

New Phytologist Supporting Information

Article title: A highly differentiated region of wheat chromosome 7AL encodes a *Pm1a* immune receptor that recognises its corresponding *AvrPm1a* effector from *Blumeria graminis*

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Article acceptance date: 1 November 2020

Fig. S1 Graphs of R gene enrichment sequencing (RenSeq) mutant read alignments to candidate contigs showing mutation positions. Histograms showing coverage depth of read alignments to candidate contigs juxtaposed. Blue graphs are mutants with IDs labelled on the left. Grey graph is parental wild-type Chinese Spring/Axminster*7A (CS/Ax7A). SNPs are denoted with red or green vertical bars that indicate a mismatch with WT CS/Ax7A occurring in almost 100% of the overlapping reads. Specific base changes are labelled. The motifs panel indicates predicted R gene motifs and how they fit into encoded protein domains and exon structure. Domains: CC, coiled-coil; P-loop, phosphate-binding loop; NB-ARC, nucleotide binding-(APAF-1, R proteins and CED-4); LRR, leucine rich repeat.

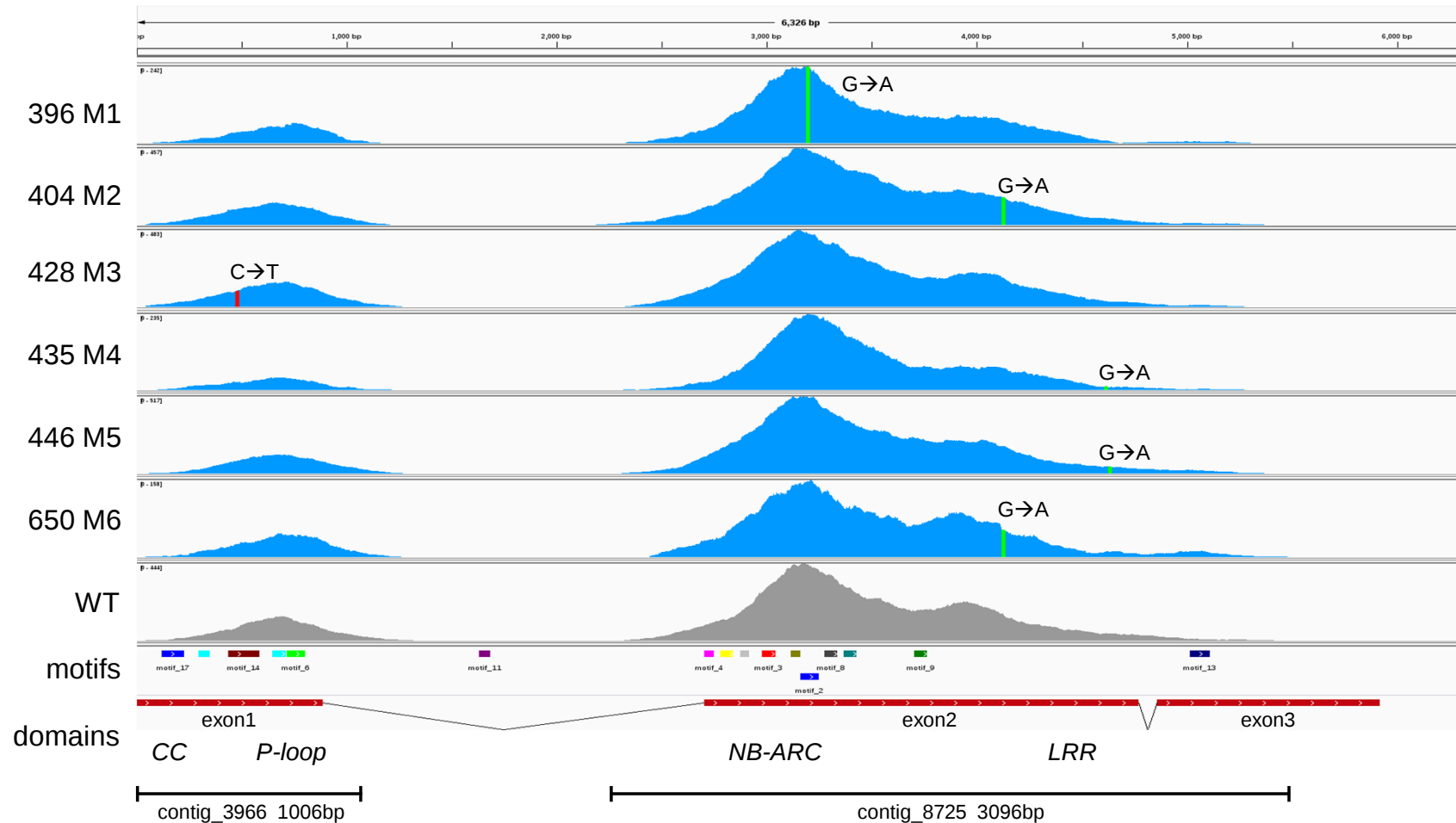


Fig. S2 Marker *Pm1aSTS1* assay showing specificity to *Pm1a*-containing *Triticum aestivum* lines. CS (Chinese Spring), Avocet R, and Kukri do not have *Pm1a*. CS/Ax7A (Chinese Spring/Axminster*7A), Thew, Norka, and Schomburgk have *Pm1a*.

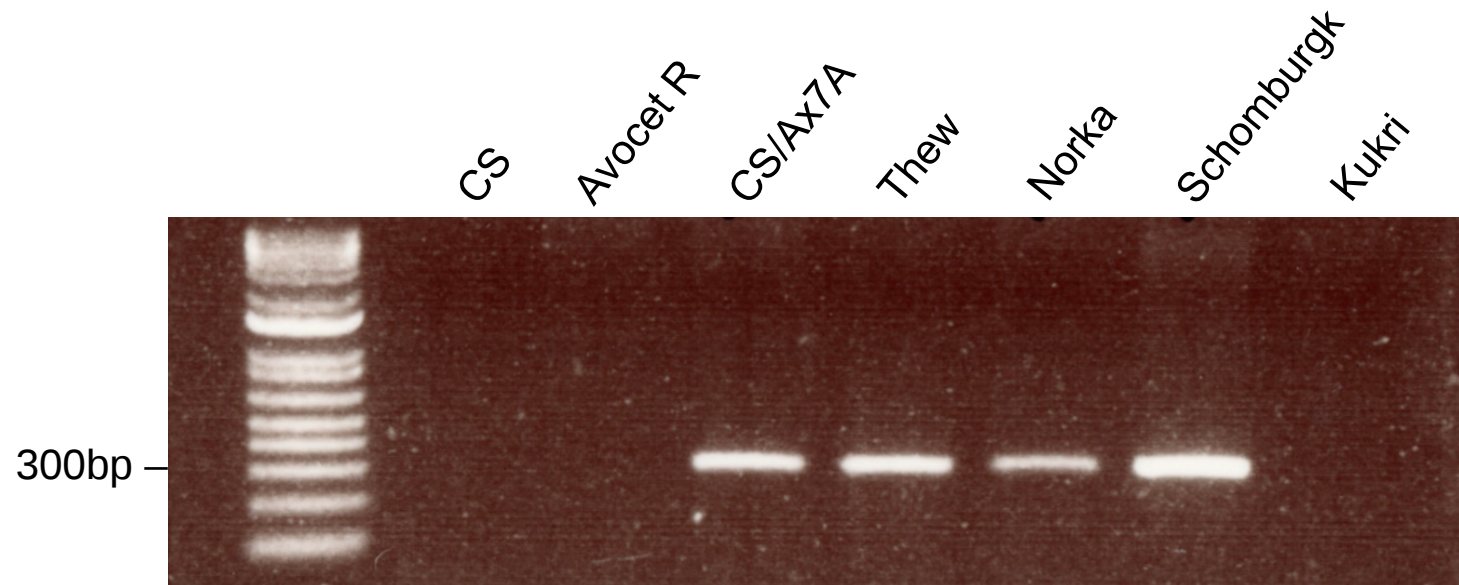


Fig. S3 Amplification of a large product based on the candidate contig from *Triticum aestivum* cv. Chinese Spring/Axminster*7A (CS/Ax7A) was absent in double mutants of *pm1* and *sr15/lr20*. PCR product used for Sanger sequencing based on the candidate contig #8725 sequence amplified in *Pm1a*-containing lines CS/Ax7A, Thew, Norka and Schomburgk, and point mutant 10487, but not in Thew and CS/Ax7A double mutants (9024-10693) presumed to be deletions.

