

## SUPPORTING INFORMATION (SI) APPENDIX

### SUPPORTING TABLES:

**S1 Table. Comparison of *P. palmivora* transcriptomes**

	<i>In planta</i> upregulated transcripts in <i>LILI/N. benthamiana</i> [1] and ARI/Marchantia	Transcripts upregulated only in ARI/Marchantia
<b>Overall</b>	784(37%)	1347
<b>Candidate Secreted Proteins</b>	219(53%)	196
<b>Candidate RXLR Effectors</b>	42(45%)	52

**S2 Table. Plants Used in This Study**

Name/Strain	Species	Reference/Source
<b>TAK1</b>	<i>Marchantia polymorpha</i>	Jim Haseloff (University of Cambridge)
<i>nop1</i>	<i>Marchantia polymorpha</i>	[2] Ishizaki et al. 2013
<i>MpSYP13A:mCitrine-MpSYP13A</i>	<i>Marchantia polymorpha</i>	[3] Kanazawa et al. 2016
<i>MpSYP13B:mCitrine-MpSYP13B</i>	<i>Marchantia polymorpha</i>	[3] Kanazawa et al. 2016
<i>35S:mCitrine-MpRab7</i>	<i>Marchantia polymorpha</i>	This Study
<i>35S:mCitrine-MpRab11A</i>	<i>Marchantia polymorpha</i>	This Study
<i>MpPRX:GUS</i>	<i>Marchantia polymorpha</i>	This Study
<i>N/A</i>	<i>Marchantia paleacea</i>	Pierre-Marc Delaux (Paul Sabatier University)
<i>N/A</i>	<i>Lunularia cruciata</i>	Pierre-Marc Delaux (Paul Sabatier University)

**S3 Table. Pathogen Strains Used in This Study**

Organism	Strain (Accession)	Reference
<i>Phytophthora infestans</i>	Pi88069-td	[4] Chaparro-Garcia et al. 2011
<i>Phytophthora palmivora</i>	ARI-tdTomato (P3914)	[5] Le Fevre et al. 2016
<i>Phytophthora palmivora</i>	ADA (P7551)	[5] Le Fevre et al. 2016
<i>Phytophthora palmivora</i>	ALOHA (P6053)	[5] Le Fevre et al. 2016
<i>Phytophthora palmivora</i>	FLIMA (P7545)	[5] Le Fevre et al. 2016
<i>Phytophthora palmivora</i>	FLIP (P7548)	[5] Le Fevre et al. 2016
<i>Phytophthora palmivora</i>	FRED (P7547)	[5] Le Fevre et al. 2016
<i>Phytophthora palmivora</i>	JAKO (P3738)	[5] Le Fevre et al. 2016
<i>Phytophthora palmivora</i>	MAZI (P6375)	[5] Le Fevre et al. 2016
<i>Phytophthora palmivora</i>	TAZI (P6802)	[5] Le Fevre et al. 2016
<i>Phytophthora palmivora</i>	STITCH (P0113)	[5] Le Fevre et al. 2016

**S4 Table. Primers Used in This Study**

Primer Name	Sequence (5-3)	Reference
<i>qRT-PCR Primers:</i>		
MpACT-qF	AGGCATCTGGTATCCACGAG	[6] Saint-Marcoux et al. 2015
MpACT-qR	ACATGGTCGTTCCCTCCAGAC	[6] Saint-Marcoux et al. 2015
MpEF1a-qF	CCGAGATCCTGACCAAGG	[6] Saint-Marcoux et al. 2015
MpEF1a-qR	GAGGTGGGTACTCAGCGAAG	[6] Saint-Marcoux et al. 2015
MpSYP13A-qF	CCAGGTTTCAGCAGTAAGCCA	This Study
MpSYP13A-qR	TTTCTCTCTACCGTTCCCG	This Study
MpSYP13B-qF	CGAAATGAGCACGAACGGGA	This Study
MpSYP13B-qR	CCAGCTTCTTCAGCAGCGAC	This Study
MpPRX-qF	ATTCGATTGCTTCCGAGGC	This Study
MpPRX-qR	AATCCACATCCCCGAAGTT	This Study
MpDIR-qF	AGACTTCGGTTTCCAGCTGA	This Study
MpDIR-qR	TATGCCTCCTTGCTTCCAC	This Study
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Ppal_03573p06550_06960qR	ATCGAGTCGTCAGTGTAGG	This Study
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Ppal_08171n02473_03033qR	GGCGGATATCTACATCACCTTC	This Study
Ppal_05494p02665_03135qF	ACCAAGGAACAACCGTTTGCC	This Study
Ppal_05494p02665_03135qR	CCTTGCCGCTTATGAGTAGAGT	This Study
Ppal_06531n05739_06188qF	GACTGATCAAGCACACCACACT	This Study
Ppal_06531n05739_06188qR	AGCTGGTCAGGAATCTCTCTGT	This Study
Ppal_03660p04152_04550qF	CAGTTCTTACAAGCGTCATGC	This Study
Ppal_03660p04152_04550qR	TCTGTCCGATCGTTGTTTTG	This Study
Ppal_18465n22905_2647qF	TGCTTTTGTGGAGTGTACTGCT	This Study
Ppal_18465n22905_2647qR	CGTTAAATCCGCTGTTATGTCA	This Study
PpEF1a-qF	CAAGATCCCGTTCGTGCCTA	[5] Le Fevre et al. 2016
PpEF1a-qR	GCGTTCAGGTTGTCAAGAGC	[5] Le Fevre et al. 2016
PpWS21-qF	CTCCAGAACGTGTACATTCG	[1] Evangelisti et al. 2017
PpWS21-qR	TGGCACCCCTTCTCCTCGG	[1] Evangelisti et al. 2017
PpCdc14-qF	TCTGCACGAGTTCAGCATT	[5] Le Fevre et al. 2016
PpCdc14-qR	CACCACTAGCGTCACGTTCT	[5] Le Fevre et al. 2016
PpHmp1-qF	TGCCATTCTTGATCTGCCGT	[5] Le Fevre et al. 2016
PpHmp1-qR	GACGATGCGAAAAGGGCTTC	[5] Le Fevre et al. 2016
REX1-qF	TCTCTTATCCAGACGAGCAACA	[1] Evangelisti et al. 2017
REX1-qR	TGACGTAGCCCTTGTAGATTGA	[1] Evangelisti et al. 2017
REX3-qF	AGTCCAAGAAGGATTTGACGAC	[1] Evangelisti et al. 2017
REX3-qR	TTCTCTAACGCATTAGCCTTCC	[1] Evangelisti et al. 2017
REX4-qF	CAGAACGACAACGAATGGTATC	[1] Evangelisti et al. 2017
REX4-qR	TCGGCAATAAGCCTTTAAATTG	[1] Evangelisti et al. 2017
PiWS21-qF	CTCCAAAACGTGTACATCCGTAAGTGC	This Study
PiWS21-qR	CCTTAGCACCCCTTCTCCTCAGCACC	This Study
PiCdc14-qF	TCTGCACGAGTTCAGCACTATGAAC	This Study
PiCdc14-qR	CCTGCAAAGGCTATGAACTGGGTG	This Study
PiHmp1-qF	CATGATGGCTGTATGGTCGGTGAGG	[7] Schoina et al. 2017
PiHmp1-qR	TTAGCTAACATTCAAGCGAGCATGAAG	[7] Schoina et al. 2017
PiAVR3a-qF	CGCCATAAACTTTGCAACCA	[7] Schoina et al. 2017
PiAVR3a-qR	TGCCGGCTGAATCGTGTAT	[7] Schoina et al. 2017
PiAVRblb2-qF	CGTCGCAGCATTCCCAAT	[7] Schoina et al. 2017
PiAVRblb2-qR	GCCACAGTGTCAAGGAGATGTCTT	[7] Schoina et al. 2017
<i>Cloning Primers:</i>		
MpRab7-B1F	AAAAAGCAGGCTcATGTCAGCTCGTAAACGAACTCTG	This Study
MpRab7-B2R	AGAAAGCTGGGTTTCAGCATTACAGACAGATGACT	This Study
MpRab11A-B1F	AAAAAGCAGGCTcATGGCTTATAGATCCGACGATG	This Study
MpRab11A-B2R	AGAAAGCTGGGTTTACGCTGAGCAACATCCTACT	This Study

## SUPPORTING METHODS

### Histochemical Staining

GUS staining was performed in mock (water) or infected (*P. palmivora* ARI-td) plants 4 days post inoculation (dpi) essentially as described in [1], except that *M. polymorpha* thalli (TAK1 and *MpPRX*:GUS) were vacuum infiltrated with the GUS staining solution. Thalli were de-stained in a solution containing 70% ethanol and 20-30% glycerol. De-stained thalli were sectioned using a vibratome and imaged using the 3D display option on a Keyence digital microscope. DAB (diaminobenzidine) staining for the detection of H<sub>2</sub>O<sub>2</sub> was performed on mock (water) or infected (*P. palmivora* ARI-td) plants 4 days post inoculation (dpi) as described in [8]. Three-week old *M. polymorpha* plants were used for all histochemical staining experiments.

