

**Supplementary Table 1: The 10 closest *MpMRI* homologs of *Arabidopsis thaliana*.** Shown is the output of a pBlast using the *MpMRI* (*Mapoly0051s0094*) amino acid sequence as query against the Araport11 database (carried out on <https://www.arabidopsis.org/Blast/> using standard settings). (a) Comparison of full-length amino acid sequences; (b) Comparison of kinase domain only.

<b>(a) Full-length amino acid sequences</b>			
<b>Gene</b>	<b>% identity (region spanning)</b>	<b>% similarity (region spanning)</b>	<b>E-value</b>
AT2G30740	70 (254/358)	79 (286/358)	e-147
AT3G59350	69 (253/364)	79 (290/364)	e-145
AT2G43230	69 (250/361)	79 (288/361)	e-144
AT2G47060	69 (254/363)	77 (283/363)	e-143
AT1G06700	68 (248/360)	77 (280/360)	e-141
AT3G17410	73 (248/338)	81 (274/338)	e-141
AT3G62220	67 (246/366)	78 (289/366)	e-141
AT1G48210	67 (245/365)	76 (280/365)	e-138
AT2G30730	69 (225/323)	82 (265/323)	e-133
<b>AT2G41970 (<i>AtMRI</i>)</b>	64 (233/364)	76 (278/364)	e-133
<b>(b) Kinase domain amino acid sequences</b>			
<b>Gene</b>	<b>% identity (region spanning)</b>	<b>% similarity (region spanning)</b>	<b>E-value</b>
AT2G43230	81 (230/283)	89 (254/283)	e-137
AT3G59350	80 (229/283)	89 (253/283)	e-137
AT2G30740	81 (230/283)	88 (251/283)	e-137
AT1G06700	81 (230/283)	88 (251/283)	e-136
AT3G17410	81 (229/281)	87 (247/281)	e-134
AT2G47060	79 (223/281)	86 (242/281)	e-130
AT3G62220	77 (218/281)	86 (244/281)	e-129
AT1G48210	77 (218/281)	85 (240/281)	e-128
AT2G30730	74 (212/283)	85 (241/283)	e-126
<b>AT2G41970 (<i>AtMRI</i>)</b>	73 (209/283)	86 (244/283)	e-124

**Supplementary Table 2: Segregation analysis of the *mri-1* allele in the *mri-1*/MRI background transformed with *proAtMRI:MpMRI-YFP*.** Shown are the observed numbers of T2 progeny - which were wild-type, heterozygous or homozygous for the *mri-1*-allele. Observed values of the transformed lines were compared by means of a chi-square test of independence with the expected values for a mendelian distribution or a male gametophytic transmission defect. [\*] marks data from Boisson-Dernier et al., 2015.

	MRI/MRI	<i>mri-1</i> /MRI	<i>mri-1</i> / <i>mri-1</i>	n	Ratio	p-value (two-tailed Chi-square test)
Observed ( <i>mri-1</i> /MRI) [*]	183	170	0	353	1 : 0.93 : 0	
Observed ( <i>mri-1</i> /MRI with <i>AtMRI-YFP</i> ) [*]	23	38	29	90	1 : 1.65 : 1.26	
Observed ( <i>mri-1</i> /MRI with <i>MpMRI-YFP</i> )	69	124	7	200	1 : 1.80 : 0.10	
Expected (Mendelian distribution)	50	100	50	200	1 : 2 : 1	<0.001
Expected (Male gametophytic defect)	100	100	0	200	1 : 1 : 0	<0.001

**Supplementary Table 3:** Structural *AtMRI* variants and the effect of their expression on male transmission of the *mri-1* allele in the progeny of heterozygous *Arabidopsis mri-1/MRI* plants. All fusion proteins are under the control of the pollen specific promoter *proLat52*. Grey, negative control. Green, positive control. Blue, overactive variant. Orange, variants related to K100. Yellow, variants related to T239. [\*] marks data from Boisson-Dernier et al., 2015.

