

Supplementary Fig. 1 | Phylogenetic analysis of core kinase domains of described resistance proteins and Pm4b. The phylogenetic tree is based on the core kinase domains delimited based on Conserved Domain Database (CDD) from NCBI. The location of the domain is indicated by the sequence range numbers. In red, the core kinase domain of Pm4b. cAPK-alpha was used as outgroup.

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	10	20	30	40	50	60	70	80	90
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Pm4b C2C	LVPAEEGRSLAPTIV	K <mark>IQMGGQ</mark>	IR <mark>R</mark> T <mark>K</mark> QG(	PQGSANPTWND	DFMLVVTEPL	EDPLVVTV	-ERISASR-		EPIGHVII
Pm4b C2D	-LGARDLLGTKNP <mark>Y</mark> V	▼AMYGDK	∙-W <mark>v</mark> r <b>t</b> rti	LVNTMMAPHWNE	QYTWDVFDLS	TVITIAV	DDCHLSSSL	GDHDAR	QQMGKVRI
Syt13 1WFM	SNHDGGCDCYV	GSVANR-TG	SV <mark>E</mark> AQTAI	LKKRQLHTTWEE	GLVLP-LAEE	ELPTATLTLTL	-TCDRFSR-	<mark>H</mark>	SVAGELRLGL
Rim1 2Q3X	LTQKPGSKSTPAP <mark>Y</mark> V	VYLLENGAC	IA <mark>K</mark> KKTR	TARKTLDPLYQQ	SLVFDESPQ-	GKVLQVIV	GDYGRMDH-	<mark>k</mark>	CFMGVAQILL
Rim2 2BWQ	-LPSREDGRPRNP <mark>Y</mark> V	K <mark>IYFLPDRSD</mark>	KN <mark>KRR</mark> TK	CVKKTLEPKWNQ'	FIYSPVHRR	EFRERMLEITL	-DQARVRE-	EE <mark>S</mark>	EFLGEILIEL
PI3KC2a 2B3R	LVTEDGADPNP <mark>Y</mark> V	K <mark>TYLLPDTHK</mark>	TS <mark>K</mark> RKTKI	SRKTRNPTFNEI	MLVYSGYSKE	TLRQRELQLSV	-SAESLRE-	<mark>N</mark>	FFLGGITLPL
Syt1 1RSY C2A	-LPALDMGGTSDP <mark>Y</mark> V	K <mark>VFLLPDKKK</mark>	<mark>k</mark> fetki	/HRKTLNPVFNE	QFTFK-VPYS	ELGGKTLVMAV	-DFDRFSK-	<mark>F</mark>	DIIGEFKVPM
Syt7 2D8K	-LPAKDFSGTSDP <mark>F</mark> V	K <mark>IYLLPDKKH</mark>	I–– <mark>K</mark> L <mark>E</mark> TKV	/KRKNLNPHWNE	FLFEGFPYE	KVVQRILYLQV <mark>I</mark>	-DYDRFSR-	<mark>N</mark>	DPIGEVSIPL
Raph 2CHD C2A	-LKPMDSNGLADP <mark>Y</mark> V	K <mark>LHLLPGASK</mark>	SN <mark>K</mark> LRTK	LRNTRNPVWNE	FLQYHGITEE	DMQRKTLRISV	-DEDKFGH-	<mark>N</mark>	EFIGETRFSL
PKCg 2UZP	-LIPMDPNGLSDP <mark>Y</mark> V	K <mark>lklipdprn</mark>	ILT <mark>KQ</mark> KTRT	CVKATLNPVWNE	FVFN-LKPG	DVE-RRLSVEV	-DWDRTSR-	<mark>N</mark>	DFMGAMSFGV
Pkca 1DSY	-LIPMDPNGLSDP <mark>Y</mark> V	K <mark>lklipdpkn</mark>	IES <mark>KQ</mark> KTKT	TIRSTLNPQWNE:	SFTFK-LKPS	DKD-RRLSVEI	-DWDRTTR-	<mark>N</mark>	DFMGSLSFGV
PKCb 1A25	-LVPMDPNGLSDP <mark>Y</mark> V	K <mark>lklipdpks</mark>	ES <mark>K</mark> QKTKI	IKCSLNPEWNE	FRFQ-LKES	DKD-RRLSVEI	V-DWDLTSR-	<mark>N</mark>	DFMGSLSFG-
Raph 3RPB C2B	-LAAMDANGYSDP <mark>F</mark> V	K <mark>lwlkpdmgk</mark>	KA <mark>K</mark> H <mark>K</mark> TQI	KKKTLNPEFNE	EFFYD-IKHS	DLAKKSLDISV	-DYDIGKS-	<mark>N</mark>	DYIGGCQLGI