### Phylogenetic Analysis

Amino acid sequences retrieved from phytozome (<https://phytozome.jgi.doe.gov/pz/portal.html>) and MarpolBase ([www.marchantia.info](http://www.marchantia.info)) were aligned using MUSCLE. The evolutionary history was inferred by the Maximum Likelihood method utilizing the Jones, Taylor & Thornton model with discrete Gamma distribution using MEGA 6 [9]. Ten thousand bootstrap replicates were conducted, and percent bootstrap values were placed on corresponding branches. The tree was drawn in MEGA 6.0

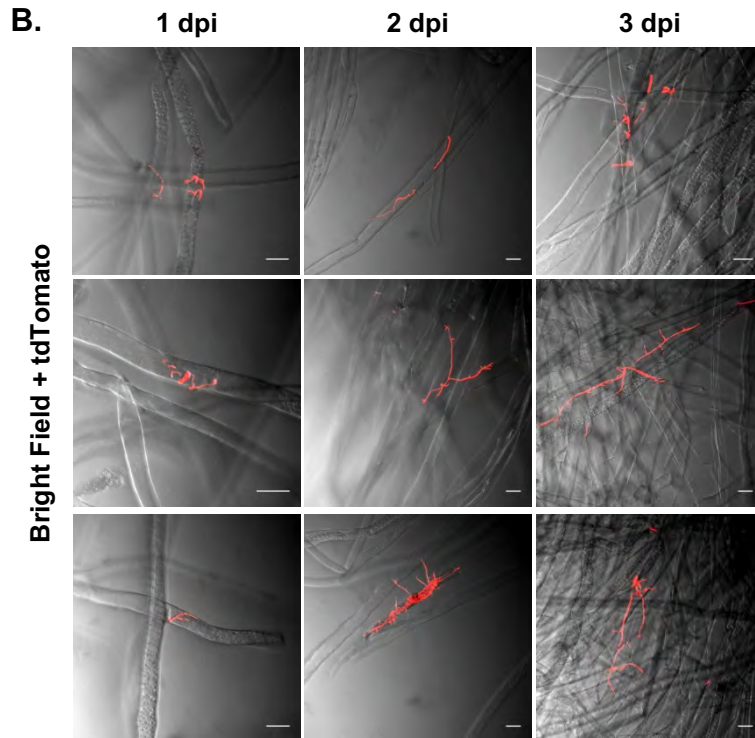
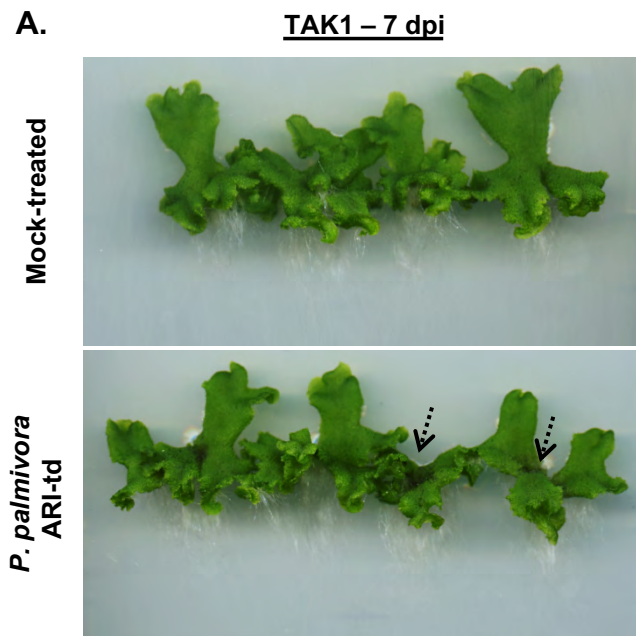
### Comparison of *P. palmivora* expression patterns in *M. polymorpha* and *N. benthamiana* pathosystems

To compare *P. palmivora* expression patterns in *M. polymorpha* and *N. benthamiana* [1] pathosystems we applied standardized analysis approach. Reads from *P. palmivora* LILI - *N. benthamiana* experiment were mapped back to the same *P. palmivora* reference genome [10] using STAR (version 2.5.2b) aligner [11]. Similar to *P. palmivora*-ARI *M. polymorpha* analysis, raw counts were obtained with FeatureCounts [12]. Uniquely mapped and properly paired reads were considered further. Differentially expressed genes were obtained applying EdgeR methodology [13,14] for both *P. palmivora*-LILI *N. benthamiana* and *P. palmivora*-ARI *M. polymorpha* datasets. In the absence of replicates in *P. palmivora*-LILI *N. benthamiana* dataset 6-18-24 hai and 48-72 hai samples were treated as pseudo-replicates for biotrophic and necrotrophic stages. To get a subset of genes up-regulated *in planta* we compared infected samples against respective *ex-planta*

stage: MZ - for *P. palmivora*-LILI *N. benthamiana* and MYC - for *P. palmivora*-ARI *M. polymorpha*. Lists of *in planta* up-regulated genes (LFC  $\geq$  2, adjusted p-value  $<$   $10^{-3}$ ) in both pathosystems were compared using UpSetR package [15].

