Characterization of the single *FERONIA* homolog in *Marchantia polymorpha* reveals an ancestral role of CrRLK1L receptor kinases in regulating cell expansion and morphological integrity

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Short title: Deciphering the ancestral role of CrRLK1L receptors in land plants

Summary

Plant cells are surrounded by a cell wall, a rigid structure rich in polysaccharides and glycoproteins. The cell wall is not only important for cell and organ shape, but crucial for intercellular communication, plant-microbe interactions, and as a barrier to the environment. In the flowering plant *Arabidopsis thaliana*, the 17 members of the *Catharanthus roseus* RLK1-like (*CrRLK1L*) receptor kinase subfamily are involved in a multitude of physiological and developmental processes involving the cell wall, including reproduction, hormone signaling, cell expansion, innate immunity, and various stress responses. Due to genetic redundancy and the fact that individual *CrRLK1L*s can have distinct and sometimes opposing functions, it is difficult to assess the primary or ancestral function of *CrRLK1L*s. To reduce genetic complexity, we characterized the single *CrRLK1L* gene of *Marchantia polymorpha*, *MpFERONIA* (*MpFER*). Plants with reduced *MpFER* levels show defects in vegetative development, i.e., rhizoid formation and cell expansion, but also affect male fertility. In contrast, *Mpfer* null mutants and overexpression lines severely affect cell integrity and morphogenesis of the gametophyte. Thus, the *CrRLK1L* gene family originated from a single gene with an ancestral function in cell expansion and the maintenance of cellular integrity. During land plant evolution, this ancestral gene diversified and was recruited to fulfil a multitude of specialized physiological and developmental roles in the formation of both gametophytic and sporophytic structures essential to the life cycle of flowering plants.

Key Words

cell wall integrity, cell elongation, *CrRLK1L* receptor kinases, *Marchantia polymorpha*, signaling pathway evolution

Introduction

The functioning of plant cells is contingent upon the cell wall acting as a barrier between the cell and its environment. This complex matrix of polysaccharides, glycoproteins, and other organic compounds defines the growth and shape of a cell and provides protection against biotic and abiotic stresses [1,2]. Moreover, its stiffness has to resist turgor pressure while also allowing cell expansion during growth. Thus, sensing and controlling cell wall integrity (CWI) is crucial for plant cells and receptor kinases (RKs) play an important role in sensing extracellular cues that activate intracellular pathways. Different RK subfamilies are defined by their extracellular domain (ECD), which is more variable than their transmembrane and kinase domains [3]. Over the last decade, the *CrRLK1L* subfamily emerged as important CWI sensors [4,5].

Functions have been assigned to 15 of the 17 *CrRLK1L* members encoded in the *A. thaliana* genome: they are required for cell elongation and cell shape, sensing environmental cues, and cell-cell communication in diverse contexts [6–22]. For instance, *FERONIA* (*AtFER*), *THESEUS1* (*AtTHE1*), *HERCULES1* (*AtHERK1*), and *AtHERK2* are required for cell elongation as *Atfer* single, *Atthe1Atherk1* double, and *Atthe1Atherk1Atherk2* triple mutants exhibit stunted growth [7,9–11]. Root hairs burst in mutants affecting *AtFER* and *[Ca²⁺]_{cyt}-ASSOCIATED PROTEIN KINASE1* (*AtCAP1*)/*ERULUS* (*AtERU*) [12,16]. Moreover, *AtFER* plays a role in powdery mildew resistance [11], innate immunity [15,22], calcium signaling [23], phytohormone signaling [10,24–26], and mechano- and heavy metal sensing [20,27]. In most contexts, the *CrRLK1L*s tend to promote cellular growth and cell elongation. In contrast, *AtTHE1* actively inhibits cell elongation in hypocotyls when cell wall perturbations occur, e.g., due to mutations in the cell wall biosynthesis machinery or upon treatment with isoxaben, a cellulose synthesis inhibitor [7,28].

In addition to regulating cellular growth during the vegetative phase of the life cycle, several *CrRLK1*Ls play a role during fertilization by controlling the growth and reception of the pollen tube. In the female gametophyte, *AtFER* is highly expressed in the two synergid cells that flank the egg cell and are important for double fertilization [6,29]. *AtFER* is crucial for pollen tube reception and the release of the two sperm cells [6,10,23,30,31], a process also involving two other, redundantly acting synergid-expressed *CrRLK1*Ls, *AtHERK1* and *ANJEA* (*AtANJ*) [17]. In contrast to these synergid-expressed *CrRLK1*Ls, four pollen-expressed *CrRLK1*Ls, named *ANXUR1* (*AtANX1*), *AtANX2*, *BUDDHA'S PAPER SEAL1* (*AtBUPS1*) and *AtBUPS2* [8,14,18,32], are redundantly required for pollen tube integrity and tip growth.

To transduce extracellular cues, *CrRLK1*Ls bind secreted peptides of the RAPID ALKALINIZATION FACTOR (RALF) family [22,32–35], which induce complex formation with members of LORELEI (LRE) family of GPI-anchored proteins [32,35–37]. Hence, it was suggested that at least some *CrRLK1*Ls act as CWI sensors that coordinate cell elongation in response to changes in the cell wall [38–43].

The 17 *A. thaliana* *CrRLK1*Ls act partially redundantly and can have opposite effects on cellular growth (reviewed in [19,41,43,44]). To investigate the original function of *CrRLK1*Ls, we characterized them in a system with reduced genetic complexity: the liverwort *Marchantia polymorpha* encodes a single *CrRLK1*L [45,46]. Liverworts represent an early diverging land plant lineage and have been hypothesized to retain, at least in part, characteristics of the earliest land plants [45,47–52]. The presence of similar types of gene families in all plant lineages suggests that the differences in development evolved by co-opting and modifying existing developmental programs and genetic networks, rather than through the evolution of novel genes [47,48,53]. *M. polymorpha* is thus an

ideal system to study the ancestral role of genes and how it was modulated and diversified during land plant evolution [45,54,55].