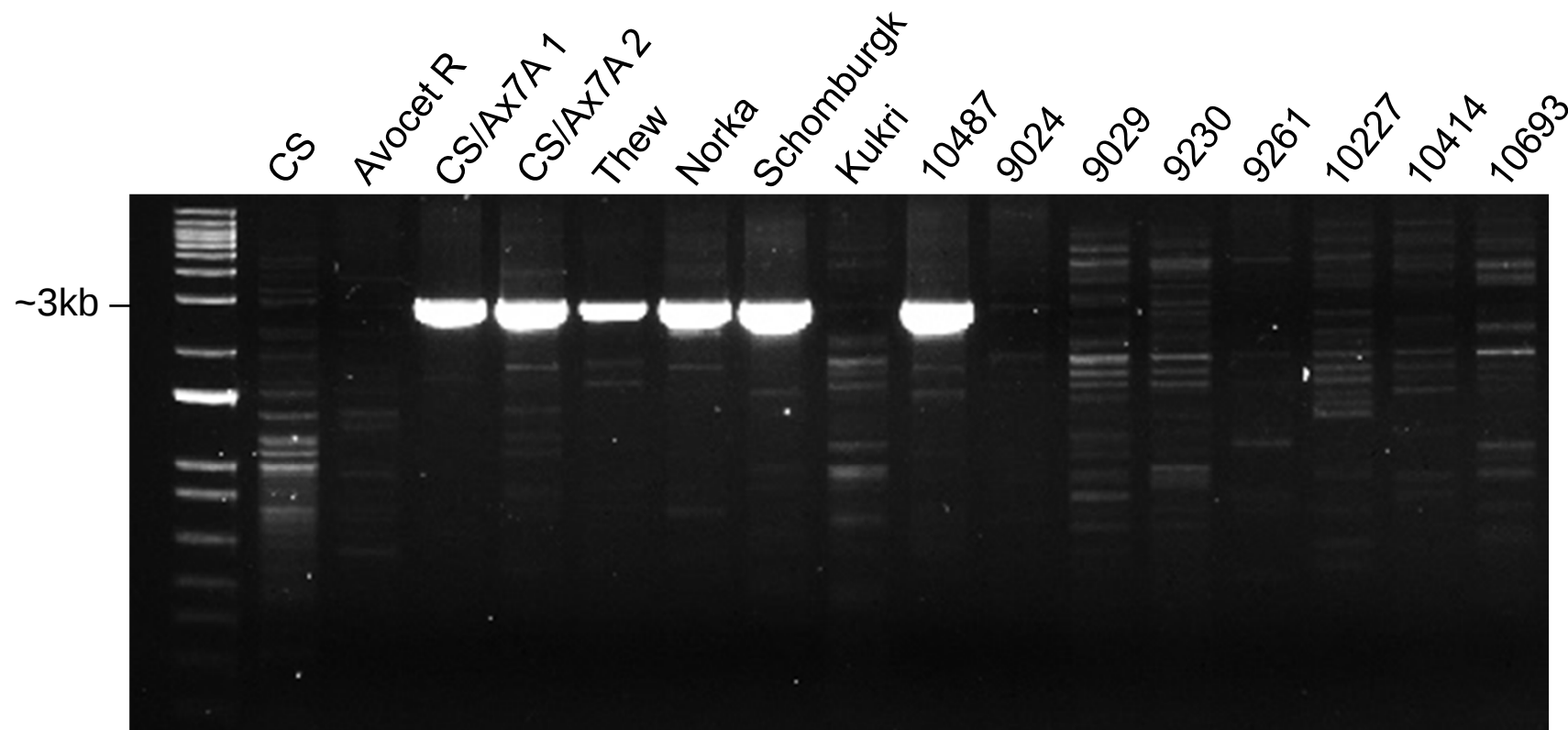


Fig. S4 Clear differentiation of resistant and susceptible *Triticum aestivum* seedlings in recombinant inbred line populations inoculated with *Blumeria graminis* f. sp. *tritici* (*Bgt*). Three-week-old seedlings at 10 days post-inoculation with the Canberra *Bgt* population. L, susceptible; R, resistant.

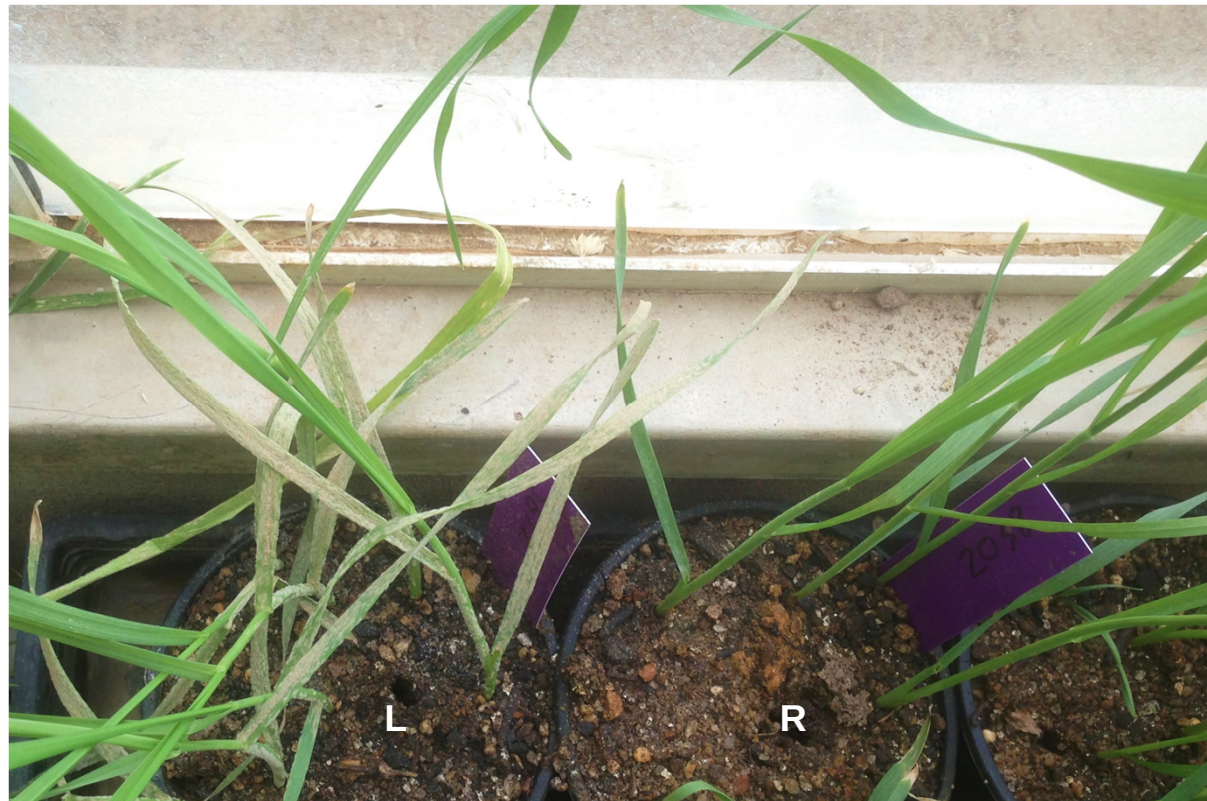


Fig. S5 Bi-parental mapping population *Blumeria graminis* f. sp. *tritici* (Bgt) 96224 (*avrPm1a*) × *B.g. triticales* THUN-12 (*AvrPm1a*) segregates on the *Triticum aestivum* near isogenic line (NIL) Axminster/8*Chancellor containing *Pm1a*. **(a)** Virulence phenotype of the isolates 96224 and THUN-12 on the near-isogenic line (NIL) Axminster/8*Chancellor and the control Chancellor at 10 days post-inoculation from three independent infection experiments. **(b)** Leaf coverage (LC) according to the following scoring: 0 (0-10% LC), 0.25 (10-40% LC), 0.5 (40-60% LC) and 1 (60-10% LC) of the parental isolates 96224 and THUN-12 on Axminster/8*Chancellor on 15 detached leaf segments. The infection score of the 15 detached leaf segments are summarised as boxplots. Individual datapoints are depicted as black dots. Datapoints are jittered for better readability **(c)** Distribution of LC according to the same scoring system as in (b) of 118 F₁ progeny of the cross 96224 × THUN-12 on Axminster/8*Chancellor.