***MpPRX* (Mapoly0106s0049) Promoter + 5' UTR:**

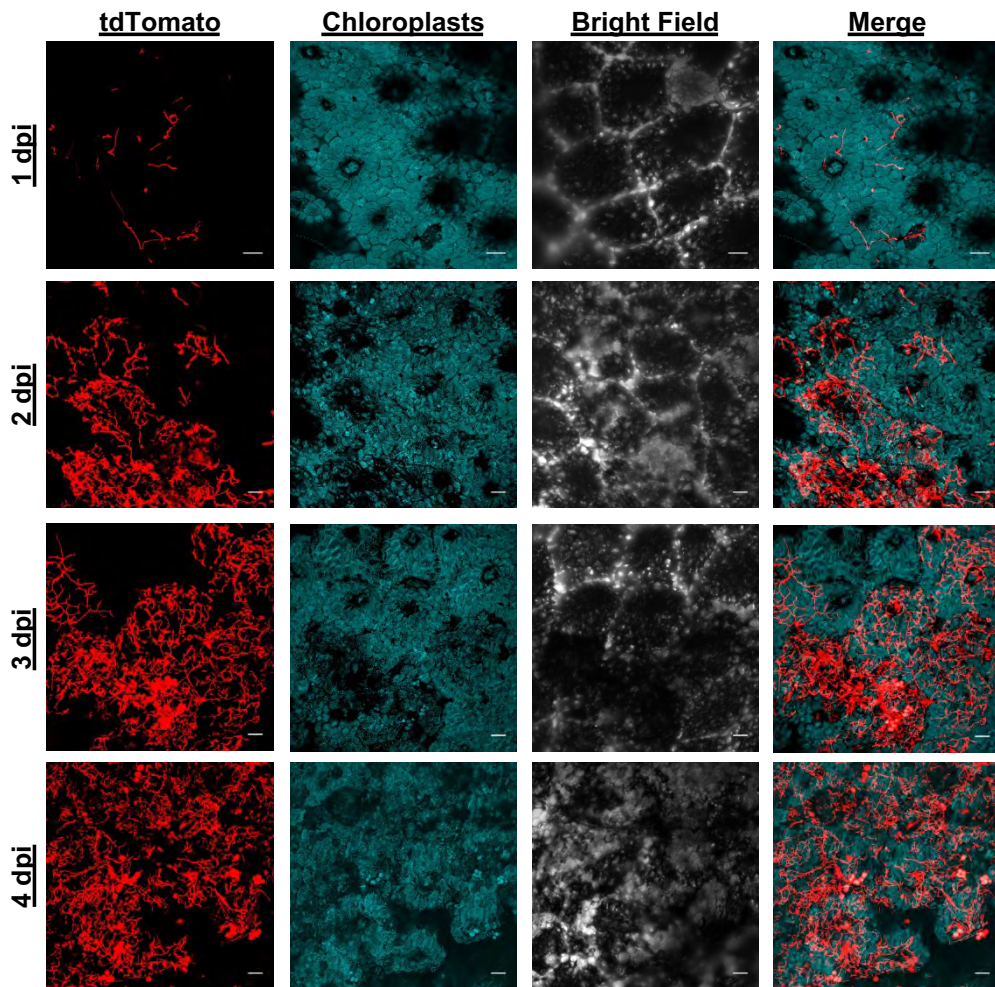
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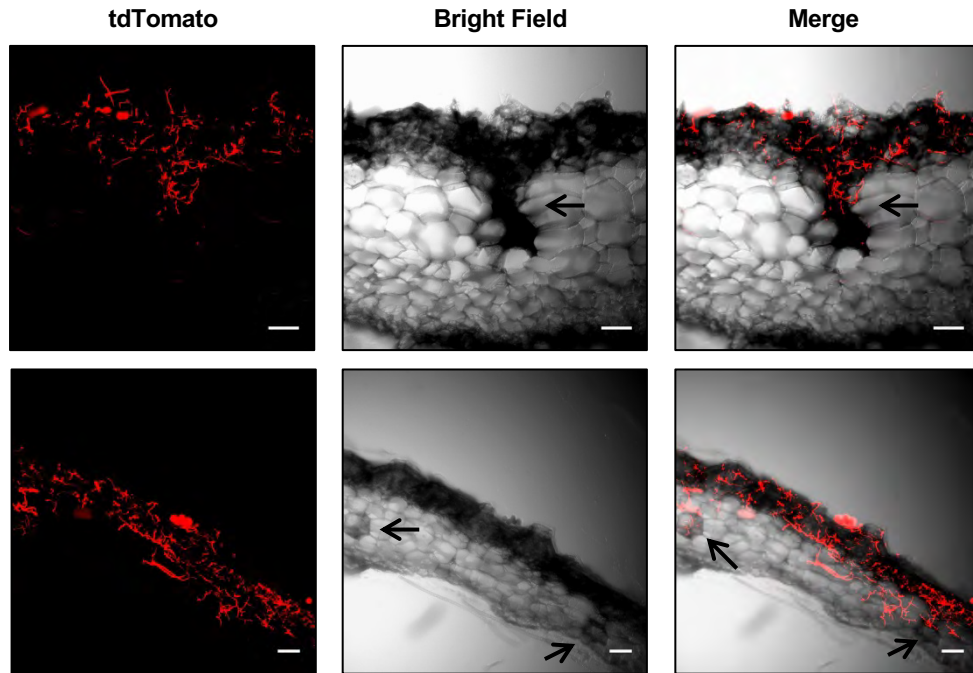
**S1 Fig. Rhizoid inoculations do not reliably lead to colonization**

**(A)** Disease symptoms at 7 days post inoculation (dpi) of 3-week-old *M. polymorpha* (TAK1) plants that were inoculated with *P. palmivora* ARI-td zoospores or water directly onto rhizoids.

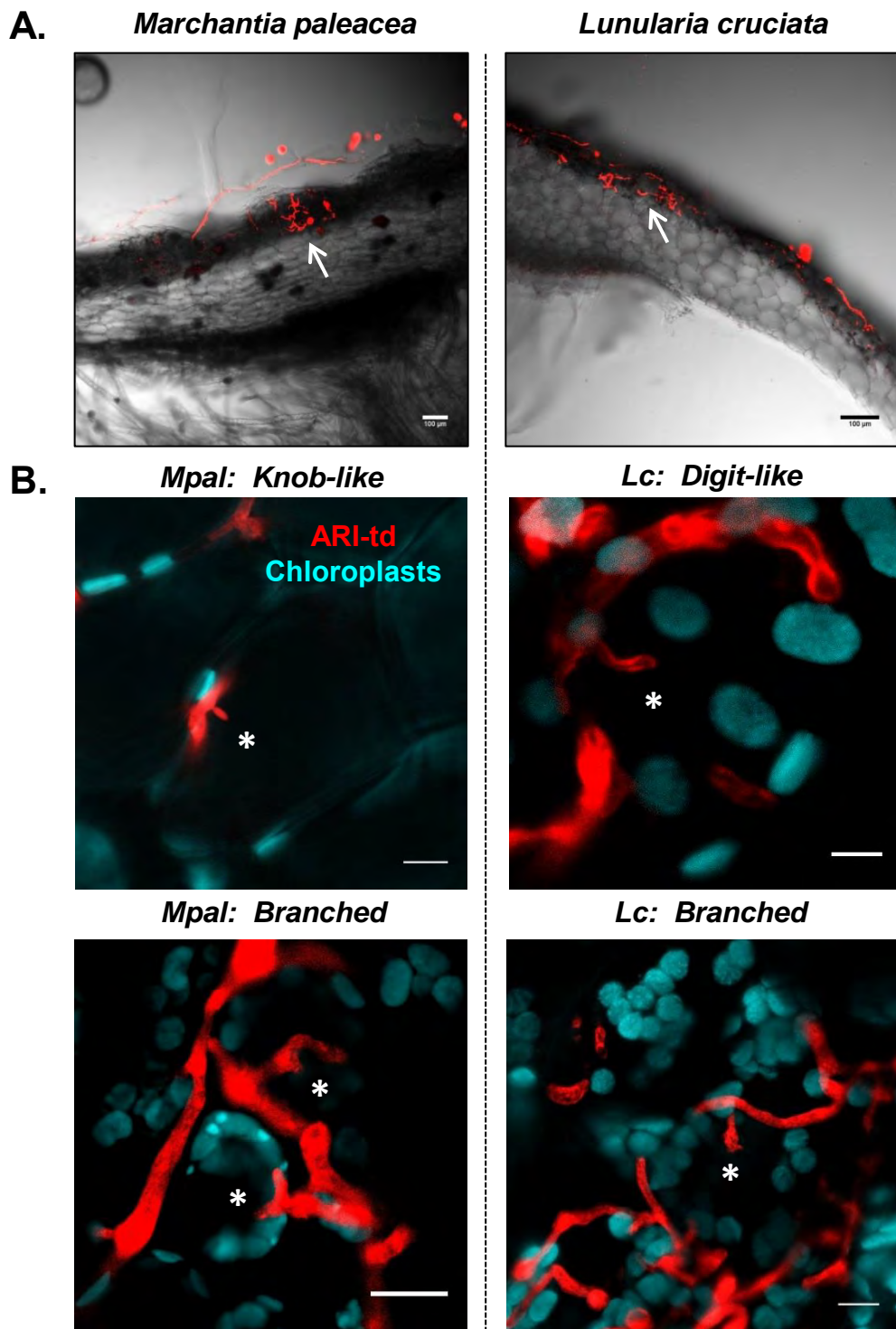
**(B)** Confocal fluorescence microscopy demonstrating *P. palmivora* ARI-td growth on 3-week-old *M. polymorpha* rhizoids from 1-3 dpi. Micrographs represent z-stack projections of merged bright field and tdTomato channels. Scale bars = 50  $\mu$ m.



**S2 Fig. *P. palmivora* colonizes TAK1 thalli.** Confocal fluorescence microscopy demonstrating *P. palmivora* ARI-td growth on 3-week-old *M. polymorpha* thalli from 1-4 days post inoculation (dpi). Micrographs represent z-stack projections. The merged micrographs display red pathogen fluorescence (tdTomato) overlaid on top of chlorophyll autofluorescence (turquoise). Scale bars = 100  $\mu$ m.



**S3 Fig. *P. palmivora* hyphae access the storage region during necrotrophy.** Confocal fluorescence microscopy demonstrating the co-occurrence of *P. palmivora* ARI-td hyphae and necrotrophic disease symptoms in the storage region of *M. polymorpha* thalli at 7 days post inoculation (dpi). Red pathogen fluorescence is merged with bright field images. Micrographs represent z-stacked images. Scale bars = 100  $\mu$ m.