Transgene	MRI/MRI	<i>mri-1</i> /MRI	<i>mri-1/mri-1</i>
<i>AtANX1</i> -YFP	47	49	0
<i>AtMRI</i> -YFP [*]	23	38	29
<i>AtMRI</i> <sup>R240C</sup> -YFP	28	43	17
<i>AtMRI</i> <sup>K100N</sup> -CFP	23	49	13
<i>AtMRI</i> <sup>K100E</sup> -CFP	29	50	12
<i>AtMRI</i> <sup>T239A</sup> -CFP	31	47	18
<i>AtMRI</i> <sup>T239E</sup> -CFP	25	47	20

**Supplementary Table 4: Pollen tube growth inhibition upon expression of *AtMRI*, *MpMRI* and their R240C structural variants.** Shown is the effect of hemizygous transgene expression on the percentage of fluorescent pollen grains and tubes. Each entry represents an independent transgenic line.

Transgene		Fluorescent pollen grains [%]	n	Fluorescent pollen tubes [%]	n
<i>proAtMRI:MpMRI</i> -YFP (hemiz.)	# 1	48	104	55	698
	# 2	48	71	54	693
	# 3	50	64	48	728
<i>proAtMRI:MpMRI<sup>R240C</sup></i> -YFP (hemiz.)	# 1	52	386	43	317
	# 2	56	424	41	250
	# 3	53	489	37	412
<i>proLat52:AtMRI</i> -CFP (hemiz.)		58	110	16	438
<i>proLat52:AtMRI<sup>R240C</sup></i> -CFP (hemiz.)		39	337	0	250

**Supplementary Table 5: Genetically modified *Arabidopsis thaliana* lines.** Lines are separated into mutant and transgenic lines. (a) Mutant lines, including targeted genetic locus, plant ecotype and molecular markers. (b) Transgenic lines, including transformed construct, genotyping primers and plant selection markers.

(a) Mutant lines	Targeted locus	Ecotype	Molecular markers
<i>mri-1</i> /MRI ( <i>qrt</i> ), (CSHL_GT21229)	AT2G41970	Ler originally but outcrossed 4 times with Col-0 (Boisson-Dernier et al., 2015).	PCR (MRI): ABD681/ABD682
			PCR ( <i>mri-1</i> ): ABD682/ABD747
			Selection <i>mri-1</i> : Kanamycin
<i>rbohH-3 rbohJ-3</i> , (SALK_136917; SALK_050665)	AT5G60010 AT3G45810	Col-0	See Boisson-Dernier et al., 2013
(b) Transgenic lines	Transformed construct	Genotyping primers	Plant selection markers
Col-0 with <i>proAt</i> MRI: <i>At</i> MRI <sup>R240C</sup> -YFP	pABD85	Construct: ABD766/ABD736	Construct: Basta
Col-0 with <i>proAt</i> MRI: <i>Mp</i> MRI-YFP	pJW20	Construct: ABD766/JW35	Construct: Basta
Col-0 with <i>proAt</i> MRI: <i>Mp</i> MRI <sup>R240C</sup> -YFP	pJW36	Construct: ABD766/JW35	Construct: Basta
Col-0 with <i>proLat52:At</i> MRI <sup>R240C</sup> -CFP	pABD49	Construct: ABD766/JW35	Construct: Basta
<i>mri-1</i> ( <i>qrt</i> ) with <i>proLat52:At</i> MRI <sup>R240C</sup> -CFP	pABD49	<i>mri-1</i> : ABD682/ABD747	Mutant: Kanamycin
		Construct: ABD766/JW35	Construct: Basta
<i>mri-1</i> ( <i>qrt</i> ) with <i>proAt</i> MRI: <i>At</i> MRI-YFP	pABD84	<i>mri-1</i> : ABD682/ABD747	Mutant: Kanamycin
		Construct: ABD766/ABD736	Construct: Basta
<i>mri-1</i> ( <i>qrt</i> ) with <i>proAt</i> MRI: <i>Mp</i> MRI-YFP	pJW20	<i>mri-1</i> : ABD682/ABD747	Mutant: Kanamycin
		Construct: ABD766/JW35	Construct: Basta
<i>mri-1</i> ( <i>qrt</i> ) with <i>proLat52:At</i> ANX1-YFP	pABD39	<i>mri-1</i> : ABD682/ABD747	Mutant: Kanamycin
		Construct: ABD546/ABD630	Construct: Basta
<i>mri-1</i> ( <i>qrt</i> ) or <i>rbohH-3 rbohJ-3</i> with <i>proLat52:At</i> MRI-YFP	pABD46	Construct: ABD680/ABD630	Construct: Basta
<i>mri-1</i> ( <i>qrt</i> ) or <i>rbohH-3 rbohJ-3</i> with <i>proLat52:At</i> MRI <sup>R240C</sup> -YFP	pABD48	Construct: ABD680/ABD630	Construct: Basta
<i>mri-1</i> ( <i>qrt</i> ) or <i>rbohH-3 rbohJ-3</i> with <i>proLat52:At</i> MRI <sup>K100N</sup> -YFP	pABD88	Construct: ABD680/ABD630	Construct: Basta
<i>mri-1</i> ( <i>qrt</i> ) or <i>rbohH-3 rbohJ-3</i> with <i>proLat52:At</i> MRI <sup>K100E</sup> -YFP	pABD108	Construct: ABD680/ABD630	Construct: Basta
<i>mri-1</i> ( <i>qrt</i> ) or <i>rbohH-3 rbohJ-3</i> with <i>proLat52:At</i> MRI <sup>T239A</sup> -YFP	pABD89	Construct: ABD680/ABD630	Construct: Basta
<i>mri-1</i> ( <i>qrt</i> ) or <i>rbohH-3 rbohJ-3</i> with <i>proLat52:At</i> MRI <sup>T239E</sup> -YFP	pABD90	Construct: ABD680/ABD630	Construct: Basta
<i>mri-1</i> ( <i>qrt</i> ) or <i>rbohH-3 rbohJ-3</i> with <i>proLat52:At</i> MRI <sup>R240M</sup> -YFP	pABD109	Construct: ABD680/ABD630	Construct: Basta
<i>mri-1</i> ( <i>qrt</i> ) or <i>rbohH-3 rbohJ-3</i> with <i>proLat52:At</i> MRI <sup>R240A</sup> -YFP	pABD110	Construct: ABD680/ABD630	Construct: Basta
<i>mri-1</i> ( <i>qrt</i> ) or <i>rbohH-3 rbohJ-3</i> with <i>proLat52:At</i> MRI <sup>R240S</sup> -YFP	pABD111	Construct: ABD680/ABD630	Construct: Basta
<i>rbohH-3 rbohJ-3</i> with <i>proAt</i> ACA9-GFP-RbohH	pABD18	See Boisson-Dernier et al., 2013	Construct: Hygromycin