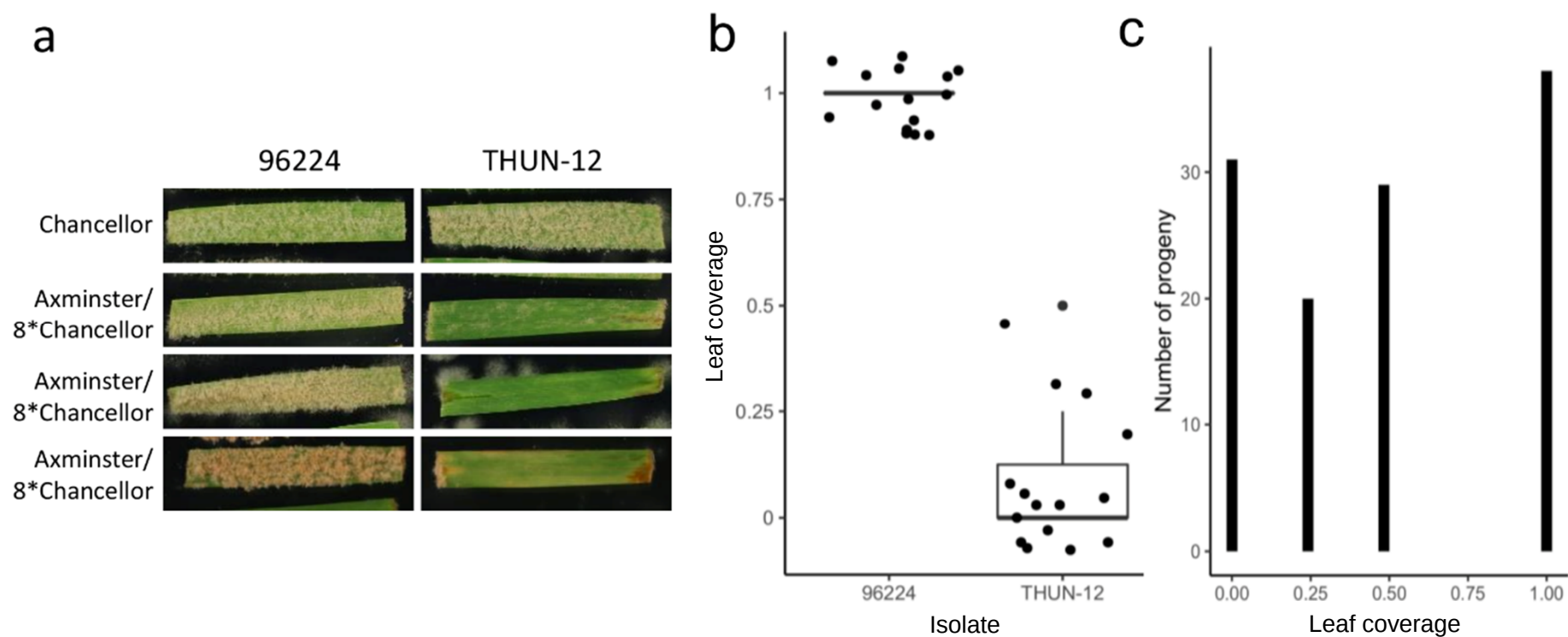


Fig. S6 *Agrobacterium* mediated transient co-expression of *BgtE*-5612 or *BgtE*-20015 with *Pm1a* and verification of protein presence in *Nicotiana benthamiana*. **(a)** The effector candidates *BgtE*-5612_96224, *BgtE*-5612_THUN12, *BgtE*-20015_96224 and *BgtE*-20015_THUN12 were co-infiltrated with *Pm1a* (OD=1.2, ratio 1:4 R:effector) in *N. benthamiana*. Co-infiltration of *Pm3f*^{L456P/Y458H} and *AvrPm3*^{a2/f2} was used as a positive control known to give weak hypersensitive response (HR) (Bourras *et al.* 2019). HR was assessed by fluorescence imaging (Fusion FX imager, see methods) 5 days after infiltration. **(b-c)** Western blot detection of BGTE-20015_THUN12 and BGTE-20015_96224 (b) or PM1a (c) C-terminally fused with a hemagglutinin (HA) epitope tag (upper panel) and Ponceau staining of RuBisCO as a loading control (lower panel). **(d)** C-terminal HA-tagged *BgtE*-5612 were co-infiltrated with *Pm1a* (OD=1.2, ratio 1:4 R:effector) in *N. benthamiana*. Co-infiltration of *Pm3f*^{L456P/Y458H} and *AvrPm3*^{a2/f2} was used as a positive control. HR was assessed by fluorescence imaging (Fusion FX imager, see methods) 5 days after infiltration.

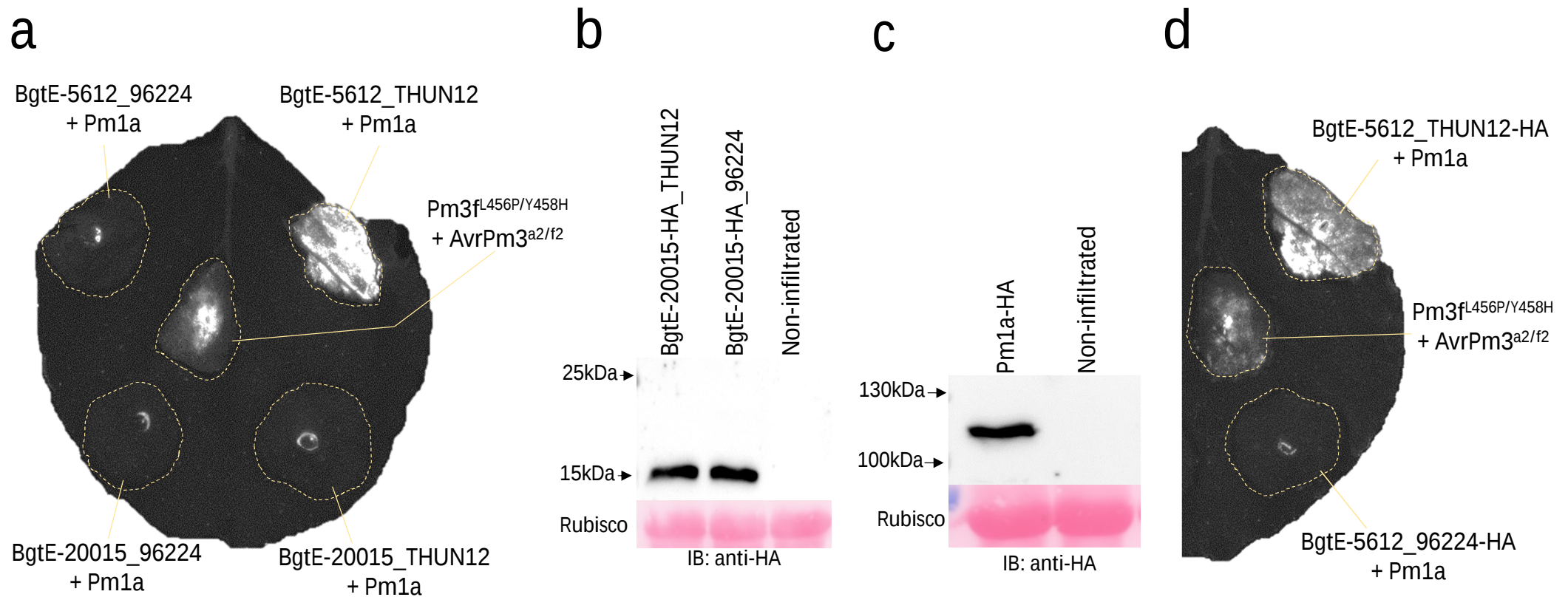


Fig. S8 Expression of the candidate effector family E004 gene members in the two *Blumeria graminis* isolates *B.g. triticales* THUN-12 and *B.g. tritici* 96224 based on RNAseq data. Expression levels are depicted as the mean of rpkm (reads per kilo base per million mapped reads) of three biological replicates with standard error bars shown. Expression level is shown at 2 days post-inoculation on susceptible triticale cv. Timbo and *Triticum aestivum* cv. Chinese Spring for THUN-12 (upper panel) and 96224 (lower panel), respectively. RNAseq data were described in previous studies (Praz *et al.*, 2018, Menardo *et al.*, 2016).