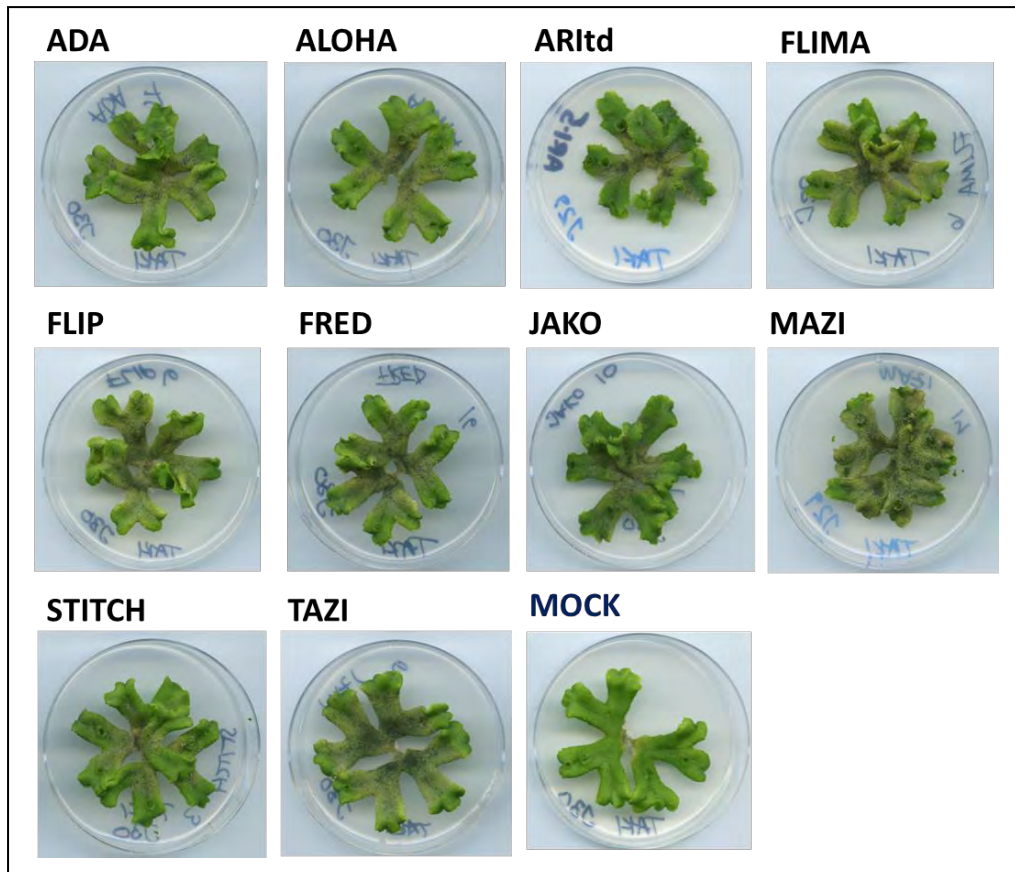
**S4 Fig. Intracellular colonization of the photosynthetic layer in *Marchantia paleacea* and *Lunularia cruciata*.**

**(A)** Confocal fluorescence microscopy of sectioned thalli of *M. paleacea* and *L. cruciata* colonized by *P. palmivora* ARI-td at 7 days post inoculation (dpi). Z-stacked images represent red pathogen fluorescence (tdTomato) merged with the bright field channel. Colonized air chambers are denoted by arrows. Scale bars = 100 µm

**(B)** Confocal fluorescence microscopy demonstrating haustoria morphology in *P. palmivora* ARI-td colonized *M. paleacea* (*Mpal*) and *L. cruciata* (*Lc*) thalli at 3 dpi. Z-stacked images display red pathogen fluorescence (ARI-td) merged with plastid autofluorescence. Scale bars = 10 µm.

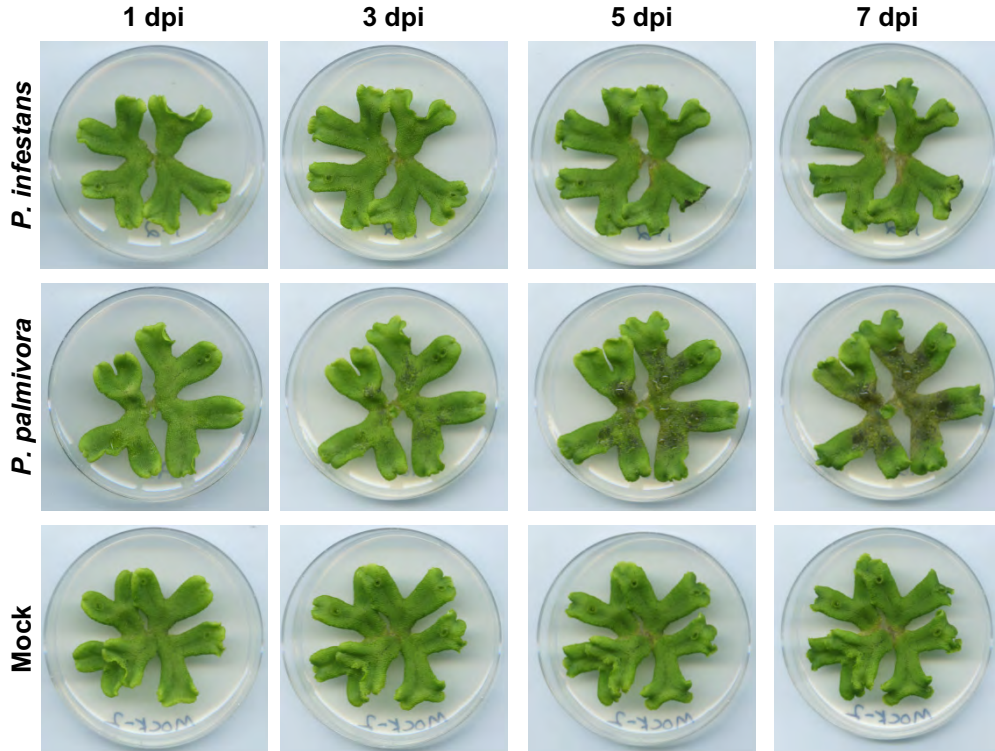


### TAK1 – 7 dpi



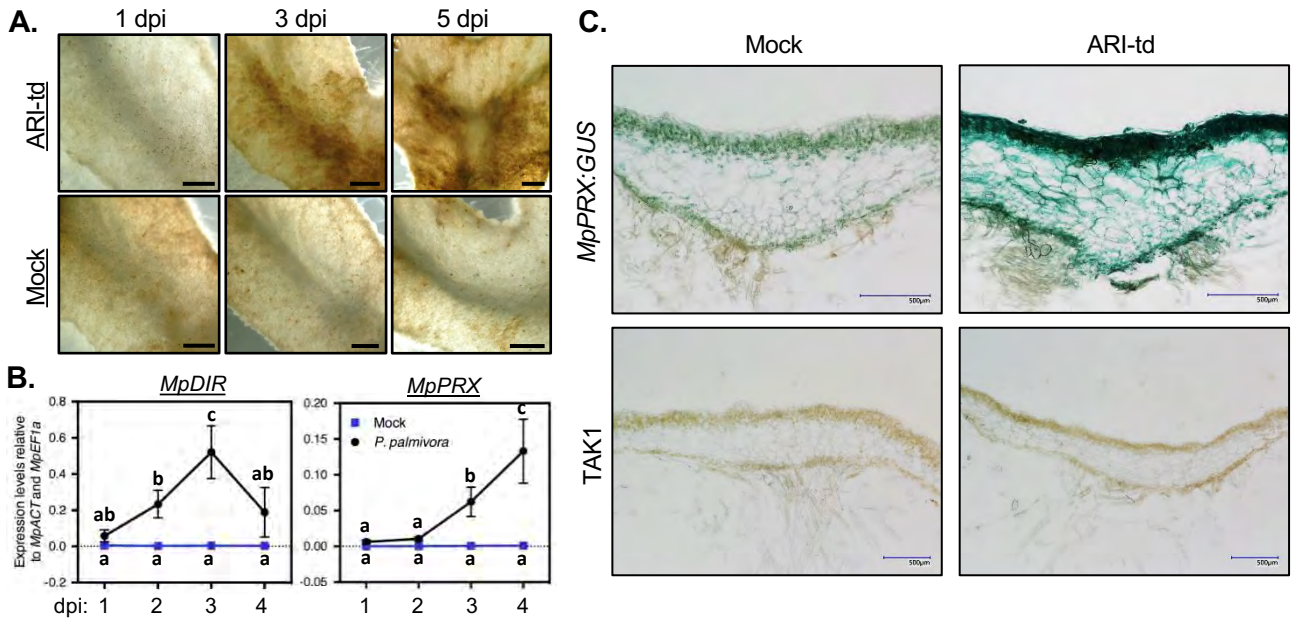
#### **S5 Fig. *P. palmivora* strains vary in aggressiveness in TAK1.**

Disease symptoms of *M. polymorpha* TAK1 plants inoculated with water (mock) or zoospores of *P. palmivora* strains 7 days post inoculation (dpi). Images displayed are representative of consistent symptoms observed from 8-16 infected plants per strain.