**Supplementary Table 6: Genetically modified *Marchantia polymorpha* lines.** Lines are separated into mutant and transgenic lines. (a) Mutant lines, including targeted genetic locus, plant ecotype and molecular markers. (b) Transgenic lines, including transformed construct, genotyping primers and plant selection markers.

(a) Mutant lines	Targeted locus	Ecotype	Molecular markers
<i>Mpmri-1</i> (Honkanen et al., 2016; [1])	<i>MpMRI</i>	Tak-2	PCR ( <i>Mpmri-1</i> ): JW60/JW63 Selection (T-DNA): Hygromycin
<i>Mpfer-1</i> (Honkanen et al., 2016; [1])	<i>MpFER</i>	Tak-1	PCR ( <i>MpFER</i> ): JW52/JW59 PCR ( <i>Mpfer-1</i> ): JW52/JW64 Selection (T-DNA): Hygromycin
(b) Transgenic lines	Transgene	Genotyping primers	Plant selection markers
<i>Mpmri-1</i> with <i>proMpEF1α:AtMRI-RFP</i>	pJW10	<i>Mpmri-1</i> : JW60/JW63 Construct: JW17/ABD736	Construct: Chlorsulfuron Mutant: Hygromycin
<i>Mpmri-1</i> with <i>proMpEF1α:MpMRI-RFP</i>	pJW28	<i>Mpmri-1</i> : JW60/JW63 Construct: JW17/JW35	Construct: Chlorsulfuron Mutant: Hygromycin
<i>Mpfer-1</i> (or Tak-2 x Tak-1) with <i>proMpEF1α:MpMRI<sup>R240C</sup>-RFP</i>	pJW51	<i>Mpfer-1</i> : JW52/JW64 Construct: JW17/JW35	Construct: Chlorsulfuron Mutant: Hygromycin
Tak-2 x Tak-1 with <i>proMpEF1α:AtMRI-3xCitrine</i>	pJW09	Construct: JW17/ABD736	Construct: (Gentamicin)
Tak-2 x Tak-1 with <i>proMpEF1α:MpMRI-3xCitrine</i>	pJW27	Construct: JW17/JW35	Construct: (Gentamicin)

**Supplementary Table 7: Vectors used in this study.** Vectors are separated into Gateway entry vectors/clones (a), destination vectors (b) and final expression constructs (c).