We refer to the a single *CrRLK1L* family member encoded in the *M. polymorpha* genome, Mp4g15890 (Mapoly0869s0001), as Mp*FERONIA* (Mp*FER*) (aka Mp*THESEUS*, Mp*THE*) [44–46,56]. An Mp*fer/the* mutant was identified in a large T-DNA screen for defective rhizoid formation, developing short and irregularly shaped rhizoids with brown tips, indicating rhizoid rupture [56]. In the same screen, a mutant affecting the ortholog of the *A. thaliana* receptor-like cytoplasmic kinase *MARIS* (At*MRI*), known to act downstream of At*FER*, was identified with a similar phenotype (named Mpmri aka Mppti, Mp7g17560 [Mapoly0051s0094]) [56,57]. As in *A. thaliana*, a dominant active version of Mp*MRI* can partially rescue the Mp*fer* rhizoid phenotype [57]. This suggests that at least some of the machinery associated with cell elongation and CWI sensing is conserved between *A. thaliana* and *M. polymorpha*.

Here, we report that *CrRLK1L* function is required in a variety of aspects of *M. polymorpha* gametophyte development. Phylogenetic analyses suggest that *CrRLK1L* genes first appeared in the common ancestor of land plants. The characterization of lines with reduced Mp*FER* levels indicates that, in addition to its function in rhizoid formation, Mp*FER* controls cell size and organ growth and is involved in male gametogenesis and fertility. Analysis of the Mp*FER* expression pattern points to an involvement in female sexual organogenesis and sporophyte development. Our data suggests that the broad involvement of *CrRLK1L* function in the development and physiology of land plants is an ancestral and conserved characteristic, although the functions of *CrRLK1Ls* were extended and adapted to control additional developmental processes in the sporophyte in the course of land plant evolution.

Results

CrRLK1L is conserved in land plants and arose in this lineage

The *M. polymorpha* genome encodes a single CrRLK1L homolog (Mp4g15890 [Mapoly0869s0001]) [44–46]. Like other CrRLK1L members, this gene encodes an intracellular serine/threonine kinase domain, a transmembrane domain, and an extracellular malectin-like domain with two malectin domains (Figure 1A). Given that the ECD of CrRLK1Ls in *A. thaliana* is crucial for their function and, in contrast to the kinase domain, not interchangeable [58], we performed a phylogenetic analysis with the amino acid sequences of the predicted malectin-like domain of Mp4g15890, the five members of the moss *Physcomitrella patens*, the two of the lycophyte *Selaginella moellendorffii*, and the 17 CrRLK1Ls of *A. thaliana* (Figure 1B). The *M. polymorpha* CrRLK1L member grouped together with the CrRLK1Ls of basal land plants and was in the same clade as AtFER, while AtTHE1 was in a different one (Figure 1B). Based on this phylogenetic position and the fact that the malectin-like domain of the *M. polymorpha* CrRLK1L shared the highest amino acid identity with AtFER (Figure S1A), we named it MpFER [45,46,57], but it is also known as MpTHE [56].

Interestingly, no significant similarity to the MpFER malectin-like domain was found when compared to available genomic data of six Chlorophyte algae (*Chlamydomonas reinhardtii*, *Dunaliella salina*, *Volvox carteri*, *Coccomyxa subellipsoidea*, *Micromonas pusilla*, *Ostreococcus lucimarinus*) and expression data of six Charophycean algae (*Klebsormidium nitens*, *Nitella mirabilis*, *Coleochaete orbicularis*, *Spirogyra pratensis*, *Mesostigma viride*, *Closterium peracerosum–strigosum–littorale* complex). In *Closterium* complex, an RK named CpRLK1 expressed during sexual reproduction was suggested to be a CrRLK1L homolog [59]. However, comparing the full sequence or the ECD of CpRLK1 with the predicted *M. polymorpha* proteome, higher identity to another RK was

found (Figures S1B and S1C). Moreover, no RALF orthologs have been reported in algae, suggesting the origin of the *CrRLK1L* pathway in land plants [45].

To further support MpFER as a member of the *CrRLK1L* subfamily, the three-dimensional structure of its ECD was modelled [60] using the structure of the AtFER ECD (PDB 6a5b.1A, [35]) as a template (Figure 1C). Overall, the predicted structure of the MpFER ECD is highly similar to that of AtFER, keeping an almost identical number of secondary structures (7 alpha-helices and 33 beta-sheets in the MpFER ECD [35] and 8 alpha-helix and 34 beta-sheets in the AtFER ECD, respectively), and a similar spatial disposition for 32 of the beta-sheet structures and 6 of the alpha-helices (Figure 1C). When comparing the structural superposition of the AtFER and MpFER ECDs (Figures 1D and S1D), we observed that most conserved parts of the malectin-like domain are in the core of the protein, while the more variable parts are located peripherally. Moreover, the 3D structure of the *CpRLK1* ECD could not be properly modelled using the AtFER ECD (Figures S1D and S1E). Taken together, these results suggest that the malectin-like domain of MpFER could interact with similar proteins as AtFER does in *A. thaliana*, and may thus be involved in similar pathways. Sequence comparison of the MpFER kinase domain showed a conservation of the activation loop and a Lys important for catalytic activity (Figure 1E). Furthermore, several determined phosphorylation sites found in AtFER, AtERU, and *CrRLK1* were conserved in MpFER (Figure 1E) [13,33,61,62]. Thus, we propose MpFER to be basal and orthologous to all other land plant *CrRLK1L*s, and that the *CrRLK1L* family arose as plants conquered land.

MpFER is broadly expressed throughout the *M. polymorpha* life cycle

In *A. thaliana*, the expression patterns of the 17 *CrRLK1L* members span vegetative and reproductive stages and both generations of the life cycle (reviewed in [43]), pointing to

the importance of *CrRLK1Ls* for basic cellular functions. To assess whether this pattern is reflected in the land plant with the most ancestral characteristics, a promoter fragment of 3.2 kb (*proMpFER*) was used to drive expression of a triple yellow fluorescent protein (trpVNS) with a nuclear localization signal (*proMpFER:trpVNS*) and transformed into *M. polymorpha* sporelings. All transformants expressing the trpVNS fluorescent protein (45 of 48 independent lines) exhibited an indistinguishable expression pattern (Figure 2). During vegetative stages of gametophyte development, expression was observed in most cells of the thallus (Figure 2A). In concordance with the importance of *MpFER* for rhizoids [56], high trpVNS expression was observed in these tip-growing cells (Figure 2B). While trpVNS was expressed in gemma cups, no expression was observed in mature gemmae, possibly due to their dormancy (Figure 2C).