**S6 Fig. *P. infestans* does not cause disease symptoms on TAK1**

Disease progression of *M. polymorpha* TAK1 plants inoculated with water, *P. palmivora* (ARI-td) zoospores or *P. infestans* (Pi88069-td) zoospores. Images display consistent disease symptoms (n=8) at 1, 3, 5, and 7 days post inoculation (dpi).

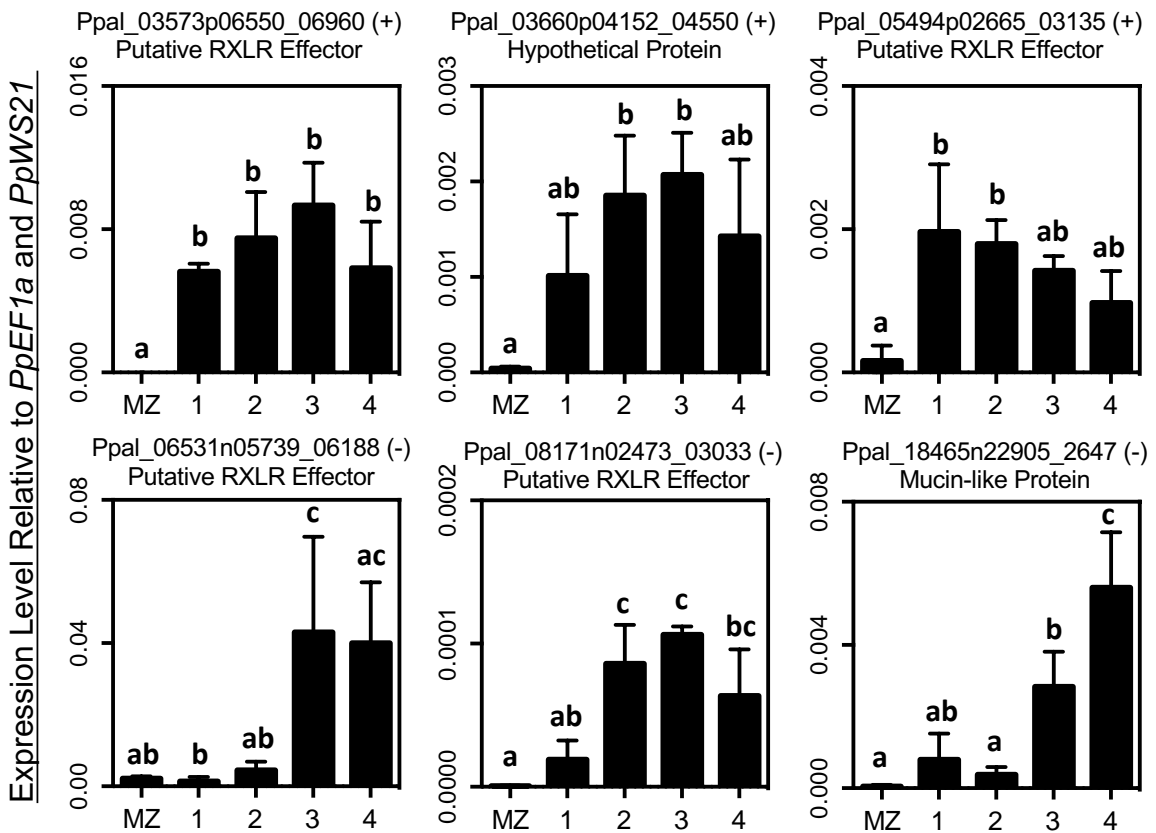


**S7 Fig. Host responses to colonization are activated in *Marchantia* air chambers.**

(A) Histochemical staining of mock-treated or *P. palmivora* ARI-td infected *M. polymorpha* TAK1 using DAB (diaminobenzidine) to detect hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) accumulation at 1, 3, and 5 days post inoculation (dpi). Scale bars = 1000 μm.

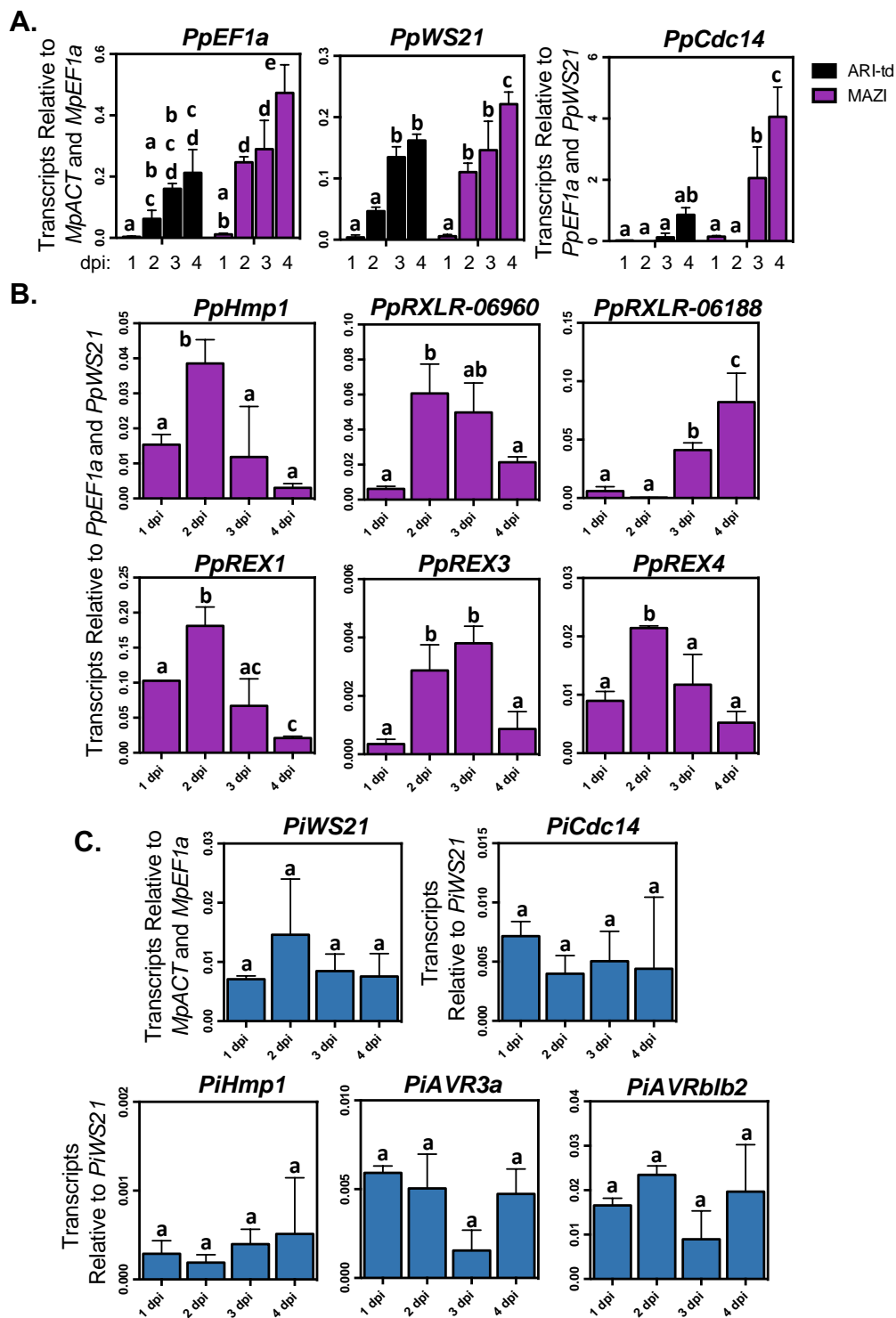
(B) qRT-PCR analysis of *MpDIR* and *MpPRX* transcripts in mock-treated (blue) and infected (*P. palmivora* ARI-td, black) *M. polymorpha* TAK1 from 1-4 dpi. Expression was quantified relative to endogenous *MpACT* and *MpEF1a* controls. Different letters indicate statistically significant differences in transcript abundance (ANOVA, Tukey's HSD,  $p < 0.05$ ).