(a) Gateway entry vectors and clones

Vector ID	Full name	Purpose	Bacterial selection marker	Vector type
pABD20	<i>At</i> ANX1 (without stop) in pDONR221	Cloning of <i>At</i> ANX1 ORF	Kanamycin	Entry clone
pABD43	<i>At</i> MRI (without stop) in pDONR207	Cloning of <i>At</i> MRI ORF	Gentamicin	Entry clone
pABD44	<i>At</i> MRI <sup>R240C</sup> (without stop) in pDONR207	Cloning of <i>At</i> MRI <sup>R240C</sup>	Gentamicin	Entry clone
pABD74	<i>At</i> MRI <sup>K100N</sup> (without stop) in pDONR207	Cloning of <i>At</i> MRI <sup>K100N</sup>	Gentamicin	Entry clone
pABD75	<i>At</i> MRI <sup>T239A</sup> (without stop) in pDONR207	Cloning of <i>At</i> MRI <sup>T239A</sup>	Gentamicin	Entry clone
pABD76	<i>At</i> MRI <sup>T239E</sup> (without stop) in pDONR207	Cloning of <i>At</i> MRI <sup>T239E</sup>	Gentamicin	Entry clone
pABD100	<i>At</i> MRI <sup>K100E</sup> (without stop) in pDONR207	Cloning of <i>At</i> MRI <sup>K100E</sup>	Gentamicin	Entry clone
pABD101	<i>At</i> MRI <sup>R240A</sup> (without stop) in pDONR207	Cloning of <i>At</i> MRI <sup>R240A</sup>	Gentamicin	Entry clone
pABD102	<i>At</i> MRI <sup>R240M</sup> (without stop) in pDONR207	Cloning of <i>At</i> MRI <sup>R240M</sup>	Gentamicin	Entry clone
pABD103	<i>At</i> MRI <sup>R240S</sup> (without stop) in pDONR207	Cloning of <i>At</i> MRI <sup>R240S</sup>	Gentamicin	Entry clone
pJW18	<i>Mp</i> MRI (without stop) in pDONR207	Cloning of <i>Mp</i> MRI	Gentamicin	Entry clone
pJW35	<i>Mp</i> MRI <sup>R240C</sup> (without stop) in pDONR207	Cloning of <i>Mp</i> MRI <sup>R240C</sup>	Gentamicin	Entry clone

(b) Gateway destination vectors

Vector ID	Full name	Purpose	Bacterial selection marker	Plant selection marker
pABD34	<i>proLat52:GW-YFP</i>	Pollen-specific expression in <i>Arabidopsis</i>	Spectinomycin	Basta
pABD35	<i>proLat52:GW-CFP</i>	Pollen-specific expression in <i>Arabidopsis</i>	Spectinomycin	Basta
pABD83	<i>proAtMRI:GW-YFP</i>	pollen- and root hair-specific expression in <i>Arabidopsis</i>	Spectinomycin	Basta
pABD106	<i>proMpEF1α:GW-3xCitrine</i> (pMpGWB224; Ishizaki et al., 2015)	ubiquitous expression in <i>Marchantia</i>	Spectinomycin	Gentamicin
pABD107	<i>proMpEF1α:GW-RFP</i> (pMpGWB327; Ishizaki et al., 2015)	ubiquitous expression in <i>Marchantia</i>	Spectinomycin	Chlorsulfuron