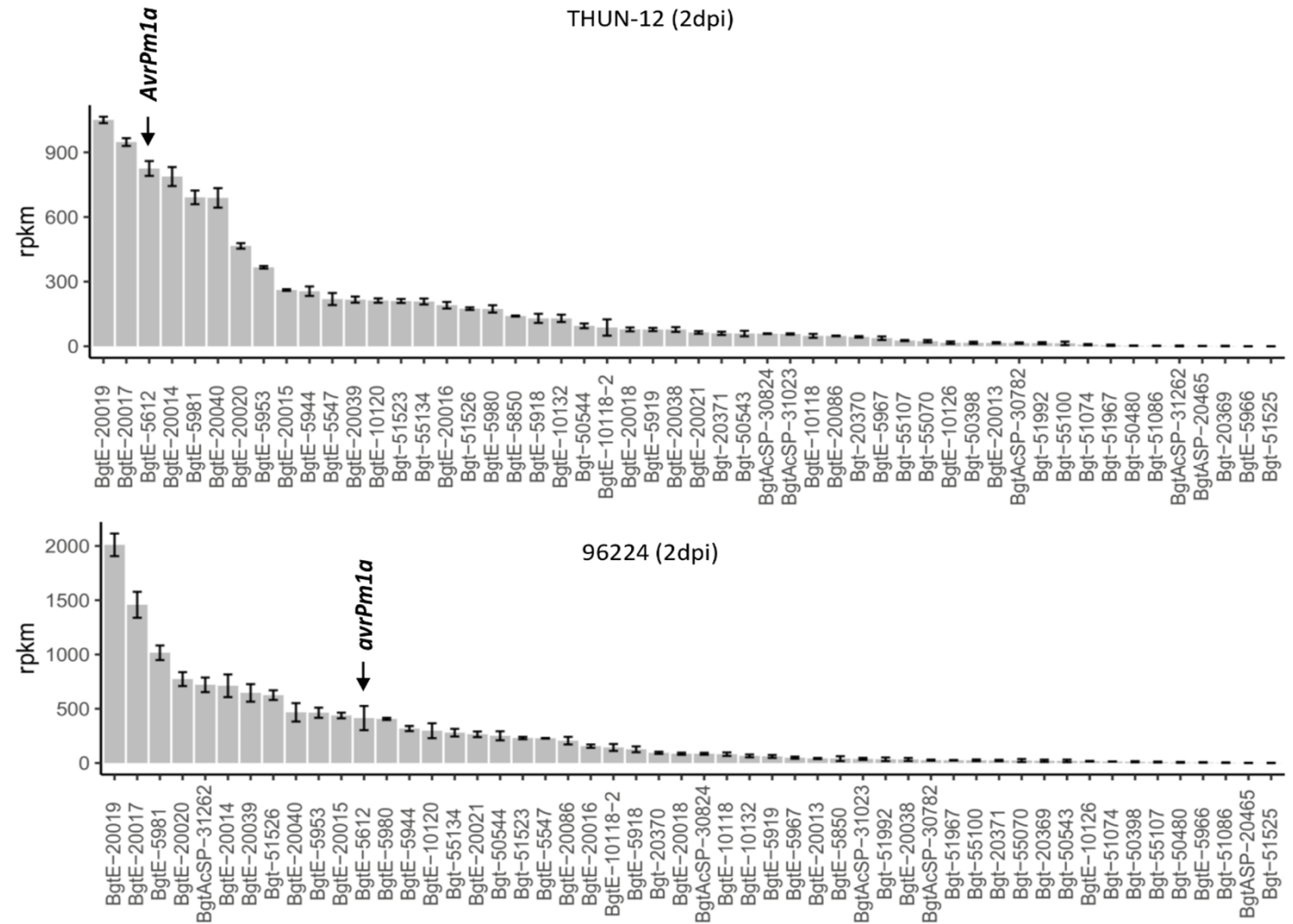


Fig. S9 Cytological examination of *Triticum aestivum* chromosome 7A in cv. Chinese Spring (CS) and lines carrying *Pm1a*. **(a)** Chromosome 7A FISH karyotypes of CS and four *Pm1a*-carrying lines (CS/Ax7A, Kenya W744, Thew, Norka). Oligo-pSc119.2 and Oligo-pTa535 were labelled with 6-carboxyfluorescein (6-FAM) and 6-carboxytetramethylrhodamine (Tamra), generating green and red signals, respectively, and allowing us to identify individual chromosomes. Chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI) and fluoresced blue. Arrows point to the weak pSc119 green signal at the distal region of 7AL in all four *Pm1a*-carrying lines but was absent in CS. **(b)** Flow-sorted chromosome was confirmed to be 7A of CS/Ax7A based on the above karyotype.

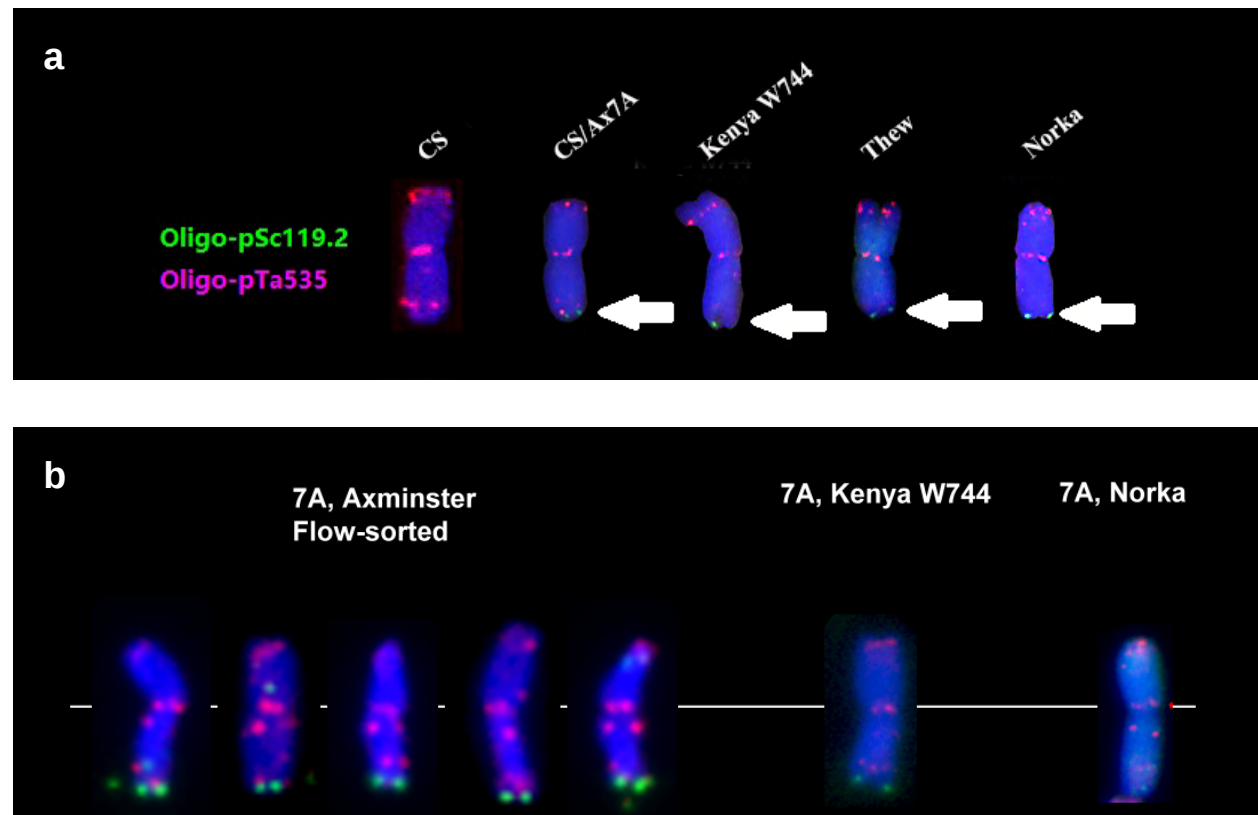


Fig. S10 *Triticum aestivum* cvs. Chinese Spring (CS) vs. Axminster sequence divergence in distal chromosome 7A.
(a) SNP density based on Axminster read alignments in 5 Mb windows along chromosome 7A of CS reference assembly (IWGSC RefSeq v1.0). The region bearing *Pm1* is located at the distal end of 7AL.
(b) Alignment scores of IWGSC RefSeq v1.0 annotated genes to Axminster 7A contigs drop sharply at distal end of 7AL. Ordered by position in CS chromosome 7A.