(C) GUS staining of mock-treated and infected (*P. palmivora* ARI-td) *MpPRX:GUS* and wild-type TAK1 thalli at 4 dpi. Thalli were sectioned using a vibratome and images were collected using the 3D display option on a Keyence digital microscope. Scale bars = 500 μm.



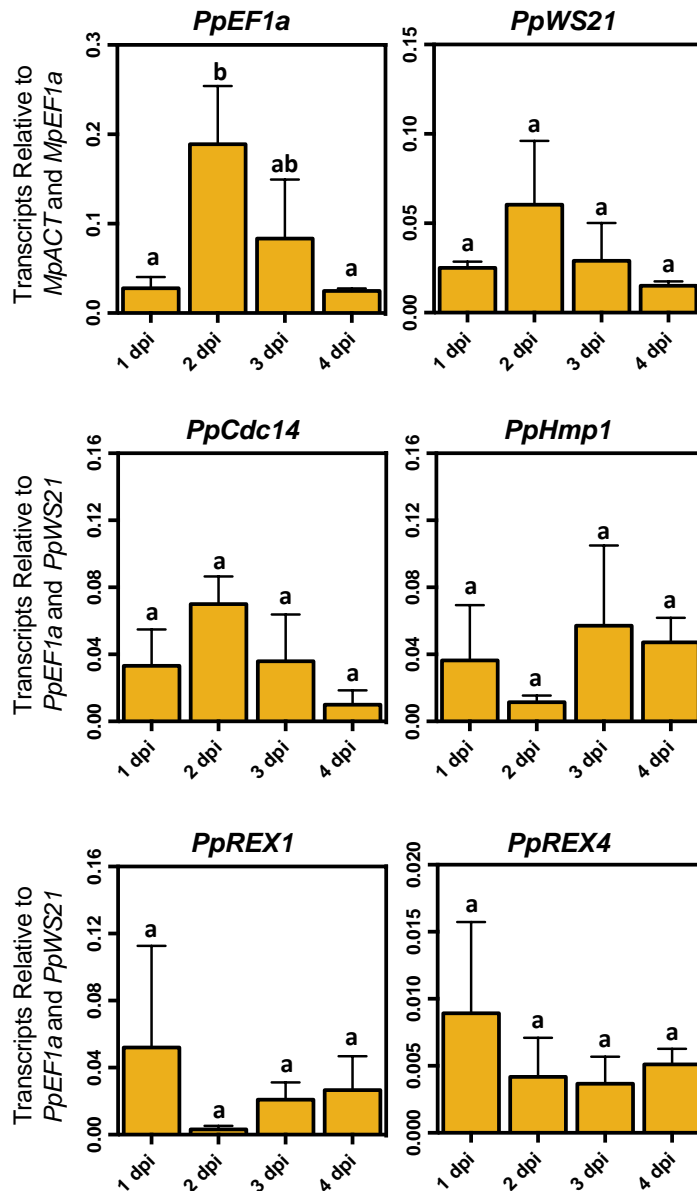
### S8 Fig. Validation of colonization-induced *P. palmivora* genes

qRT-PCR analysis of *P. palmivora* (ARI-td) genes identified by RNA-seq analyses. Expression levels were quantified in an axenically propagated MZ (mycelia + zoospores) sample and during the colonization of *M. polymorpha* thalli from 1-4 days post inoculation (dpi). Expression levels were quantified relative to internal controls *PpEF1a* and *PpWS21*. Different letters indicate statistically significant differences in expression levels (ANOVA, Tukey' HSD,  $p < 0.05$ ). Performed twice with similar results.



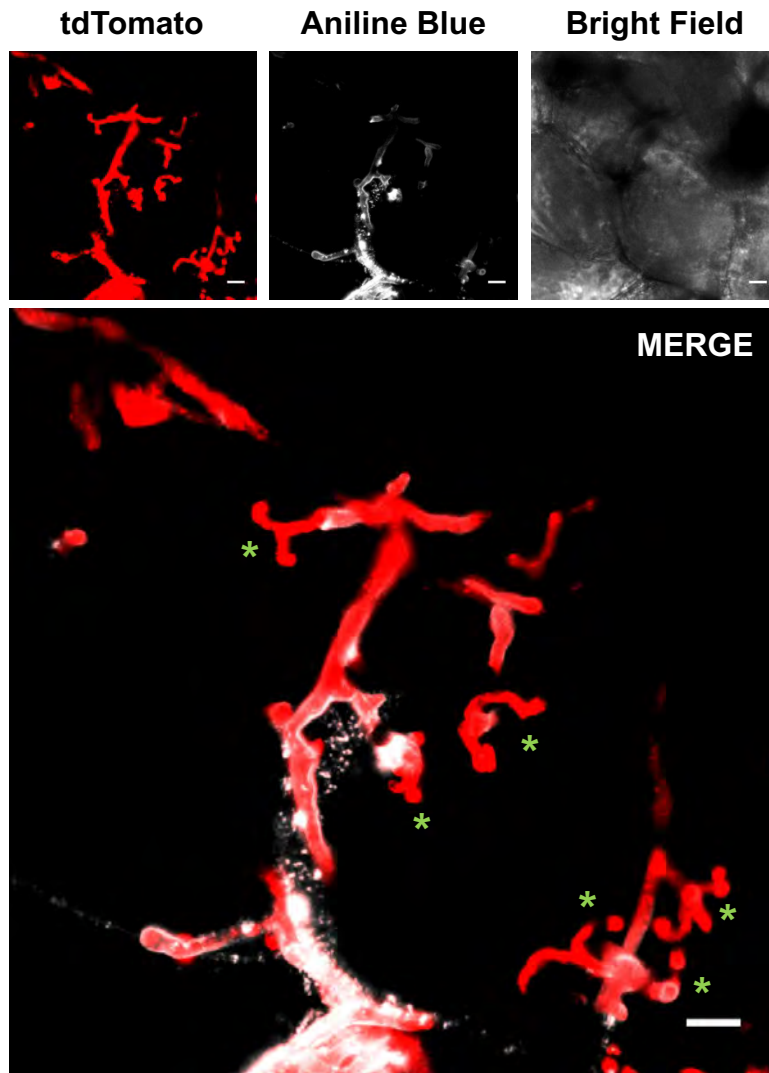
**S9 Fig. Expression analysis of *Phytophthora* lifestyle markers during compatible and incompatible interactions with *Marchantia* thalli.**

**(A)** Comparative qRT-PCR analysis of pathogen biomass (*PpEF1a* and *PpWS21*) and sporulation (*PpCdc14*) during interactions between 3-week-old *M. polymorpha* thalli and *P. palmivora* ARI-td (black) or MAZI (magenta) from 1-4 days post inoculation (dpi). **(B)** Expression analysis of haustoria-associated *PpHmp1* and RXLR effectors (*PpREX1,3,4*) in the *P. palmivora* isolate MAZI during compatible interactions with *M. polymorpha* thalli. Additional RXLR effectors *PpRXLR-06960* and *PpRXLR-06188* represent the *P. palmivora* loci Ppal\_03573p06550\_06960 (+) and Ppal\_06531n05739\_06188 (-), respectively. **(C)** Expression analysis of *P. infestans* biomass (*PiWS21*), sporulation (*PiCdc14*), haustoria-associated (*PiHmp1*), and RXLR effector genes (*PiAVR3a* and *PiAVRblb2*) during incompatible interactions with *M. polymorpha* thalli. Different letters indicate statistically significant differences in transcript levels (ANOVA, Tukey's HSD,  $p < 0.05$ ). Performed twice with similar results.