(c) Binary and Gateway expression vectors

Vector ID	Full name	Entry clone and destination vector	Bacterial selection marker	Plant selection marker
pABD18	<i>proAtACA9-GFP-RbohH</i>	n/a	Kanamycin	Hygromycin
pABD39	<i>proLat52:AtANX1-YFP</i>	pABD20/pABD34	Spectinomycin	Basta
pABD46	<i>proLat52:AtMRI-YFP</i>	pABD43/pABD34	Spectinomycin	Basta

pABD47	<i>proLat52:AtMRI</i> -CFP	pABD43/pABD35	Spectinomycin	Basta
pABD48	<i>proLat52:AtMRI<sup>R240C</sup></i> -YFP	pABD44/pABD34	Spectinomycin	Basta
pABD49	<i>proLat52:AtMRI<sup>R240C</sup></i> -CFP	pABD44/pABD35	Spectinomycin	Basta
pABD84	<i>proAtMRI:AtMRI</i> -YFP	pABD43/pABD83	Spectinomycin	Basta
pABD85	<i>proAtMRI:AtMRI<sup>R240C</sup></i> -YFP	pABD44/pABD83	Spectinomycin	Basta
pABD88	<i>proLat52:AtMRI<sup>K100N</sup></i> -CFP	pABD74/pABD35	Spectinomycin	Basta
pABD89	<i>proLat52:AtMRI<sup>T239A</sup></i> -CFP	pABD75/pABD35	Spectinomycin	Basta
pABD90	<i>proLat52:AtMRI<sup>T239E</sup></i> -CFP	pABD76/pABD35	Spectinomycin	Basta
pABD108	<i>proLat52:AtMRI<sup>K100E</sup></i> -CFP	pABD100/pABD35	Spectinomycin	Basta
pABD109	<i>proLat52:AtMRI<sup>R240A</sup></i> -CFP	pABD101/pABD35	Spectinomycin	Basta
pABD110	<i>proLat52:AtMRI<sup>R240M</sup></i> -CFP	pABD102/pABD35	Spectinomycin	Basta
pABD111	<i>proLat52:AtMRI<sup>R240S</sup></i> -CFP	pABD103/pABD35	Spectinomycin	Basta
pJW09	<i>proMpEF1α:AtMRI</i> -3xCitrine	pABD43/pABD106	Spectinomycin	Gentamicin
pJW10	<i>proMpEF1α:AtMRI</i> -RFP	pABD43/pABD107	Spectinomycin	Chlorsulfuron
pJW20	<i>proAtMRI:MpMRI</i> -YFP	pJW18/pABD83	Spectinomycin	Basta
pJW27	<i>proMpEF1α:MpMRI</i> -3xCitrine	pJW18/pABD106	Spectinomycin	Gentamicin
pJW28	<i>proMpEF1α:MpMRI</i> -RFP	pJW18/pABD107	Spectinomycin	Chlorsulfuron
pJW36	<i>proAtMRI:MpMRI<sup>R240C</sup></i> -YFP	pJW35/pABD83	Spectinomycin	Basta
pJW51	<i>proMpEF1α:MpMRI<sup>R240C</sup></i> -RFP	pJW35/pABD107	Spectinomycin	Chlorsulfuron