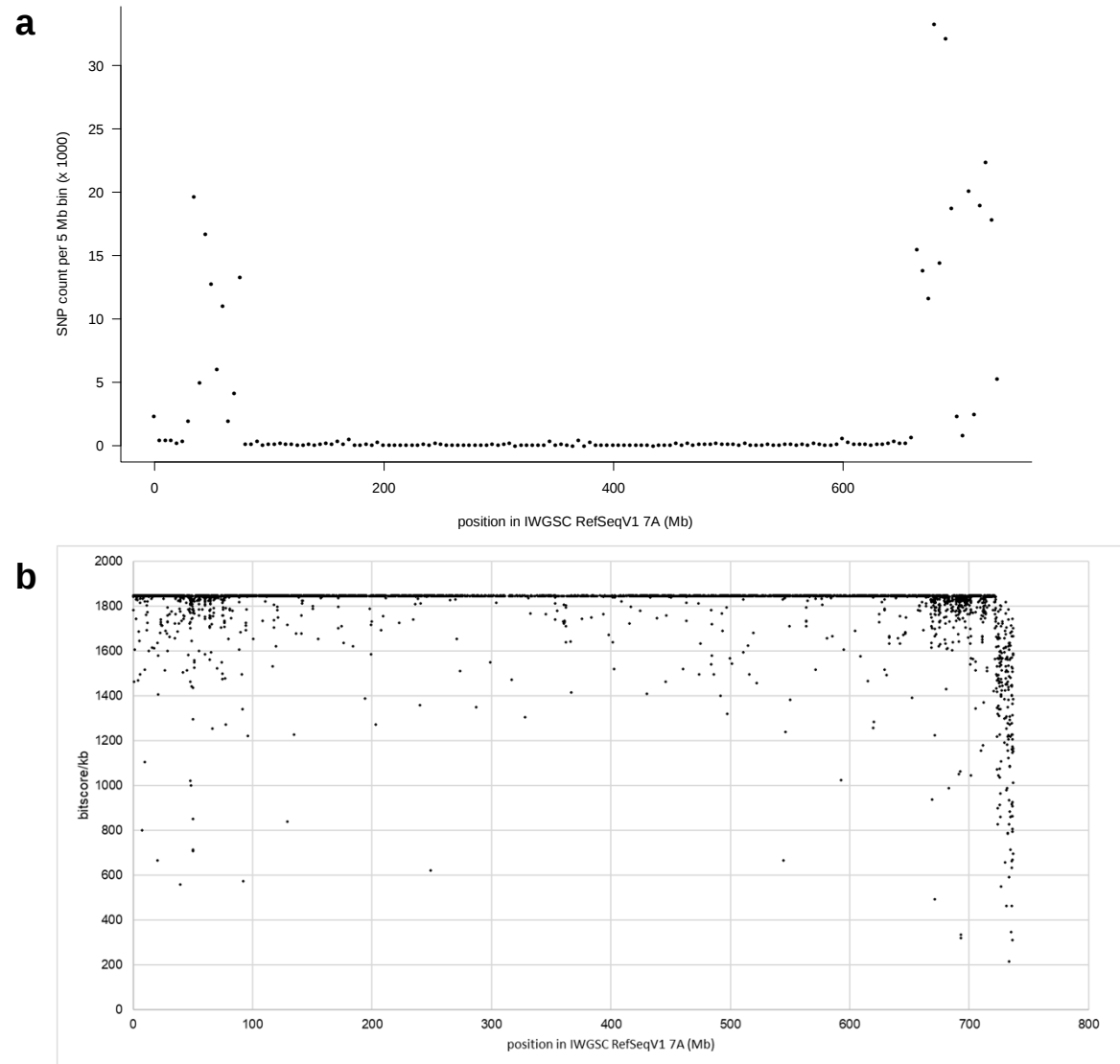


Fig. S11 Contig length distributions from assembly of *Triticum aestivum* cv. Axminster flow-sorted chromosome 7A with and without contaminants. Number of contigs (y-axis) belonging to each length (kb) bin (x-axis). Blue bars show counts for raw assembly, orange bars show counts for assembly with probable chromosomes 2A and 3A derived contigs removed. Most contamination appeared to be shorter contigs as N50 improved 3-fold with contaminants removed and combined length moved closer to the true size of chromosome 7A (table inset with lengths in bp).

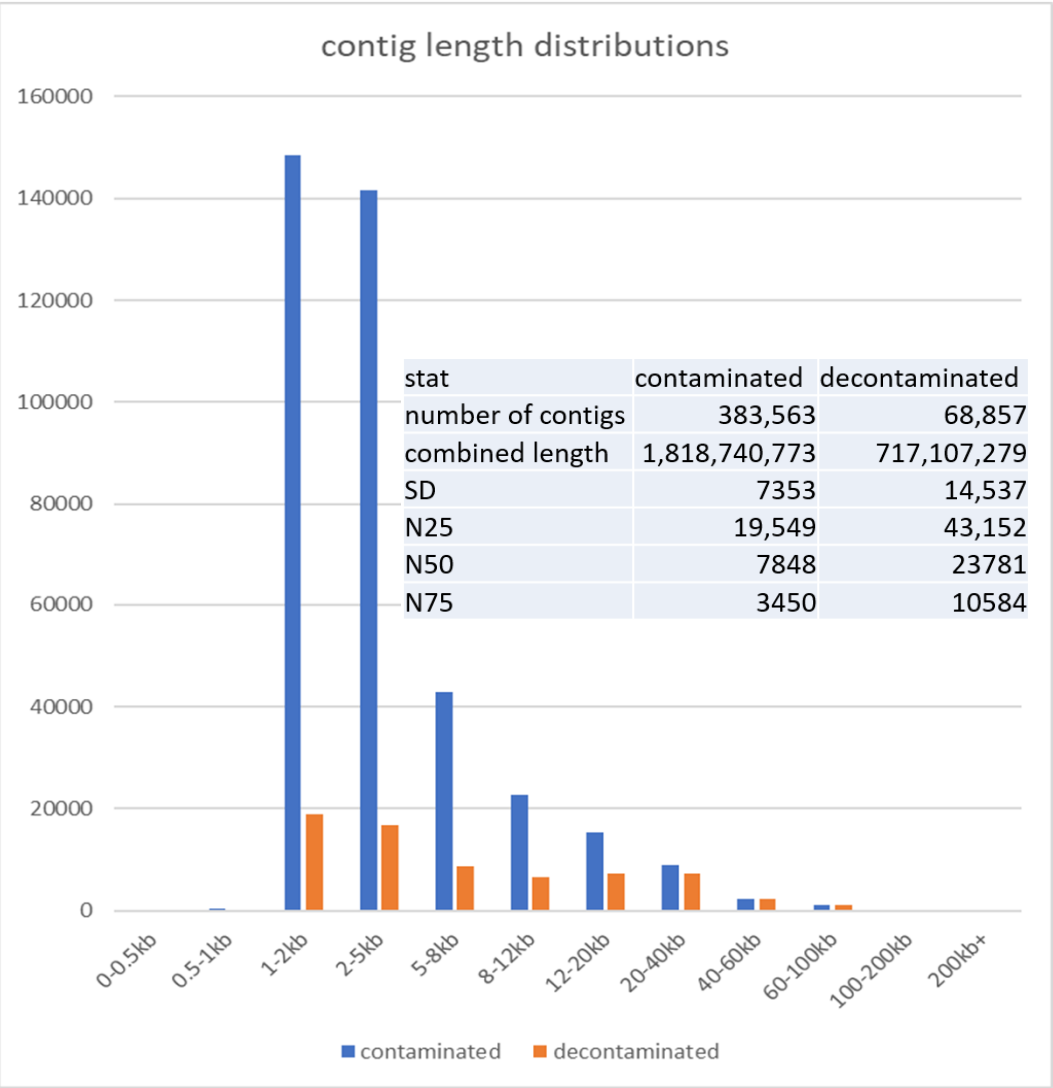
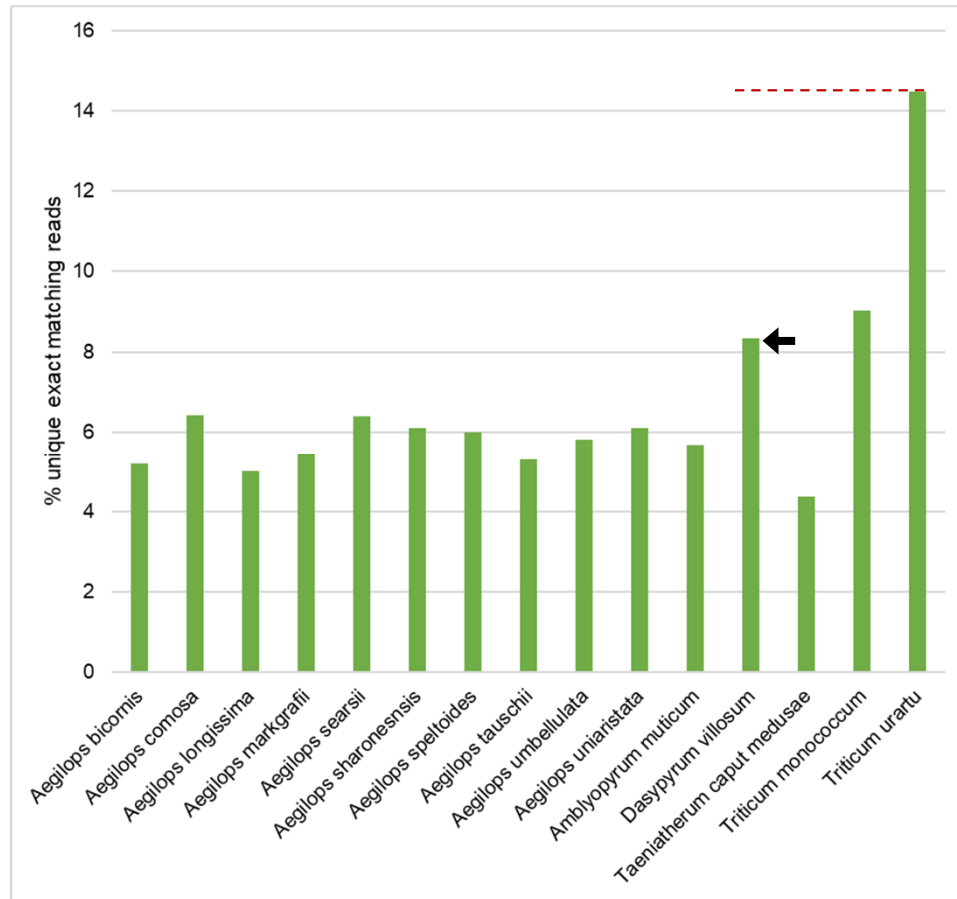