In mature female sexual organs, strong expression was observed in the entire archegoniophore (Figure 2D). The archegonia expressed trpVNS in most cells, except for the egg cell (Figure 2E). However, after fertilization, *proMpFER:trpVNS* became active in the zygote (Figure 2F). Inside the sporogonium, the sporogenous cells and subsequently the developing spores, but not the elater cells, strongly expressed trpVNS (Figure 2I). In male sexual organs, trpVNS was broadly detected in the antheridial splash platform (Figure 2G) and, specifically, in the spermatogenous tissue of the antheridia and the non-reproductive jacket cells surrounding them at different developmental stages (Figure 2H). Taken together, *MpFER* is expressed in most tissues during vegetative stages of gametophyte development as well as in the antheridia and archegonia during the sexual reproduction. Interestingly, the *proMpFER* promoter is not active in mature gemmae and the unfertilized, quiescent egg cell, although expression is initiated after dormancy and in the zygote. In concordance with the collectively ubiquitous expression of *CrRLK1Ls* in *A.*

thaliana, the MpFER expression pattern reinforces the importance of this family for basic cellular functions in land plants.

MpFER controls cell expansion during vegetative gametophyte development

To analyze the function of MpFER, three independent artificial microRNA constructs targeting MpFER were designed (amiR-MpFER), based on the endogenous microRNA MpmiR160 (Figure S2, [63]) and driven by the ubiquitous *pro*MpEF1 promoter [64]. While amiR-MpFER1 and amiR-MpFER2 target sites in the ECD coding sequence, amiR-MpFER3 targets the sequence encoding the kinase domain (Figures 3A and 3B). For each amiR-MpFER construct, several independent transgenic lines were obtained that showed a reduction in thallus size (Figures 3C, 3D and S3A). MpFER levels were quantified by qRT-PCR in two independent amiR-MpFER2 and amiR-MpFER3 lines each, which showed a similar phenotype (Figure 3E). MpFER expression ranged from 10% to 20% of the wild-type level in all amiR-MpFER lines analyzed (Figure 3E).

Previously, the T-DNA insertion mutant *Mpfer-1* was identified in a screen for mutants with defective rhizoids ([56], referred to as *Mpthe*). As the T-DNA inserted into the 3' UTR of the MpFER gene (Figure 3A; [56]), MpFER expression was analyzed by qRT-PCR but it showed no reduction compared to the wild type (Figure 3F), suggesting that the T-DNA insertion does not affect MpFER transcription. However, thallus size of *Mpfer-1* mutants is similar to that of amiR-MpFER lines (Figures 3C and 3D), suggesting that MpFER function is partially affected by the T-DNA insertion.

Rhizoids are tip-growing cells analogous to the root hairs in flowering plants (angiosperms). Reduced MpFER activity strongly impaired rhizoid formation and the rhizoids collapsed (Figure 3C). This had also been reported for the *Mpfer-1* mutant [56] and is in concordance with the function of AtFER and AtANX1/2 in tip-growing root hairs

[12,16,33,65] and pollen tubes [8,14], respectively. As *CrRLK1L* members are important for cell expansion in angiosperms [7,9,11,19,34,66,67], cell size of epidermal thallus cells in fully differentiated regions with minimal growth was measured in *Mpfer-1* and amiR-*MpFER* lines, showing a reduction in cell area as compared to the wild type (Figures 3G, S3B and S3C). Although amiR-*MpFER* lines produced more intact rhizoids than *Mpfer-1* mutants (Figure 3C), the average epidermal cell area was similarly reduced, although the distribution of cell areas differed between amiR-*MpFER* lines and *Mpfer-1* (Figures 3G, S3B and S3C).

In conclusion, *MpFER* has a fundamental role in rhizoid formation and growth during vegetative development. These results suggest an ancestral and conserved function of the *CrRLK1L*s in polar cell growth and cell expansion.

***MpFER* is important for the morphological integrity of the gametophyte**

Given that *Mpfer-1* and amiR-*MpFER* lines retain some *MpFER* activity, we generated *Mpfer* knock-out mutants with the goal to unveil potentially hidden *CrRLK1L* functions by generating a plant without any *CrRLK1L* activity. Using CRISPR/Cas9, two sites in the ECD-coding sequence of *MpFER* were targeted (Figure 4A). At least 10 independent lines were selected and analyzed for each target site. All plants with severely affected thallus development contained a frame shift mutation at the respective target site (Figures 4A-4C) while *MpFER* was not affected in transgenic plants with normal development.

The thallus area of the newly generated, amorphic knock-out mutants was more strongly reduced compared to *Mpfer-1* [56], confirming that, like the amiR-*MpFER* lines, *Mpfer-1* is a hypomorphic mutant (Figures 4C and 4D). In contrast, rhizoid integrity was similarly affected in hypo- and amorphic *Mpfer* alleles, suggesting that rhizoid formation is more sensitive to reduced *MpFER* activity than thallus growth (Figures 4E-4F). Despite of the

strong impact on thallus development, both hypo- and amorphic *Mpfer* mutants form gemma cups.

The strong disruption of thallus growth in amorphic *Mpfer* mutants prompted us to investigate whether cells of the thallus died in the absence of *MpFER* activity. Indeed, using trypan blue staining, we detected dead cells in these mutants, indicating a loss of morphological integrity (Figure 4G).