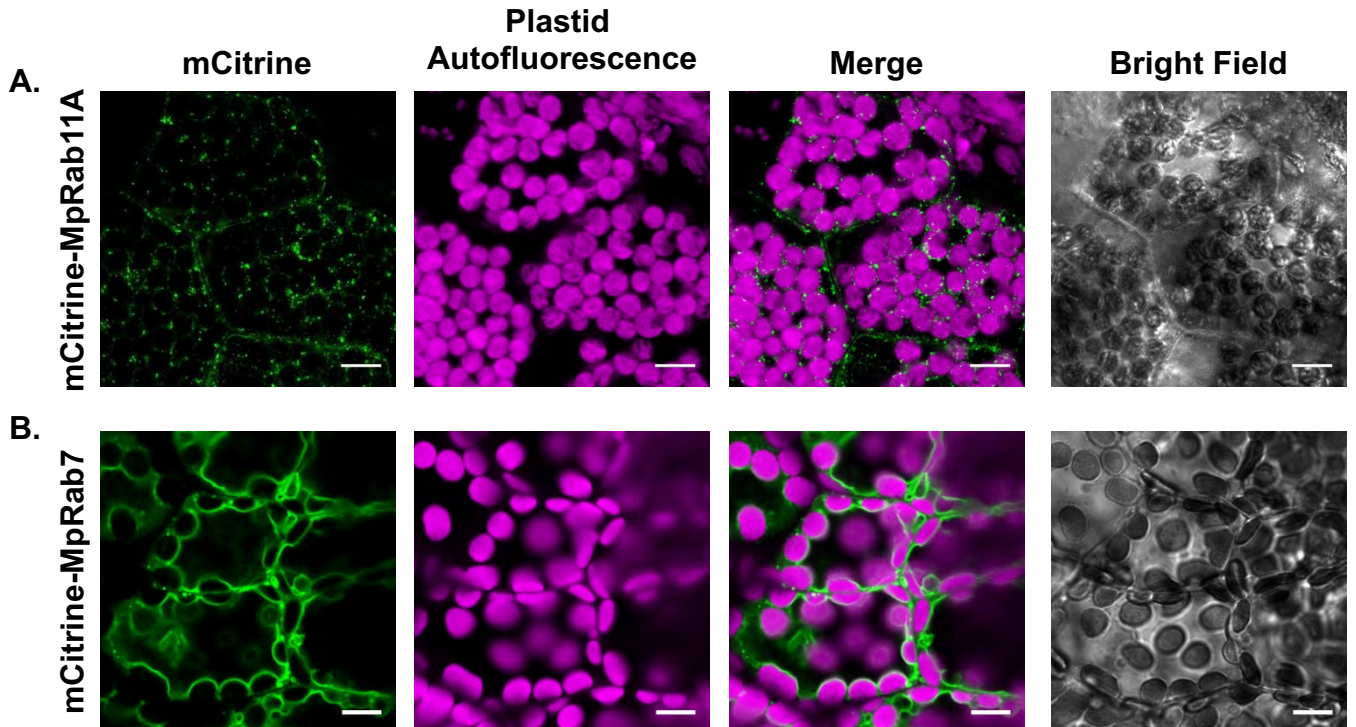
**S10 Fig. Expression analysis of *P. palmivora* lifestyle markers during incompatible interactions with *Marchantia* rhizoids.**

qRT-PCR expression analysis of pathogen biomass (*PpEF1a* and *PpWS21*), sporulation (*PpCdc14*), haustoria development (*PpHmp1*), and RXLR effectors (*PpREX1* and *PpREX4*) during interactions between 3-week-old *M. polymorpha* rhizoids and *P. palmivora* ARI-td from 1-4 days post inoculation (dpi). Different letters indicate statistically significant differences in transcript levels (ANOVA, Tukey's HSD, p < 0.05). The data are representative of two experimental replicates.



**S11 Fig. Callose does not envelope *P. palmivora* infection structures**

Confocal fluorescence microscopy of aniline blue stained *M. polymorpha* TAK1 thalli infected with *P. palmivora* ARI-td at 3 days post inoculation (dpi). Images represent z-stack projections displaying red fluorescence from the pathogen (tdTomato), callose deposition through aniline blue staining (white), bright field, or tdTomato merged with aniline blue. Asterisks (\*) denote intracellular infection structures that are not enveloped by callose.