Fig. S12 Proportions of reads from diploid *Triticeae* species exactly matching to the flow-sorted chromosome 7A assembly of *Triticum aestivum* cv. Axminster. Values are percentage of reads exactly matching (edit distance = 0) out of the total uniquely mapped reads per species. Red dashed lines indicate levels of *T. urartu*, an ancestral benchmark from the A-genome donor. Black arrows indicate levels for *Dasypyrum villosum*, a speculative donor. (a) Reads aligned to whole set of Axminster 7A contigs. (b) Reads aligned to exclusive set of contigs assigned to distal Axminster 7AL. *D. villosum* levels approach those of *T. urartu*.

a



b

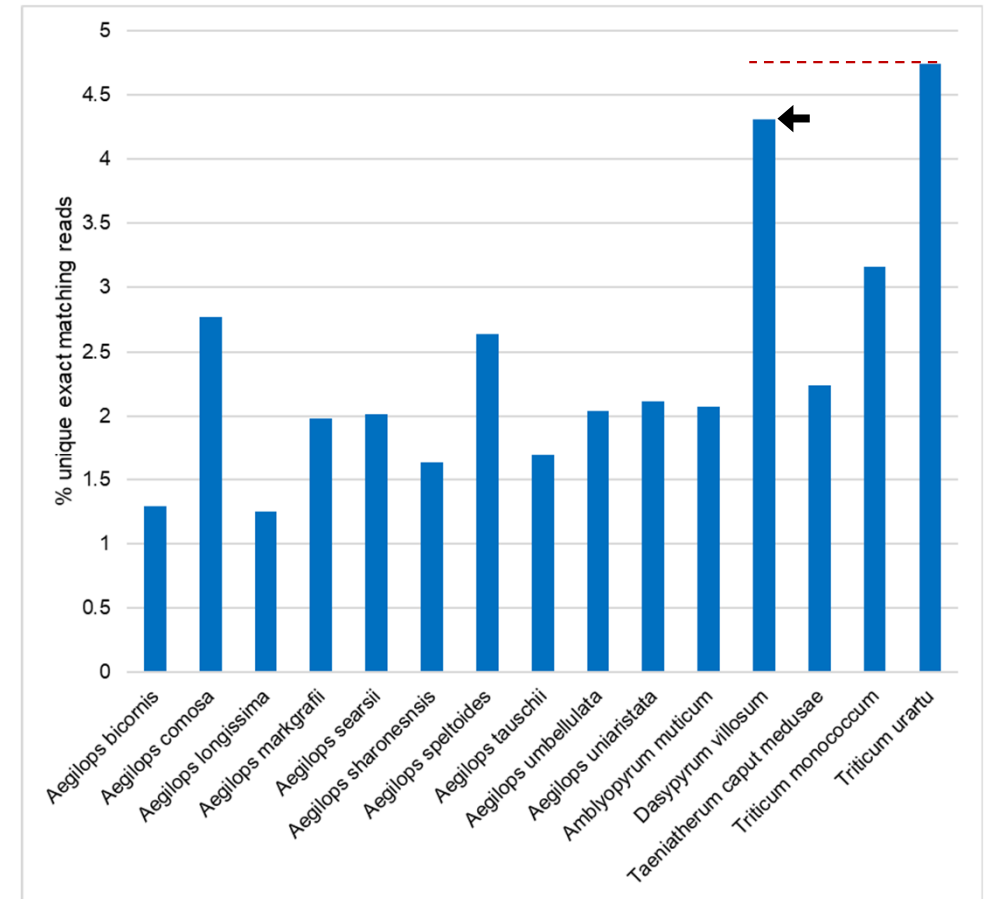


Fig. S13 Protein alignment of the candidate effector family E004 members in the reference *Blumeria graminis* f. sp. *tritici* isolate 96224. The effector family definition is based on Müller *et al.* (2019). Proteins were aligned using the Clustal algorithm and conserved amino acid residues are coloured according to the ClustalX colour scheme (blue: hydrophobic; red: positively charged; purple: negatively charged; green: polar uncharged; yellow: proline; pink: cysteine; orange: glycine). The Y/FxC motif and conserved C-terminal cysteine are indicated by vertical black boxes. *AvrPm1a* (BGTE-5612) is indicated by a horizontal black box.

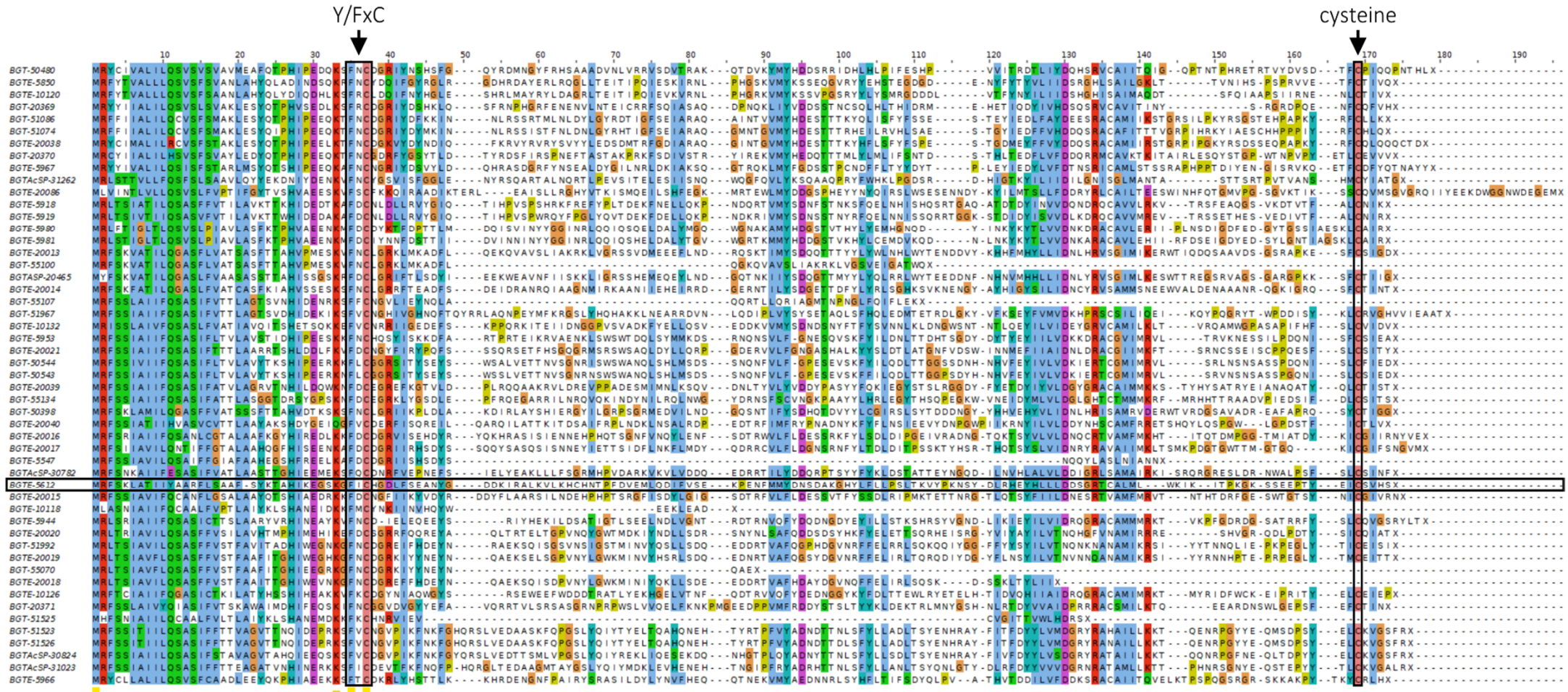


Fig. S14 (a) Alignment of *Blumeria graminis* f. sp. *tritici* AVR proteins and three family members of AVRPM1A. Protein sequences were aligned according to the intron position. Signal peptide, the Y/FxC motif, C-terminal cysteine and intron position are indicated. Intron size in basepairs (bp) is indicated for each protein. Number of amino acid (aa) residues between the intron and the first (part of Y/FxC motif) and the second cysteine are also indicated above the protein sequence. **(b)** Modelling of the 2D protein structure of AVRPM1A_THUN-12 and AVRPM2 without signal peptide using the Quick2D toolkit. The Y/FxC motif, C-terminal cysteine and intron position are indicated. Polymorphic amino acids between AVRPM1A_THUN-12 and AVRPM1A_96224 are highlighted in orange. **(c)** Structural modelling of AVRPM1A_THUN-12 and AVRPM2 using IntFold5.0. Domains predicted by the IntFold algorithm are depicted as follows: red indicates a predicted β -strand, blue indicates a predicted α -helix. The AVRPM1A protein models to the following two templates; a ribonuclease crystallized from *Aspergillus phoenicis* (PDB id =1rds) (Nonaka *et al.*, 1993) and the ribonuclease F1 of *Fusarium moniliforme* (PDB id =1fus) (Vassilyev *et al.*, 1993). AVRPM2 models to BEC1054 a RNase-like effector from barley powdery mildew (*B.g. hordei*) and family member of AVRPM2 (PDB id = 6fmb) (Pennington *et al.*, 2019) as well as a ribonuclease from *Hericum erinaceus* (PDB id = 5GY6) and the ribonuclease from *Aspergillus phoenicis* (PDB id =1rds).