Some reproductive but not all CWI sensing functions of *CrRLK1Ls* are conserved in land plants

In *A. thaliana*, at least seven *CrRLK1L* family members play a role in reproduction, with *AtANX1/2* and *AtBUPS1/2* being important for pollen tube growth [8,14,18,32] and *AtFER*, *AtHERK1*, and *AtANJ* for pollen tube reception by the synergid cells [6,17]. To characterize the function of *MpFER* in reproductive development, amiR-*MpFER3-2* lines were transferred to sexual organ-inducing conditions [68]. The number of antheridiophores produced was significantly less in amiR-*MpFER* lines compared to the wild type (Figures 5A and S3D) and the antheridiophore splash platforms were also smaller (Figure 5B). The reduction in *MpFER* expression in antheridiophores of amiR-*MpFER* lines was confirmed by qRT-PCR and results were comparable to those using thallus tissue (Figure 5C). A pronounced reduction in spermatogenous tissue was observed in many antheridia isolated from amiR-*MpFER3-2* lines (Figures 5D and 5E). However, although the plants were grown under optimal conditions in axenic culture boxes, the effects on reproductive structures could be partly indirect due to the reduction in plant size and/or rhizoid function.

To determine male fertility of these amiR-*MpFER* lines with reduced spermatogenous tissue, we performed crosses with wild-type female plants. The spermatocyte

concentration harvested from wild-type antheridia was almost 3-fold higher than from
amiR-MpFER lines and was adjusted using a hemocytometer. In just one of the crosses
(n=20), a single sporophyte was formed, resulting in 0.05 ± 0.05 (mean $\pm$ SE) sporophytes
per archegoniophore, whereas the same females crossed with wild-type males yielded
 9.95 ± 2.33 (mean $\pm$ SE) sporophytes per archegoniophore. In summary, MpFER plays
a role in antheridiophore development and spermatocytes of plants with reduced MpFER
activity are poorly fertile. Thus, CrRLK1Ls have a conserved role in reproduction in
addition to their role in cell expansion and integrity during vegetative development.
Some *A. thaliana* CrRLK1Ls are involved in surveying CWI and in inhibiting growth when
CWI is impaired. Cell wall defects caused either by mutations affecting cellulose
biosynthesis or by treatment with isoxaben, a cellulose synthesis inhibitor, can be
suppressed by a mutation in *AtTHE1* [7,28]. To investigate whether MpFER also acts as
CWI sensor, we treated wild-type, *Mpfer*, and amiR-MpFER3-2 gemmae with isoxaben
(Figure 5F). As in the wild type, isoxaben affected the development of gemmae with
reduced MpFER activity, all growing progressively less with increasing isoxaben
concentration (Figure 5F). These results suggest that the function of *AtTHE1* in
repressing cellular growth when CWI is impaired appeared later in the course of land
plant evolution or was lost in the *Marchantia* lineage.

The CrRLK1L signaling pathway is conserved in land plants

As downregulation of MpFER or AtFER produce similar phenotypes concerning polarized
growth and cell expansion in *M. polymorpha* and *A. thaliana*, respectively, interspecific
complementation was attempted. First, the coding sequence of MpFER fused to the
Green Fluorescent Protein (GFP) gene under the control of the viral 35S promoter
(*pro35S:MpFER-GFP*) was transformed into heterozygous *Atfer-2* mutants. Then, GFP

expression at the cell periphery and complementation of the bursting root hair and reduced rosette size phenotypes were assessed in transgenic plants (Figure 6A). *Atfer-2* homozygotes expressing the MpFER-GFP protein look similar to *Atfer-2* mutants, indicating that MpFER does not complement these *Atfer-2* loss-of-function phenotypes (Figure 6A). In a complementary experiment, the coding sequence of AtFER fused to the Citrine gene under the control of the MpEF1 promoter (*proMpEF1:AtFER-Cit*) was transformed into amiR-MpFER3-2 plants. Ten independent lines with Citrine expression at the cell periphery were phenotypically characterized (Figures 6B and 6C). All had bursting rhizoids and a reduced thallus size, similar to the parental amiR-MpFER3-2 line, suggesting that AtFER does not rescue the vegetative phenotypes produced by down-regulation of MpFER.

In *A. thaliana*, it was shown that AtFER forms a complex with AtRALF peptides and the LRE/LLG co-receptors [35]. As no interspecific complementation was observed, the conservation of binding surfaces between CrRLK1L, RALF, and LRE homologs were analyzed. The *M. polymorpha* genome encodes three RALF peptides (MpRALF: Mp1g27120 [Mapoly0002s0166]; MpRALF2: Mp2g21670 [Mapoly0040s0047]; MpRALF3: Mp7g07270 [Mapoly0076s0067]) [44,45]. We performed a phylogenetic analysis using the amino acid sequences of the predicted mature peptides of *A. thaliana* and *M. polymorpha* RALFs (Figure S4A). All MpRALF peptides clustered together with AtRALFs known to interact with CrRLK1L receptors, including AtRALF1 and AtRALF23, which serve as AtFER ligands [22,33] (Figure S4A). The MpRALFs shared the four conserved Cys residues as well as the YXXY and YY motifs with the AtRALF1 subgroup (Figure S4B). However, only MpRALF1 and MpRALF3 had an RRXL motif important for S1P cleavage (Figures S4B and S4C)[69], consistent with the presence of one S1P ortholog in basal plants (Mp8g07990 [Mapoly0155s0018], Figure S4D). There are two

MpLRE/LLG proteins encoded in the *M. polymorpha* genome (Figure S5A). Both the MpLRE1 (Mp5g09600 [Mapoly0048s0110]) and MpLRE2 (Mp4g22100 [Mapoly0090s0020]) proteins showed conservation of the eight Cys and the ND motif distinctive of the family (Figure S5B); however, MpLRE1 did not contain a GPI anchoring site (Figure S5B). Structure prediction using AtLLG2 as a template [35] showed a conserved general structure (Figures S5C and S5D). Some of the MpRALF and MpLRE family members show a similar expression pattern as MpFER (Figure S5F), suggesting that the corresponding proteins could form a complex similar to that described in *A. thaliana*.

To gain insights into the formation of a putative CrRLK1L signaling complex, the MpFER/MpRALF/MpLRE complex was modelled. The general structure appeared conserved (Figure S5E); however, analysis of conserved sites between *M. polymorpha* and *A. thaliana* showed a low amino acid conservation in the interacting surfaces of the complex subunits (Figure 6D). This suggests that the lack of interspecific complementation may be due to differences in amino acids that are important for complex formation, and that the proteins forming the complex have diverged, following distinct routes of co-evolution in the two lineages.