Syt4 1W15	-LPKSDVSGLSDP <mark>Y</mark> V	K <mark>VNLYHAKKR</mark>	LS <mark>K</mark> KKTHV	/KKCTPNAVFNE	LFVFD-IPCE	SLEEISVEFLV	-DSERGSR-	<mark>N</mark>	EVIGRLVLGA
Syt1 1K5W C2B	-LKKMDVGGLSDP <mark>Y</mark> V	K <mark>IHLMQNGKR</mark>	LK <mark>K</mark> K <mark>K</mark> TTI	KKNTLNPYYNE	SFSFE-VPFE	QIQKVQVVVTV <mark>I</mark>	-DYDKIGK-	<mark>N</mark>	DAIGKVFVGY
Syt7 3N5A	-LKAMDIGGTSDP <mark>Y</mark> V	K <mark>VWLMYKDKR</mark>	VE <mark>K</mark> K <mark>K</mark> TV	KKRNLNPIFNE:	SFAFD-IPTE	KLRETTIIITV	-DKDKLSR-	<mark>N</mark>	DVIGKIYLSW
<b>b</b>	/I AWAVGI AKWI DNI RRWRNP V TVI VI		PTAFLY-VVMIG	MYYNRERPKIPAG-MDIRIS(		P	SSRPFVIRARY		T LI GDE AAOGER I OAL VSWR
MCTP16_2028_0000 MLR IVNN   MCTP14_017-774 FERLVSI   MCTP14_017-774 FERLVSI   SCTP14_014-1017 FERLVSI   SCTP14_0171-012 FERLVSI   SCTP14_0171-012 FERLVSI   SCTP14_0170-012 FERLVSI   MCTP10_010-1012 FERLVSI   MCTP10_010-1012 FERLVSI   MCTP10_000-1014 FERLVSI   MCTP2_000-1014 FERLVSI   MCTP2_000-1011 FERLWSI   MCTP2_000-1012 FERLWSI   MCTP2_000-1011 FERLWSI   MCTP2_000-1012 FERLWSI   MCTP2_000-1011 FERLWSI   MCTP3_074-773 FERLWSI   MCTP4_000-000 FWRFT1   MCTP4_000-0000 FWRFT1	VAGAMVDUVRWDUTREMMENTSTLUV FORWIDAWKWDUTREMMENTSTLUV LSRAATIARVIHGIRTWHPPTTVLU JSRAATIARVIHGIRTWHPPTTVLU JSRAATIARVIHGIRTWHPPTTVLU JSRAATAVEVENTSTF FSGLICTGE/FDDUCRWKPPETTAI JSRAATAVEVENTSTF FSGLICTGE/FDDUCRWKPPETTULI LSRAFTVGKWPEDICSWRNPITTLU LSRAFTVGKWPEDICSWRNPITTLU LSRAFTVGKWPEDICSWRNPITTLU LSRAFTVGKWPEDICSWRNPITTLU LSRAFTVGKWPEDICSWRNPITTLU LSRAFTVGKWPEDICSWRNPITTLU LSRAFTVGKWPEDICWRNPITTLU	IL VVML IVE PDL. IL VVML IVE PX: IL VZ FVVC PX: IL VZ IL VX C PHLVI IL VZ IL VX C PHLVI IL FL IL VX C PELLI IL FL IL VX C PELLI VX FFL IL VX PELLI VX FFL IL VX PELLI VX FFL IL VX PELLI IL FL IL VX PELLI IL VX PELIC VX IL VX VX PELIC VX IL VX VX PELIC VX IL VX VX PELIC VX I	VPTLAFY-LFVI6/   VPTAFY-LFVI6/   PTVFWY-AFLL/2   PTVFWY-AFLL/2   PTVFWY-AFLL/2   PMULLY-TAAVO   PWSUC-FML00   PTFLY-WFL60   PTFLY-WFL60   PTFLY-WFL60   PTFLY-WFL70   PTFLY-WFL70   PTFLY-WFL70   PTFLY-FL70	WWW FERSEAL PHIDDELS UPF GESPHAPH-IMDILS MERRAGOPH-IMDILS MERRAGOPH-MDARIS MERRAGOPH-MDARIS MULTERRAGOPH-MDARIS MULTERRAGOPH-MDARIS MULTERRAGOPH-MDARIS MULTERRAGOPH-MDARIS MULTERRAGOPH-MDARIS MULTERRAGOPH-MDARIS MULTERRAGOPH-MDARIS MULTERRAGOPH-MDARIS MULTERRAGOPH-MDARIS MULTERRAGOPH-MDARIS MULTERRAGOPH-MDARIS MULTERRAGOPH-INDILS MULTERRAGOPH-INDILS MULTERRAGOPH-INDILS MULTERRAGOPH-INDILS MULTERRAGOPH-INDILS MULTERRAGOPH-INDILS MULTERRAGOPH-INDILS MULTERRAGOPH-INDILS MULTERRAGOPH-ILVDLLLUM	ADA ABROBEL DEE