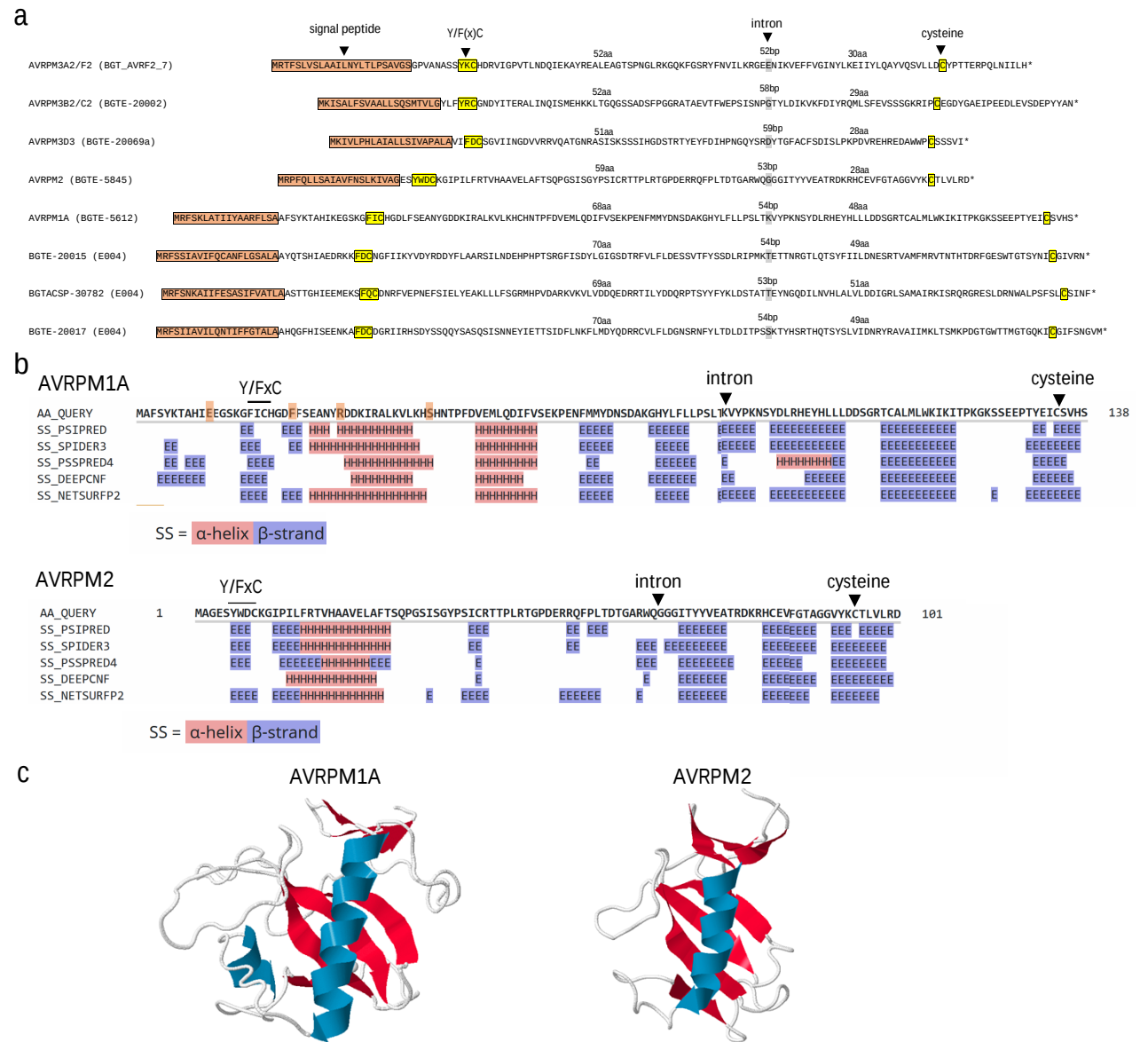


Table S1 Summary of candidate effector genes and the encoded proteins in the genetic confidence interval underlying the QTL on Bgt_chr-06 in the reference *Blumeria graminis* f. sp. *tritici* isolate 96224.

Gene Name ¹	SNPs ²	AA ³	Effector family ⁴	Pfam ⁵	Signal peptide ⁶	Size ⁷	Expression 96224 ⁸	Expression THUN-12 ⁹	logFC ¹⁰
BgtE-20040	-	-	E004	-	yes (1-21)	159	466.6	688.6	0.50
BgtE-20016	-	-	E004	-	yes (1-21)	163	155.1	190.4	0.23
BgtE-20017	-	-	E004	-	yes (1-21)	163	1457.8	947.7	-0.69
BgtE-5547	-	-	E004	-	yes (1-21)	61	22.1	20.4	-0.13
BgtAcSP-30782	2	-	E004	-	yes (1-21)	160	26.3	14.5	-0.89
BgtE-5612	5	K28E,L40F, G47R,C60S	E004	-	yes (1-18)	155	414.1	825.2	0.95
Bgt-20015	1	T94A	E004	-	yes (1-21)	160	437.6	261.2	-0.81

¹Gene name of effector candidates in *B.g. tritici* 96224 reference isolate

²Number of SNPs in isolate THUN-12 compared to isolate 96224 based on Illumina resequencing data

³Amino acid changes in isolate THUN-12 compared to isolate 96224

⁴Candidate effector family, based on effector family definition in Müller *et al.* (2019)

⁵Pfam domain annotation of mature peptide of effector proteins candidates

⁶Signal peptide prediction based on SignalP3.0, amino acids forming the signal peptide are indicated in brackets

⁷Protein size as number of amino acids

⁸Mean rpkm value of three independent biological replicates of RNAseq data of the isolate *B.g. tritici* 96224 at two days post infection (2dpi)

⁹Mean rpkm value of three independent biological replicates of RNAseq data of the isolate *B.g. triticae* THUN-12 at 2dpi

¹⁰Log-fold change of expression of isolate THUN-12 in comparison to isolate 96224, logFC-values above 1.5 or below -1.5 are considered significant

Table S2. Primers used to clone gene-synthesized *Pm1a* and *Pm1a*-HA into *Agrobacterium* compatible expression vector pIPKb004

Description	Forward primer 5'-3'	Forward site	Reverse primer 5'-3'	Reverse site	Annealing temp. (°C)	Polymerase	Amplicon length (bp)
amplification of Pm1a-fragmentA ¹	CTATCTCTCTCGAGCTTTCG CAGATC	Vector overlap	ATTAAGCTCACTGGCGTAAAT TCGATG	Intron1	65°C	Platinum SuperFi (Invitrogen)	2637
Amplification of Pm1a-fragmentB ¹	GACCATCGAATTTACGCCAG TGAG	Intron1	GATCGGGGAAATTCGAGTCAT CAC	Vector overlap	65°C	Platinum SuperFi (Invitrogen)	2262
Amplification of Pm1a-fragmentB ¹ introducing HA epitope tag	GACCATCGAATTTACGCCAG TGAG	Intron1	TTAAGCGTAATCTGGAACATC GTATGGGTAGATCACTTGGTC TTCTTGCTGC	Exon3	65°C	Platinum SuperFi (Invitrogen)	2254
Amplification of Pm1a-fragmentB-HA reintroducing vector overlap	GACCATCGAATTTACGCCAG TGAG	Intron1	GATCGGGGAAATTCGAGTCAT CACCACTTTGTACATTAAGCG TAATCTGGAACATCGTATGG	Exon3-HA	65°C	Platinum SuperFi (Invitrogen)	2289

¹ template sequence is listed in Supplementary Data File S1

Table S3 Accession numbers of immune receptor proteins used to construct the phylogenetic tree in Figure S7.