Because interspecific complementation of the amiR-MpFER phenotypes was unsuccessful, we asked whether suppression using a downstream component of CrRLK1L signaling identified in *A. thaliana* was possible. AtMRI, a receptor-like cytoplasmic kinase, acts downstream of AtFER or AtANX1/2 in the regulation of polar tip growth [70,71]. The dominant AtMRI^{R240C} mutation can suppress pollen tube rupture in the *Atanx1/Atanx2* double mutant and the root hair defects of *Atfer-4* [70,71]. A mutation in MpMRI (aka MpPTI) with a similar bursting rhizoid phenotype as observed in *Mpfer-1*, was identified in the same genetic screen for *M. polymorpha* mutants with defective

rhizoids [56]. To test whether MpMRI is a conserved downstream component of the MpFER signal transduction pathway, a dominant-active form of the protein equivalent to AtMRI^{R240C} was transformed into the amiR-MpFER3-2 line, driven by 2 kb of the endogenous promoter (*proMpMRI:MpMRI^{R240C}*). Several independent lines showed a partial restoration of rhizoid growth; however, we also observed defects in thallus development of lines with higher levels of MpMRI expression, mainly abnormalities in the epidermis (Figures 6E and S6A-S6E). This suggest that MpMRI acts downstream of MpFER in the signal transduction pathway controlling polarized growth since the origin of land plants, in agreement with a recent report on the functional characterization of MpMRI [57].

Overexpression of MpFER affects morphological integrity

Because overexpressing AtFER in *M. polymorpha* did neither lead to any obvious phenotypes nor complementation of amiR-MpFER lines (Figures 6B and 6C), we also overexpressed AtFER and MpFER in the wild type. As in the amiR-MpFER background, overexpression of AtFER (*proMpEF1:AtFER-Cit*) in a wild-type background had no effect, consistent with the idea that AtFER is unable to form a complex with the corresponding *M. polymorpha* proteins. However, when expressing an MpFER-Citrine fusion protein (*proMpEF1:MpFER-Cit*) in wild-type plants, thallus development was strongly affected (Figure 7A). As expected, MpFER-Cit localized to the membrane (Figure 7B), and higher protein levels correlated with more severe phenotype (Figures 7A and 7C). Scanning electron microscopy of epidermis of plant expressing high levels of MpFER showed defects in the formation of air chambers and air pores (Figure 7D), similar to what we observed when expressing the dominant-active MpMRI^{R240C} (Figure S6A-S6C). Moreover, overexpression of MpFER affects the number of lobes in the antheridial

receptacle, producing only 4 instead of 8 (Figure S6F), supporting the importance of MpFER during sexual development. Considering the importance of MpFER for normal rhizoid formation, we also analyzed rhizoids in the *proMpEF1:MpFER-Cit* lines: while rhizoid morphology looked normal, a decrease in rhizoid number was observed (Figures 7E and 7F).

Taken together, the results obtained by overexpressing MpFER support the role of MpFER in cell expansion and maintaining tissue integrity during plant morphogenesis.

Discussion

During land plant evolution, many developmental aspects have changed in order to adapt to new environments, producing the enormous diversity observed in the plant kingdom. However, the control and maintenance of CWI remained a key aspect for the biology of a plant cell. CWI sensing is not only important for vegetative growth by cell expansion, but it is also essential for sexual reproduction and defense responses.

The *CrRLK1L* family was first characterized for its importance during angiosperm fertilization, a process comprising aspects of polar cell elongation, CWI control, and cell-cell communication [6,30,31]. Lately, different members of this family were found to carry out diametrically opposite roles in diverse aspects of plant development, which has made it difficult to define the core or ancestral function of this gene family comprising 17-members in *A. thaliana* [6–12,14–20,32].

Here, we report the characterization of the single *CrRLK1L* gene encoded in the genome of *M. polymorpha*. Structurally, the MpFER ECD and kinase domain share similarities with previous characterized *CrRLK1L* members, including the malectin-like domain and conserved phosphorylation sites. Conservation of RALF and LRE members in *M. polymorpha* suggests that MpFER also forms a complex at the plasma membrane [35].

While modeling predicted that the general structure of the complex was conserved, the interaction surfaces seem to have diverged and co-evolved independently in the respective lineages.

We observed a broad involvement of *MpFER* at both vegetative and reproductive stages of development: During vegetative growth of the gametophyte, *MpFER* is required for rhizoid formation and cell expansion but it also plays a role in male gametogenesis and is expressed in female reproductive organs. These findings suggest that the *CrRLK1Ls* have, also in ancestral land plants, held roles in various aspects of both vegetative and reproductive development. Thus, the role of *CrRLK1Ls* in angiosperm reproduction does not represent a derived feature from a purely vegetative function in cell elongation in bryophytes, but constitutes a conserved feature of this gene family.

Moreover, the almost ubiquitous expression of *MpFER* in *M. polymorpha* is consistent with the collective expression of different *CrRLK1L* members in essentially all tissues and organs of *A. thaliana* [43]. Thus, gene duplication allowed the expansion of the *CrRLK1L* family in angiosperms, diversifying their expression patterns and functions but also leading to genetic redundancy [8,17,32,43].

CrRLK1L members are important sensors of CWI during polarized growth, both in pollen tubes [8,32] and root hairs [16]. In early divergent land plants, rhizoids are tip-growing cell with a function analogous to that of root hairs, important for taking up nutrients and water [56,72]. Based on the conservation of many genes controlling the development and growth of tip-growing cells forming rooting systems, it was suggested that these pathways were active in the earliest land plants that existed about 470 million years ago [56]. That *MpFER* also controls rhizoid integrity points to the importance of the *CrRLK1L* pathway for polarized growth since the origin of land plants. Moreover, the fact that amorphic *Mpfer* mutants contain many dead cells demonstrates the importance of the *CrRLK1L* pathway

for cellular integrity during vegetative growth. This aspect has not yet been reported for *A. thaliana* *CrRLK1L* mutants, possibly due to genetic redundancy among family members.