EDVV X003 ALPOEL DEE EDVF X003 ALPOEL DEE FONT AEL AHPOEL DEE FONT AET VFPOEL DEE FONT AET VFPOEL DEE FONT ADAL HAFEEL NEE FONT MEA ASPOEL DEE FONT ADAL MAPOEL DEE FONT ADAL	/P P P (P YEDTVLWYPDQVHPDEI P P P P P P P P P P P P P P P P P P P	SINGPEUVELEY SIKSOVLKERY TROPEVVELEY TROPEVVELEY TSROPOVLKERY TSROPOVLKERY TSROPOVLKERY TSROUTKERY TSROUTKERY TSROUVKERY TSROUVKERY TSROUVKERY TSROUVKERY TSROUVKERY TSROUVKERY TSROUVKERY TSROUVKERY TSROUVKERY TSROUVKERY TSROUVKERY TSROUVKERY TSROUVKERY TSROUVKERY TSROUVKERY	DICLINVGARVO DRLRRVGARVO DRLRALAGRAO DRLRSVCGRLO DRVRSVGRLO DRVRSVGRIO DRLRSVAGRIO DRLRSVAGRIO DRLRSVAGRIO DRLRSVGGRVO DRLRSVGGRVO DRLRSVGGRVO DRLRSVGGRVO DRLRSVGGRVO DRLRSIGGRVO DRLRSIAGRIO	T LOG VAAOGER MAALU TINE T LOG VAAOGER MAALU TINE T LOD VAAOGER VSALLSWN T LOD VAAOGER VSALLSWN T VGDU AAOGER VGALLSWN T VOOD AAOGER VGALLSWN T VVOOD AAOGER VGALLSWN T VVOOD AAOGER VGALLSWN T VI GOLATOGER F GALLSWN T VI GOLATOGER F GALLSWN T VVOOD AATOGER FLAST LLSWN
ALT-12.009-1060 D-FRATI   ALT-146_046-1049 D-FRATI   ALT-146_04-107 D-FRATI   ALT-14_017 D-FRATI   CTP14_014-107 D-FRATI   CMCTP14_014-107 D-FRATI   CMCTP14_014-107 D-FRATI   MAD_V2519-746 D-FRATI   MCTP0_01012 D-FRATI   MCTP0_01012 D-FRATI   MCTP0_010700 D-FRATI   MCTP2_030-1016 D-FRATI   MCTP6_057-1029 D-FRATI   MCTP6_057-1029 D-FRATI   MCTP6_057-1029 D-FRATI   MCTP6_157-1776 D-FRATI   MCTP4_1544-776 D-FRATI   MCTP4_1543-7445 G-DDQLJ	EF001CFFVALVLYVPTRWVA   EFV01CFFVALVLYVPTRWVA   LFU1FCFV3CGV1CFVSWKLIL   EFV1ECFV3CGV1CFVFKVFLI   IFMTLS_VV3VLYLPFKVFLI   IFMTLS_VV3VLYLPFKVFLI   IFMTLS_VV3VLYLPFKVFLI   IFMTCLS_V3VLYLPFKVFLI   IFMTCLS_V3VLYLPFKVFLI   IFMTCLS_V3VLYLPFKVFLI   IFMTCLS_V3VLYLPFKVFLI   IFMTCS_V1FMALYLPFKLIAN   IFV1CFLIAAIVLYLPFKUVAI   IFV1CFLIAAIVLYLPFKUVAI   IFV1CFLIAAIVLYLPFC0VAI   IFV1CFLIAAIVLYL	AGGY THE LARGENT FRO F FELAEVWARE REVEVE BGGY THEREROL LAGUTUL REPREROL LAGUTUL REPREROL LAGUTUL REPREROL SGYTY MERIFERS. K LAGITUL REPREROL SGYTY MERIFERS. K LAGITUL REPRESS. LAGITUL REPRESS. LAGITUL REPRESS. LAGITUL REPRESS. LAGITUL REPRESS. LAGITUL REPRESS. TAGLEVICE THERESS. TAGLEVICE THE LENCE	LINE TASULTFRALE VKRSPVLNFFRRLE SOPSMOLNFFRRLE SOPSMOLNFFRRLE (LPSRGLSFFRRLE (LPSRGLSFFRRLE (LPS LPSNFFRRLE KLPSVPVNFFRRLE KLPSVPVNFFRRLE (LPSVPLNFFRRLE (LPSVPLNFFRRLE (LPSVPLNFFRRLE (LPSVPLNFFRRLE KLPSVPLNFFRRLE (LPSVPLNFFRRLE KLPSVPLNFFRRLE (LPSVPLNFFRRLE KLPSVPLNFFRRLE (LPSVPLNFFRRLE KLPSVPLNFFRRLE (LPSVPLNFFRRLE)	SISDRLH SISDRLM SRADSIL SRADSIL SRADSLL SRADSLL SKADCML SISTDSLL AKTDCML SISTDSLL AKTDCML AKSDBLL AKTDCML SISDLL SISTDLMF TNEVLF-					