Protein accession or DOI	R gene
This study	<i>Pm1a</i>
AAC49408	<i>Prf</i>
AAC97933	<i>Mi-1</i>
AAF36987	<i>Hrt1</i>
AAF42831.1	<i>RPP13-Rld-2</i>
AAF42832.1	<i>RPP13-Nd-1</i>
AAG31014	<i>Sw-5b</i>
AAG37354	<i>Mla1</i>
AAO16000	<i>Mla13</i>
AAO43441	<i>Mla12</i>
AAQ10735	<i>Tm-2</i>
AAQ10736	<i>Tm-2²</i>
AAQ55540	<i>Mla7</i>
AAQ55541	<i>Mla10</i>
AAQ96158	<i>Pm3b</i>
AAR19096	<i>Rpg1-b</i>
AAS49213	<i>3gG2</i>
AAS79233	<i>Rp3</i>
AAT08955	<i>Ha-NTIR11g</i>
AAW48299	<i>R3a</i>
AAX31149	<i>Rxo1</i>
AAX89382	<i>Rps1k-1 and/or Rps1k-2</i>
AAZ21626	<i>Pm3a</i>
AAZ21627	<i>Pm3d</i>
AAZ33493.1	<i>Pi54 (Syn. Pik-k(h))</i>
AAZ23113	<i>Pm3f</i>
AAZ95005	<i>Rpi-blb2</i>
ABB78077.1	<i>Pm3c</i>
ABB78078.1	<i>Pm3e</i>
ABB78079.1	<i>Pm3g</i>
ABB88855	<i>Pi9</i>
ABB91438	<i>Fom-2</i>
ABC73398	<i>Piz-t</i>
ABC94599	<i>Pi2</i>
ABE68835	<i>CaMi</i>
ABS29034	<i>Lr1</i>
ABY58665.1	<i>Pm3k (TdPm3)</i>
ABY58667.1	<i>TtPm3</i>
ACB72455	<i>Pc</i>
ACI25288.1	<i>Rpi-sto1</i>

ACI25289.1	<i>Rpi-pta1</i>
ACJ66594	<i>Rpi-vnt1.1</i>
ACJ66595.1	<i>Rpi-vnt1.2</i>
ACJ66596	<i>Rpi-vnt1.3 (Syn. Rpi-phu1)</i>
ACN56757.1	<i>RPP13-UKID80</i>
ACN56765.1	<i>RPP13-UKID36</i>
ACN56766.1	<i>RPP13-UKID34</i>
ACN56776.1	<i>RPP13-UKID5</i>
ACN79513	<i>Pid3</i>
ACU65454	<i>R2-like</i>
ACU65455	<i>Rpi-abpt</i>
ACU65456	<i>R2</i>
ACU65457	<i>Rpi-blb3</i>
ACZ65484	<i>Mla2</i>
ACZ65485	<i>Mla3</i>
ACZ65486	<i>Mla8</i>
ACZ65487	<i>Mla9</i>
ACZ65490	<i>Mla18-2</i>
ACZ65492	<i>Mla22</i>
ACZ65493	<i>Mla23</i>
ACZ65495	<i>Mla27-1</i>
ACZ65496	<i>Mla27-2</i>
ACZ65497	<i>Mla28</i>
ACZ65500	<i>Mla32</i>
ACZ65501	<i>Mla34</i>
ACZ65502	<i>Mla35-1</i>
ADB07392	<i>Bph14</i>
ADF29624	<i>Pi36</i>
ADK47521	<i>Rdg2a</i>
ADU57957	<i>CYR1</i>
ADX06722	<i>TmMla1</i>
AEC47890	<i>R3b</i>
AER13157	<i>Rpp4C4</i>
AFM35701	<i>Pi25</i>
AGI99538	<i>RSG3-301</i>
AGQ17386	<i>Sr33</i>
AGP75918	<i>Sr35</i>
AGT37271	<i>RPP7</i>
AGY30894	<i>Pm8</i>
AGY30895	<i>Pm3-1B</i>
AIB02970	<i>Ph-3</i>
AIC32313	<i>Rpg1r</i>

AIU36098	VAT
AKS24975.1	Pi50
ALO61074	Sr50
AMY98955	Rpi-amr3i
ANJ02805	R8 (Syn. Rpi-smira2)
ANZ78204	Pvr4
AOR08328	Tsw
APF29096	PigmR (Syn. PigmR6)
ARO38245.1	Lr22a
ATE88995	Sr13
AUO29720	Pm60
AVK42833	Sr21
AVR54589	Pm21
AYD60116	Pm17
AYV61514	Sr46
BAA76282	Pib
BAC67706	Rcy1
BAH20862	Pit
BAJ25849	Pb1
BAJ33559	L^3
BAJ33561	L^1
BAJ33562	L^{1a}
BAJ33563	L^{1c}
BAJ33564	L^2
BAJ33565	L^{2b}
BAJ33566	L^4
BAM17521	N'
BAN59294	Pii
CAB50786	Rx1
CAB56299	Rx2
CAC29241	Mla6
CAL64731	Rcq1
CUM44200.1	Sr22
CUM44213.1	Sr45
CZT14023.1	Pm2
NP_001067618	NLS1
NP_001172592	Pish
NP_001233995	Hero A
Q9ZSD1	RGC2B (Syn. Dm3)
https://doi.org/10.1046/j.1365-313X.2002.029005569.x	NbPrf (Niben101Scf00650g02002XLOC)

Table S4 Primers used for PCR and sequencing of *Pm1a*.

Description	Forward primer 5'-3'	Forward site	Reverse primer 5'-3'	Reverse site	Annealing temp. (°C)	Polymerase	Amplicon length (bp)
STS dominant marker <i>Pm1aSTS1</i>	CAATATAAACTTCAGATGTTCT ATTCTCAAAC	Intron1	CTACATTGGCTATGCGTGTAG TC	Exon2	55	GoTaq®	333
EST marker <i>Pm1aEST1</i>	TAGCCAATAAACCTCCAAGCGC AGC	Exon2	GAAGCTCCGTGATCCGTAGTC CATG	Exon2	59	GoTaq®	732
EST marker <i>Pm1aEST2</i>	CGGCTCTGGTCTCAAGAATACT G	Exon3	G TTCAGTTCAGTTTGTGCGCCC	Exon3	55	GoTaq®	331
Large amplicon covering contig #8725	ATACTGTTGTATGACTTTATAAG GTCTTGTGC	Intron1	G TTCAGTTCAGTTTGTGCGCCC	Exon3	66	Phusion®	2964
Primary bridging of contigs #3966 and #8725	GGGCTGGGAAAGACCAC	Exon1	CCAGTTGAGAAGGGCATAAAC	Exon2	62	Phusion®	2282
Secondary bridging of contigs #3966 and #8725 (nested PCR on 1µL 1:50 dilution of primary product)	ACCACCCTCGCCATGG	Exon1	CATGCAGTCACTTTATTAATTA TTCG	Intron1	61	Phusion®	1664
Primary amplification of Exon1	CTCCGTCATCGGCAAGC	Exon1	CTAGCCAAGAGCAGGTTTTCA CAT	Intron1	63	Phusion®	1556
Secondary amplification of Exon1 (nested PCR on 1µL 1:50 dilution of primary product)	GACATGGAGGAGTGCATCGAC	Exon1	CCGCAAGCCCACCACCAAC	Intron1	66	Phusion®	1006
Sequencing primer 1	CTCCGTCATCGGCAAGC	Exon1	n/a	n/a	n/a	n/a	n/a
Sequencing primer 2	GCATCGACCGCTTCATG	Exon1	n/a	n/a	n/a	n/a	n/a
Sequencing primer 3	n/a	n/a	TGTCTTCTTCACGAACCTGGG CC	Exon1	n/a	n/a	n/a
Sequencing primer 4	n/a	n/a	CGGGCCTTGATTGATTACAA TATAATC	Exon1	n/a	n/a	n/a
Sequencing primer 5	GAGACTATCAGTGTGCGAGCTTC C	Exon1	n/a	n/a	n/a	n/a	n/a

Sequencing primer 6	n/a	n/a	AAAGCCACCATCTAGGCAAT	Intron1	n/a	n/a	n/a
Sequencing primer 7	CAATATAAACTTCAGATGTTCT ATTCTCAAAC	Intron1	n/a	n/a	n/a	n/a	n/a
Sequencing primer 8	n/a	Exon2	GTTCAGTTCAGTTTGTGCGCC	n/a	n/a	n/a	n/a
Sequencing primer 9	GTTTATGCCCTTCTCAACTGG	Exon2	n/a	n/a	n/a	n/a	n/a
Sequencing primer 10	GGATCTCCCACACCACTTGAAG ACC	Exon2	n/a	n/a	n/a	n/a	n/a
Sequencing primer 11	n/a	n/a	GAAGCTCCGTGATCCGTAGTC CATG	Exon2	n/a	n/a	n/a
Sequencing primer 12	CTCATGGATGCAGCCTGGGGAT TTG	Exon2	n/a	n/a	n/a	n/a	n/a
Sequencing primer 13	n/a	n/a	CGTCGGAGTGCTGGCATAGC	Exon2	n/a	n/a	n/a
Sequencing primer 14	GCTATGCCAGCACTCCGACG	Exon2	n/a	n/a	n/a	n/a	n/a
Sequencing primer 15	n/a	n/a	GATTTAGAAAATAGCTTAGGT GAGCAAG	Exon3	n/a	n/a	n/a
Sequencing primer 16	n/a	n/a	ATCCGTACTCTTCAACTAATT ACAG	Exon3	n/a	n/a	n/a