**Supplementary Table 8: Oligonucleotides used in this study.**

Primer ID	Full name	Nucleotide Sequence (5' to 3')	Purpose
ABD546	ANX1-F5	GAAATTTGCAGACACGGCGGAG	Genotyping of <i>AtANX1</i> ORF
ABD630	eYFP-R	AAGCACTGCAGGCCGTAGC	Genotyping of ORF-YFP fusions
ABD681	GT21229-F1	TTCGGCTACCACGCTCCAGA	Genotyping of <i>MRI/mri-1</i>
ABD682	GT21229-R1	GGACCGGCCGTTTAGAGTT	Genotyping of <i>MRI/mri-1</i>
ABD698	mCFP-F	CGAGGAGCTGTTCACCGGGG	Genotyping of CFP- or YFP-ORF fusions
ABD699	mCFP-R	CCTCGAACTTACCTCGGCGC	Genotyping of ORF-CFP fusions
ABD747	Ds5-2	TCCGTTCCGTTTTCTGTTTTTAC	Genotyping of <i>mri-1</i> /MRI
ABD766	pA <i>MRI</i> -F	TTGTTTCCCAGCTTTATCGCGG	Genotyping of <i>proA</i> <i>MRI</i> -ORF fusions
ABD756	MRI_K100N_F	GGAGAAGCTGTTGCTATCAATAAACTTGATGCTAGTTCTTC	Site-directed mutagenesis
ABD757	MRI_K100N_R	GAAGAAGCTAGCATCAAGTTTATTGATAGCAACAGCTTCTCC	Site-directed mutagenesis
ABD758	MRI_T239A_F	GGCTAGGCTTCATTCTGCTCGTGTGTTTGGGAAC	Site-directed mutagenesis
ABD759	MRI_T239A_R	GTTCCCAAACACGAGCAGAATGAAGCCTAGCC	Site-directed mutagenesis
ABD760	MRI_T239E_F	CGCGGCTAGGCTTCATTCTGAGCGTGTGTTTGGGAACATTC	Site-directed mutagenesis
ABD761	MRI_T239E_R	GAATGTTCCCAAACACGCTCAGAATGAAGCCTAGCCGCG	Site-directed mutagenesis
ABD800	MRI_R240A_F	CTAGGCTTCATTCTACTGCTGTTTTGGGAACATTCGG	Site-directed mutagenesis
ABD801	MRI_R240A_R	CCGAATGTTCCCAAACAGCAGTAGAATGAAGCCTAG	Site-directed mutagenesis
ABD802	MRI_R240S_F	CTAGGCTTCATTCTACTTCTGTTTTGGGAACATTCGG	Site-directed mutagenesis
ABD803	MRI_R240S_R	CCGAATGTTCCCAAACAGAAAGTAGAATGAAGCCTAG	Site-directed mutagenesis
ABD804	MRI_R240M_F	GCTAGGCTTCATTCTACTATGGTTTTGGGAACATTCGGC	Site-directed mutagenesis
ABD805	MRI_R240M_R	GCCGAATGTTCCCAAACCATAGTAGAATGAAGCCTAGC	Site-directed mutagenesis
ABD808	MRI_K100E_F	GAAGCTGTTGCTATCGAAAACTTGATGCTAG	Site-directed mutagenesis
ABD809	MRI_K100E_R	CTAGCATCAAGTTTTTCGATAGCAACAGCTTC	Site-directed mutagenesis
JW17	Mp_pEF1-F	GCAGTGGAGCGTCTGGCTTA	Genotyping of <i>proMpEF1<math>\alpha</math></i> -ORF fusions
JW25	MpMRI_fwd_w/attB1	GGGGACAAGTTTGTACAAAAAAGCAGGC TTAATGGCATGGTGTGTTGCTG	Amplification of <i>MpMRI</i> ORF with attB1-site
JW26	MpMRI_rev_w/attB2	GGGGACCACTTTGTACAAGAAAGCTGGG TTCCATCCCGTGTGGGTGATC	Amplification of <i>MpMRI</i> ORF with attB2-site and without stop-codon
JW34	MpMRI_CDS_F1	GCAACTCCAAGCTGAGTGA	Genotyping of <i>MpMRI</i> ORF
JW35	MpMRI_CDS_R1	CCTTGCCACCTCCATGCAT	Genotyping of <i>MpMRI</i> ORF
JW45	Mp_rbm27_F1	CCAAGTGCGGGCAGAATCAAGT	Genotyping of male <i>Marchantia</i> plants
JW46	Mp_rbm27_R1	TTCATCGCCCGCTATCACCTTC	Genotyping of male

			Marchantia plants
JW47	Mp_rhf73_F1	TGACGACGAAGATGTGGATGAC	Genotyping of female Marchantia plants
JW48	Mp_rhf73_R1	GAAACTTGGCCGTGTGACTGA	Genotyping of female Marchantia plants
JW52	MpFER_3'UTR_R1	CACTCCCAAATGAACGCACG	Genotyping of <i>Mpfer-1</i> / <i>MpFER</i>
JW59	MpFER_3'UTR_F2	CGTGCCCTCTGTCTCCTGTTCC	Genotyping of <i>Mpfer-1</i> / <i>MpFER</i>
JW60	MpMRI_5'UTR_R1	GCGGGCCTTGACTGCCTCT	Genotyping of <i>Mpmri-1</i> / <i>MpMRI</i>
JW63	pCambia1300_RB1	CCTGCAGGCATGCAAGCTTGG	Genotyping of <i>Mpmri-1</i> / <i>MpMRI</i>
JW64	pCambia1300_RB2	GCTGGCGTAATAGCGAAGAGG	Genotyping of <i>Mpmri-1</i> / <i>MpMRI</i>