 3S^l#2 segment harboring *Pm13*; 6, Bainong 64; 7, Bainong 207; 8, Zhoumai 16; 9, Zhoumai 18; 10, Zhoumai 22; 11, Zhoumai 28; 12, Ping'an 602; 13, Ping'an 0518; 14, Ping'an 901; 15, Yanzhan 4110; 16, Aikang 58; 17, Zhengzhou 366; 18, Xinong 979; 19, Nannong 9918; 20, Tianmin 198; 21, Tianmin 369; 22, Yangmai 5; 23, Yunong 202; 24, Yumai 49-198. Arrows pointed to the polymorphic bands of *Pm13* functional molecular markers AelMLKL-1 and AelMLKL-8.