The signaling mechanisms downstream of *MpFER* in *M. polymorpha* development are still unclear. *MpMRI*, the *M. polymorpha* ortholog of *AtMRI*, which acts downstream of *AtFER* and *AtANX1/2* in *A. thaliana* during polarized growth [70], does have a similar rhizoid phenotype as *MpFER* [56,57]. The suppression of the rhizoid phenotype in *amiR-MpFER* transformants with the constitutively active form *MpMRI*^{R240C} corroborates a conserved *CrRLK1L* signaling cascade during polarized growth in *M. polymorpha* but whether this pathway also relies on Ca²⁺ and reactive oxygen species (ROS) as second messengers as it does in *A. thaliana* remains to be determined.

In *A. thaliana*, loss-of-function mutations in the motor protein *AtKINESIN-13a*, a microtubule-based motor, cause cell elongation in petals, leaves, and hypocotyls concomitant with changes in cell wall composition in leaves [73]. This effect depends on functional *AtTHE1* and does not occur in *Atkinesin-13-a/Atthe1* double mutants. Interestingly, in this case, *AtTHE1* stimulates cell elongation in response to defects in cell wall deposition [73]. Based on our results, we have no indication that *MpFER* acts as a CWI sensor and regulates growth similar to *AtTHE1* in response to cell wall disturbances. We have not observed a context in *M. polymorpha* development in which *MpFER* inhibits cell expansion. Therefore, the ability to restrict growth in response to disturbances in CWI could represent an evolutionary derived feature, as gene duplication allowed for the diversification and specialization of *CrRLK1L*s to regulate cellular growth in a more complex and context-dependent way. The capacity to flexibly regulate growth appears to be fundamentally context-dependent, as a similar discrepancy in promoting or inhibiting growth is also known for some well-studied growth promoting agents, like the

phytohormones auxin and brassinosteroid, which can have growth-inhibitory effects dependent on tissue and concentration. The reverse was noted for the phytohormones ethylene and abscisic acid, which are usually considered growth inhibitors but can also promote growth, dependent on the context [74–76].

Taken together, our results suggest an ancestral and conserved function of the *CrRLK1Ls* in polar cell growth and cell expansion, but also during sexual reproduction. In addition, we probe the relevance of *CrRLK1L* members for cell integrity and morphogenesis. In angiosperms, *CrRLK1Ls* occupy an important position at the interface of developmental and environmental inputs, which are integrated by a downstream signaling machinery that controls cell shape and polar growth to ensure normal development, *e.g.* by preventing cell rupture upon CWI defects. Whether the same downstream machinery is utilized in a similar fashion in *M. polymorpha* remains to be shown, even though the similarity in function points to a related mechanism. Even less is known about upstream aspects of *CrRLK1L* signaling. Future studies on the transcriptional regulation and the upstream components, such as the putative RALF ligand(s), in the highly simplified *M. polymorpha* system could provide fundamental insights into the molecular mechanism of the *CrRLK1L* pathway, which is so central to land plant physiology, growth, and development.

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Author contributions

MAM: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing

MR: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Funding acquisition, Writing – original draft, Writing – review & editing

AF-G: Data curation, Formal analysis, Software, Validation, Visualization, Writing – original draft, Writing – review & editing

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496

497 **Competing interests**

498 The authors have declared that no competing interests exist.

499

500 **Main figure titles and legends**

501 **Figure 1. CrRLK1Ls Are Conserved among Land Plants**

502 (A) Representation of AtFER and MpFER proteins. Malectin-like domains are
503 represented in blue and kinase domains in green. SP, signal peptide; TM, transmembrane
504 domain (yellow). Amino acids important for regulation and activity are marked in red,
505 violet, and light blue as described in Figure 1E.

506 (B) A rooted neighbor-joining tree of the amino acid sequence of the predicted malectin-
507 like domain. CrRLK1L members from *Marchantia polymorpha* (Mp), *Physcomitrella*
508 *patens* (Pp), *Selaginella moellendorffii* (Smo), and *Arabidopsis thaliana* (At) were used.
509 The numbers indicate the bootstrap values (%) from 1000 replications. The given scale
510 represents a substitution frequency of 0.1 amino acids per site.

511 (C) Cartoon representation of the predicted three-dimensional structure of the MpFER
512 malectin-like domain, showing predicted alpha-helix and beta-sheet structures.

513 (D) Structural superposition of the malectin-like domains of AtFER (blue) and MpFER
514 (green).

515 (E) Alignment of the cytoplasmic domains of MpFER, AtERU, AtFER, and CrRLK. Kinase
516 domains are in green, putative phosphorylation sites in light blue (Ser) and violet (Thr),
517 and the conserved catalytic Lys in red.

518 See also Figure S1

519

Figure 2. MpFER Is Broadly Expressed in most Tissues throughout the *M. polymorpha* Life Cycle

Expression of *proMpFER:trpVNS* in different organs of male and female *M. polymorpha* plants. Fluorescence is visualized as either green (epifluorescence microscope) or yellow (confocal microscope) color, depending on the panel.

(A to D) Bright field (left) and epifluorescence (right) images of a thallus, (A) rhizoid (C), gemmae cups (C), and an archegoniophore (D). Red arrows indicate meristematic zones, the yellow arrow gemmae. Scale bars, 0.5 mm.

(E and F) Confocal images of archegonia before (E) and 2 days after fertilization (F). Scale bar, 50 μ m.

(G) Bright field (left) and epifluorescence (right) images of an antheridiophore. Scale bar, 1 mm.

(H) Confocal image of antheridia. Scale bar, 100 μ m.

(I) Confocal image of sporophyte and spores. Scale bar, 25 μ m.

Figure 3. MpFER Controls Cell Size during Gametophyte Development

(A) Representation of the mature *MpFER* mRNA. Coding sequence are in green, locations of the amiRNA target sites in blue.

(B) Base complementary between mature amiR-*MpFER1*, amiR-*MpFER2*, and amiR-*MpFER3* with the *MpFER* transcript.