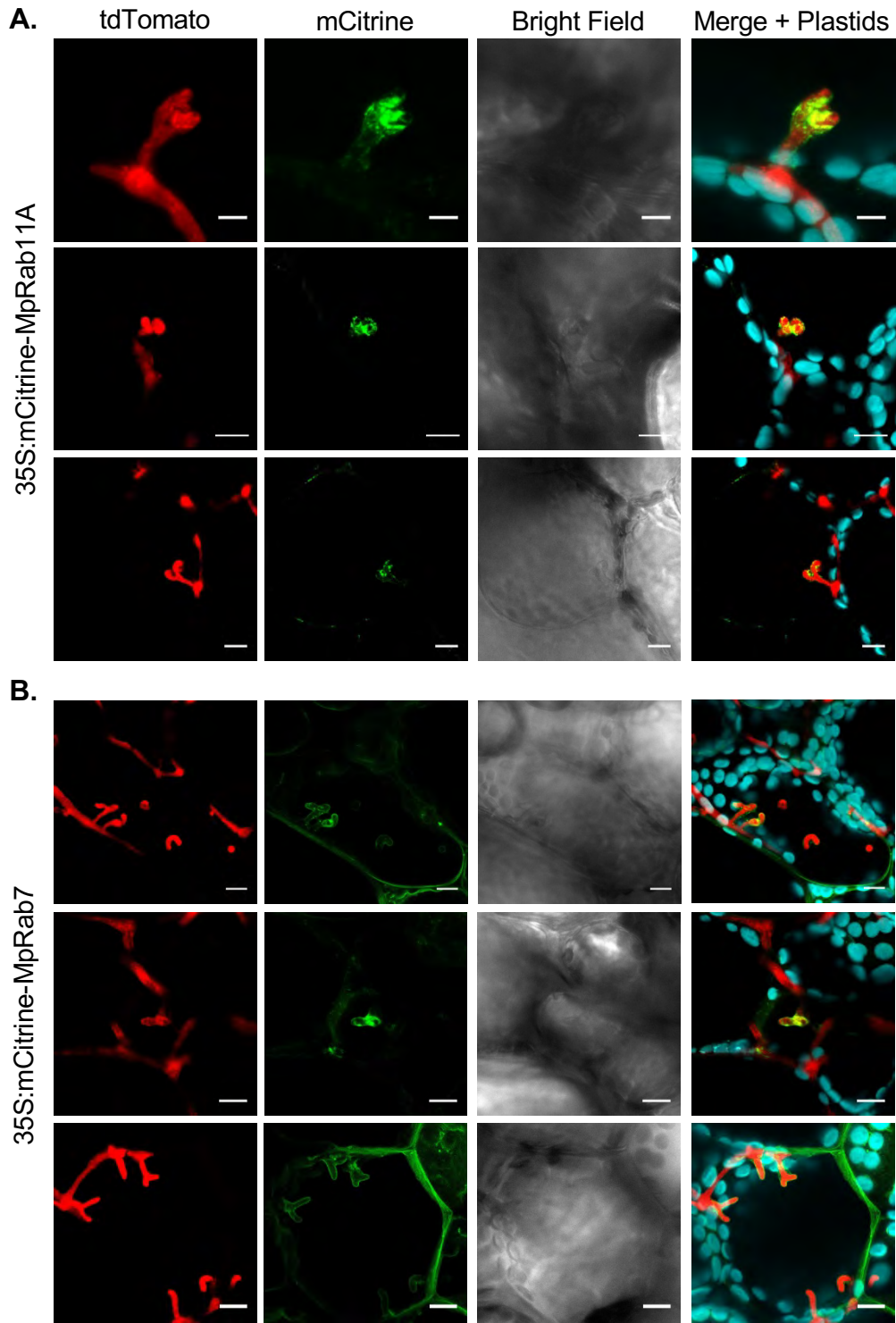


**S12 Fig. Subcellular localization of MpRab11A and MpRab7**

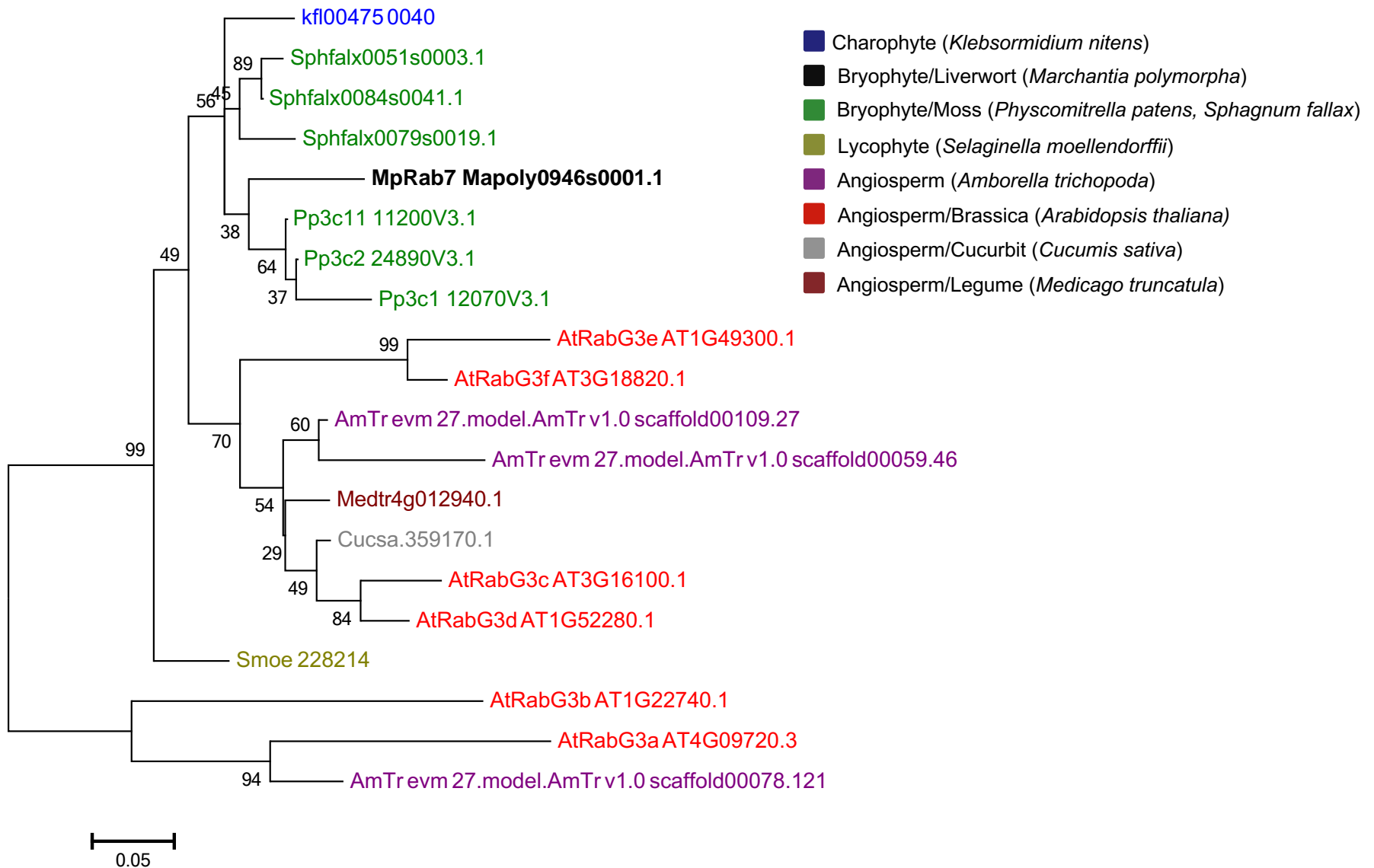
**(A)** Confocal fluorescence microscopy showing subcellular localization patterns of MpRab11A in 35S:mCitrine-MpRab11A/TAK1 gemmae. Micrographs display mCitrine fluorescence, plastid autofluorescence (magenta), both channels merged, or bright field images. Scale bars = 10  $\mu$ m.

**(B)** Confocal fluorescence microscopy showing subcellular localization patterns of MpRab7 in 35S:mCitrine-MpRab7/TAK1 gemmae. Micrographs display mCitrine fluorescence, plastid autofluorescence (magenta), both channels merged, or bright field images. Scale bars = 10  $\mu$ m.

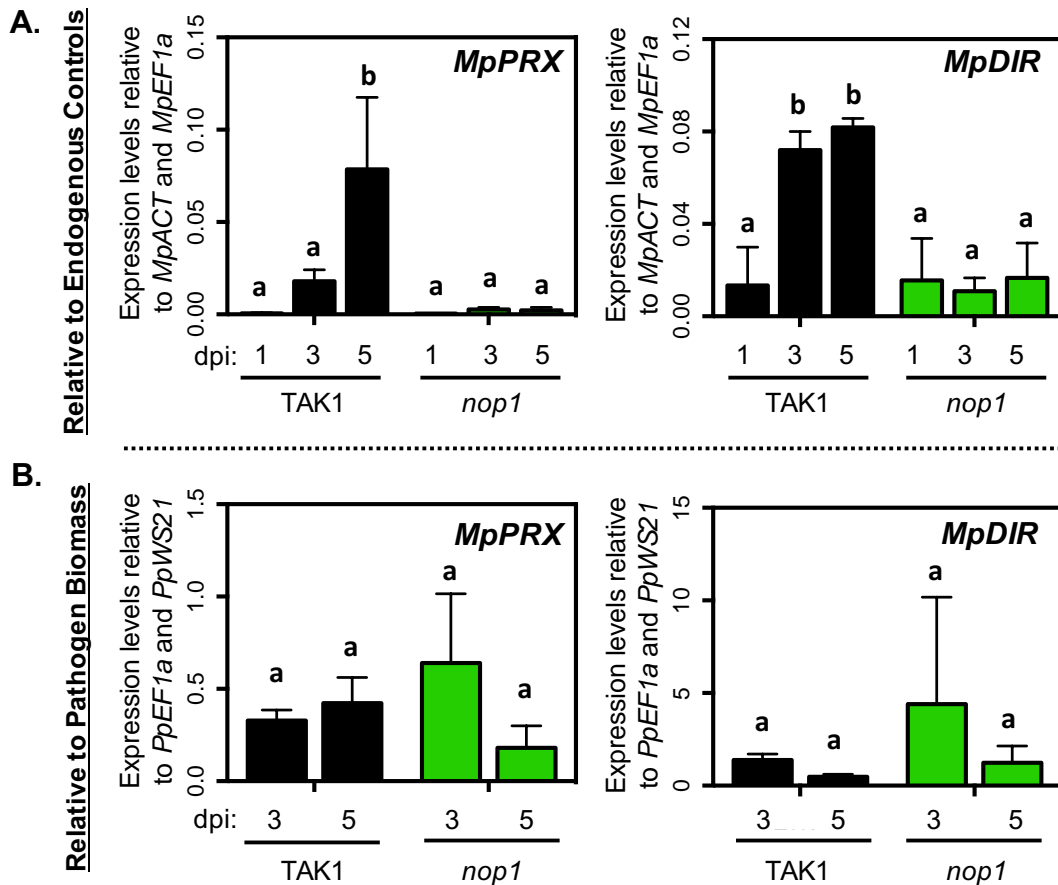




**S13 Fig. MpRab11A and MpRab7 accumulate at *P. palmivora* intracellular infection structures in living *Marchantia* cells.** Confocal fluorescence microscopy showing subcellular localization patterns of MpRab11A (**A**) and MpRab7 (**B**) in 35S:mCitrine-MpRab11A/TAK1 or 35S:mCitrine-MpRab7/TAK1 thalli infected with *P. palmivora* ARI-td at 2-3 days post inoculation (dpi). Z-stacked micrographs display tdTomato pathogen fluorescence, mCitrine fluorescence, bright field images, and a merged image displaying mCitrine, tdTomato and plastid autofluorescence (cyan), both channels merged, or bright field images. Scale bars = 10  $\mu$ m.



**S14 Fig. Rab7 phylogeny.** Maximum likelihood phylogram of Rab7 proteins from the green-plant lineage. Amino acid sequences were aligned using MUSCLE. The evolutionary history was inferred by the Maximum Likelihood method utilizing the Jones, Taylor & Thornton model with discrete Gamma distribution using MEGA 6. Ten thousand bootstrap replicates were conducted and percent bootstrap values were placed on corresponding branches.



**S15 Fig. Colonization-induced *MpPRX* and *MpDIR* expression is not compromised in *nop1* mutants.**

**(A)** qRT-PCR analysis of *MpDIR* and *MpPRX* transcripts in wild-type TAK1 and *nop1* mutants at 1, 3, and 5 days post inoculation (dpi) with *P. palmivora* ARI-td in 3 week-old plants. Expression was quantified relative to endogenous *MpACT* and *MpEF1a* controls. Different letters indicate statistically significant differences in transcript abundance (ANOVA, Tukey's HSD,  $p < 0.05$ ).

**(B)** qRT-PCR analysis of *MpDIR* and *MpPRX* transcripts in wild-type TAK1 and *nop1* mutants at 3 and 5 days post inoculation (dpi) with *P. palmivora* ARI-td in 3 week-old plants. Expression was quantified relative to pathogen biomass (*PpEF1a* and *PpWS21*). Different letters indicate statistically significant differences in transcript abundance (ANOVA, Tukey's HSD,  $p < 0.05$ ).

## SUPPORTING REFERENCES:

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