Supplementary Fig. 2 | Pm4b-C2C/C2D domain analysis for lysine-rich clusters involved in interaction with phosphoinositides. a, Sequence-based alignment of Pm4b C2C and C2D domains (first two rows) with C2 domains reported to bind phosphoinositides, for example, the C2 domain of PKCa (1DSY). Protein identification and PDB codes are located on the left. Conserved residues that form the lysine-rich cluster ( $Y_xKx_{n1}K_xKx_{n2}W(Y/L/C)x_{n3}N$ ) are depicted as white letters on dark blue background. Yellow letters in C2C and C2D domains correspond to homologues residues compared to the classical lysine-rich cluster. Pm4 C2D domain exhibits diverse amino acid substitutions, including K -> V or T disrupting the presence of conserved positive charged and aromatic residues present characteristic of the lysine-rich cluster. However, in Pm4 C2C domain, although lacking the characteristic positively charged (K) and aromatic (Y, W) amino acids present in typical lysine-rich clusters, there are substitutions by amino acids with similar physicochemical properties. In the third position, instead of a Lysine, there is an Arginine, another positively charged polar amino acid. In the fifth position, tryptophan is substituted by another nonpolar amino acid, Valine. Finally, in position sixth, Asparagine is substitute by glutamic acid, another polar and relatively small amino acid. **b**, Alignment of the terminal part of Arabidopsis MCTPs and Pm4b\_V2, underlined on purple. Transmembrane domains are depicted as red squares. The characteristic duplication present in Pm4b\_V2 is indicated in blue. The protein region displayed is indicated by the sequence range numbers.