(C) Relative expression level of *MpFER* in thallus tissue from wild-type (WT; Tak1) and two independent insertion lines of amiR-*MpFER2* and amiR-*MpFER3*, as measured by qRT-PCR. *MpEF1* was used as internal control. Shown are means $\pm$ standard errors of the means (SEM) of three biological replicates. *P < 0.01, one-way analysis of variance (ANOVA).

(D) Relative expression level of *MpFER* in WT and *Mpfer-1* thallus tissue, as measured by qRT-PCR. *MpEF1* was used as internal control. Shown are means $\pm$ SEM of three biological replicates. * $P < 0.01$, one-way ANOVA.

(E) Representative pictures of 10-day old gemmalings of WT, *Mpfer-1*, and amiR-*MpFER3-2* lines. Scale bar, 1 mm.

(F) Thallus area of WT, *Mpfer-1*, and amiR-*MpFER3-2* lines. $n = 30$, outliers are indicated as black dots.

(G) Violin plot of cell size in WT, *Mpfer-1*, and amiR-*MpFER3-2* lines. Significant differences are indicated according to the non-parametric Kruskal-Wallis test and linear regression models (** $p < 0.001$).

See also Figures S2 and S3

Figure 4. The Integrity of Thalli from *Mpfer* Knock-out Lines Is Severely Affected

(A) Representation of the mature *MpFER* mRNA with the coding sequence in green, and the location of the gRNA target sites in red.

(B) Sequences of gRNA target sites in the confirmed *Mpfer* knock-out mutants. Deletions and insertions are highlighted in red.

(C) Representative pictures of 10-day old gemmalings of WT, *Mpfer-1*, and two independent *Mpfer* knock-out mutants. Scale bar, 0.5 cm.

(D) Thallus area of 10-day old gemmalings of WT, *Mpfer-1*, and two independent *Mpfer* knock-out mutants. $n = 30$.

(E) Representative pictures of 3-day old gemmalings growing in upside-down plates of WT, *Mpfer-1*, and two independent *Mpfer* knock-out mutants. Scale bar, 500 μ m.

(F) Rhizoid number in 3-day old gemmalings of WT, *Mpfer-1*, and two independent *Mpfer* knock-out mutants. $n = 30$.

(G) Trypan blue staining of 7-days old gemmalings of WT, *Mpfer-1*, and two independent *Mpfer* knock-out mutants. Scale bar, 1 mm.

Figure 5. Conservation of CrRLK1L Function in Land Plants

(A) Number of antheridiophores produced per plant for wild-type (WT, n=9) and two independent amiR-MpFER3 lines (amiR-MpFER3-2, n = 9; amiR-MpFER3-3, n = 12). Shown are means $\pm$ SEM of two biological replicates. Means with same letter do not differ significant different with $P > 0.05$, one-way ANOVA, Duncan test.

(B) Antheridiophore splash platform size distribution of WT (n = 32), amiR-MpFER2-3 (n = 34), and amiR-MpFER3-3 (n = 11) lines. For all antheridia with a stalk >8 mm, length and width of the platform were recorded.

(C) qRT-PCR of MpFER levels in antheridiophores in a WT and amiR-MpFER3-3 line. MpEF1 was used as internal control. Shown are means $\pm$ SEM of three biological replicates. * $P < 0.01$, ANOVA.

(D and E) Mature antheridia of a WT (D) and amiR-MpFER3-3 line (E). Bright field images (left) and in cross-section (right). The spermatogenous areas are indicated in red. Scale bar, 100 μ m.

(F) Thallus area of 15-day old gemmalings of WT, *Mpfer-1* mutant, and an amiR-MpFER3-2 line growing on media containing different isoxaben concentrations (nM). n = 30, outliers are indicated as black dots. Shown are means $\pm$ SEM of two biological replicates. Means designated by the same letter do not significantly differ at $P > 0.05$, one-way ANOVA, Duncan test.

See also Figure S3

Figure 6. Interspecific Complementation of *A. thaliana* and *M. polymorpha* Plants with Reduced Levels of *FER* Activity

(A) Roots of independent *A. thaliana* lines expressing 35S:Mp*FER*-GFP in *Ler* wild-type (WT) and *Atfer-2* plants. Scale bar, 200 μ m.

(B) 10-day old gemmalings of WT and amiR-Mp*FER*3-2 *M. polymorpha* plants with and without expression of *AtFER*-Cit. Scale bar, 0.5 mm.

(C) Citrine expression in 7-day old gemmalings of WT and amiR-Mp*FER*3-2 plants with and without expression of *AtFER*-Cit. Scale bar, 100 μ m.

(D) Cartoon representation of the structural conservation of the CrRLK1L^{ECD}/LRE/RALF complex by comparing *AtFER*/Mp*FER* and *AtLLG2*/MpLRE1. Blue and green represent spatially highly conserved regions of CrRLK1L and LRE homologs, respectively. Red indicates structural regions with low spatial conservation, lilac the C-terminal part of *AtRALF*23, and black arrows the interaction regions of the three proteins.

(E) Complementation of amiR-Mp*FER* lines with a dominant-active version of Mp*MRI* under its own promoter (*pro*Mp*MRI*:*MRI*^{R240C}). Scale bar, 1 mm.

See also Figures S4-S7

Figure 7: Overexpression of Mp*FER* Affects Morphological Integrity

(A) Representative pictures of 10-day old gemmalings of wild-type (WT, Tak1) and different lines overexpressing Mp*FER* (*pro*Mp*EF1*:Mp*FER*-Cit). Scale bar, 1 mm.

(B) Citrine expression at the plasma membrane in WT and *pro*Mp*EF1*:Mp*FER*-Cit lines #5 and #9 as observed under confocal microscopy. Scale bar, 20 μ m.

(C) Western blot analysis of *pro*Mp*EF1*:Mp*FER*-Cit lines using an anti-GFP antibody. *pro*Mp*EF1*:Cit and WT lines were used as positive and negative controls, respectively. The

Ponceau membrane staining of the most intense band at 55 kDa (presumably Rubisco) was used as a loading control.