**Supplementary Fig. 3 | Co-localization analysis of Pm4b\_V1 and Pm4b\_V2 with characterized markers.** Pm4b\_V1 and Pm4b\_V2 isoforms were co-infiltrated with the plasma membrane-marker (35S:REM 1.2 m\_RFP40), the mRFP-fused cytosolic localization sequence (pGWB45538) and the ER-marker (ER-ck, CD3-95939) to examine their subcellular localization. Pm4b\_V1 mainly co-localizes with the cytosolic marker while Pm4b\_V2 with the ER marker. High Pearson correlation coefficients of Pm4b\_V1 and Pm4b\_V2 indicate their co-localization when co-expressed. On top of each boxplot, number of observations and means. Different letters indicate significant differences using ANOVA followed by Tukey honest significant difference (HSD) test (P<0.05). At least n = 10 single-scanned cell images per experiment were collected and analyzed using the same conditions of laser intensity, pinhole size, and gain levels. In the boxplots, center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by the geom\_boxplot function of the ggplot2 R package; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, individual data points are represented by dots.



AET2Gv21296800\_V1

0.87

0.75

0.99

0.99

0.92

HORVU2Hr1G126810\_V1

1

1

0.77

0 99

1

1

AET2Gv212971\_V1

ITDC2AG081930\_V1

Pm4b\_V1

Pm4\_DW\_Un-H2\_V1

Pm4\_WEW\_2B-H1\_V1

Pm4\_DW\_2B-H1\_V1

TRITDC2BG090970 V1

- AET2Gv21296200\_V1

Pm4\_WEW\_2A-H3\_V1 1 TraesCS2A01G558500\_V1

Pm4\_Tu-H1\_V1

SECCE2Rv1G0142720.1\_V1 Pm4\_DW\_Un-H1\_V1

Pm4\_Scer\_2R-H2\_V1

TraesCS2B01G621800\_V1

Pm4\_Scer\_2R-H1\_V1

Pm4\_WEW\_2A-H1\_V1

0.2



Supplementary Fig. 4 | Phylogenetic analysis of Pm4 homologues. The tree on the top corresponds to full-length predicted proteins based on Pm4b\_V1 isoform. Likewise, isoform Pm4\_V2 is displayed in the bottom. In red, Pm4b\_V1/V2. For both cases, the kinase domain of the rice Os04g30030 was used as outgroup.



**Supplementary Fig. 5 | Sequence comparison of the contig\_18057 in wheat cultivars Fed-***Pm4b* and SYMattis. Dotplot alignment of the Pm4 contig\_18057 from Fed-Pm4b (horizontal) and SYMattis (vertical). On top of the dotplot, it is displayed a schematic drawing of the Pm4 CDS. The first blue box corresponds to exons one to five. The second and third blue boxes, to exons six and seven, respectively. SYMattis contained the *Pm4* contig\_18057 sequence spanning physical positions 788'726'801-788'747'264, at the very distal end of chromosome arm 2AL. Around 27 bp downstream of the stop codon of Pm4b\_V2 lies a novel TE of the Mutator superfamily (https://www.botinst.uzh.ch/en/research/genetics/thomasWicker/trep-db.html). Since this TE lies so close to the gene, it provides downstream regulatory sequences to *Pm4*. For example, two putative poly-adenylation signals are located inside this TE.