(D) Scanning electron microscopical images of thalli from 20-day old plants of WT and *proMpEF1:MpFER-Cit* lines #5 and #9. Scale bar, 500 μ m.

(E) Representative pictures of 3-day old gemmalings growing in upside-down plates of WT and *proMpEF1:MpFER-Cit* lines #5 and #9. Scale bar, 1 mm.

(F) Number of rhizoids in 3-day old gemmalings of WT and *proMpEF1:MpFER-Cit* lines #5 and #9. n = 30.

See also Figure S6

Supplemental figure titles and legends

Figure S1. The CrRLK1L Family Appeared together with Land Plants. Related to Figure 1.

(A) Percentage of identity of the malectin-like domain, the complete ECD, and the full protein of MpFER with AtFER, AtANX1, AtANX2, and AtTHE1.

(B) Percentage of identity of the ECD and full protein of CpRLK1 with MpFER, AtFER, and Mapoly001s0111.

(C) A rooted neighbor-joining tree of the amino acid sequence of the predicted malectin-like domain was generated using ClustalW. CrRLK1L members from *Marchantia polymorpha* (Mp), *Physcomitrella patens* (Pp), *Selaginella moellendorffii* (Smoe), and *Arabidopsis thaliana* (At) were used. Algal CpRLK1 and Mapoly001s0111 were also included. The numbers indicate the bootstrap values (%) from 1000 replications. The given scale represents a substitution frequency of 0.1 amino acids per site.

(D) Qmean value for prediction of MpFER or CpRLK1 3D structures of the ECD based on the AtFER ECD. Green numbers indicate a good modelling fit, red numbers indicates a bad modelling fit.

(E) Structural superposition of the ECD of AtFER (blue) and CpRLK1 (purple).

Figure S2. Design of amiR-MpFER Precursors. Related to Figure 3.

(A) *MpmiR160* (Mapoly0002s0211, Mp1g26670) and amiR-MpFER1, amiR-MpFER2, and amiR-MpFER sequences. miRNA sequences are in red and miRNA* in blue. Cloning sequences from amiR-MpFER constructs are in bold.

(B) Drawing of the minimum free energy structure of *MpmiR160* and amiR-MpFER3 constructs predicted by the RNAfold web server (<http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi>). Red arrows indicate location and orientation of the mature miRNA in the precursor. The structures are colored by base-pairing probabilities; for unpaired regions the color denotes the probability of being unpaired.

Figure S3. Reduction in Thallus and Cell Area in Plants with Reduced MpFER Levels. Related to Figures 3 and 5.

(A) Thallus area of wild-type (WT), *Mpfer-1*, and amiR-MpFER3-2 plants at different days after putting gemmae on plates. $n = 30$, outliers are indicated as black dots. Areas were estimated using ImageJ software.

(B) Representative images from cell surface areas measured in WT, amiR-MpFER2-1, and amiR-MpFER3-2 plants. Scale bar, 50 μm .

(C) Difference in cell size between WT, amiR-MpFER2-1, and amiR-MpFER3-2 plants is significant ($p < 0.001$) based on the nonparametric Kruskal-Wallis test and a linear regression model with a highly significant interaction ($p < 0.001$).

(D) Induction of antheridiophores in WT and two independent amiR-Mp*FER3* lines. Three plants were grown in each sterile plastic box under far-red light induction.

Figure S4. MpRALF Peptides Belong to the AtRALF1-clade of the RALF Family. Related to Figure 6.

(A) A rooted neighbor-joining tree of the amino acid sequence of the predicted mature RALF peptides was generated using ClustalW. RALF members from *M. polymorpha* and *A. thaliana* were used. Red arrows indicate RALFs that are known ligands of CrRLK1Ls. The numbers indicate the bootstrap values (%) from 1000 replications. The given scale represents a substitution frequency of 0.1 amino acids per site.

(B) Amino acid sequence comparison of AtRALF1 and MpRALF1-3. Predicted signal peptides are in black, predicted mature peptides in light blue, conserved Cys in red, and predicted S1P recognition sites in violet.

(C) Processing pathway AtRALF1 by the S1P protease.

(D) A rooted neighbor-joining tree of the amino acid sequence of the S1P orthologs was generated using ClustalW. S1P members from *M. polymorpha*, *P. patens*, and *A. thaliana* were used. The numbers indicate the bootstrap values (%) from 1000 replications. The given scale represents a substitution frequency of 0.1 amino acids per site.

Figure S5. Two LORELEI-like Proteins Are Encoded in the *M. polymorpha* Genome. Related to Figure 6.

(A) A rooted neighbor-joining tree of the amino acid sequence of LRE orthologs was generated using ClustalW. LRE members from *M. polymorpha*, *P. patens*, and *A. thaliana* were used. The numbers indicate the bootstrap values (%) from 1000 replications. The given scale represents a substitution frequency of 0.1 amino acids per site.

(B) Amino acid sequence of AtLRE, MpLRE1, and MpLRE2. Conserved Cys are in light blue, the ND motif in red, and the GPI-anchoring site in green.

(C) Cartoon representation of the predicted 3-dimensional structure of MpLRE1, showing predicted alpha-helices.

(D) Structural superposition of AtLGG2 (blue) and MpLRE1 (green).

(E) Cartoon representation of the predicted 3-dimensional structure of the MpFER/MpLRE1/MpRALF1 complex, showing predicted alpha-helices and beta-sheets.

(F) Heatmap depicting relative gene expression based on RNAseq data (row-Z-score of vs normalized counts) of MpFER and the *M. polymorpha* orthologs of AtMRI, AtRALF1, and AtLRE across different tissues. Vegetative and reproductive tissues are grouped by green and orange, respectively. Averaged expression values are represented with colors of increasing red and blue intensity indicating upregulation and downregulation of gene expression, respectively.

Figure S6. Expression of MpMRI^{R240C} Suppresses the Bursted Rhizoid Phenotype of amiR-MpFER3 Lines but Leads to Aberrant Epidermis Development. Related to Figures 6 and 7.

(A to C) Epidemical pictures of thalli from the wild-type (WT, Tak-1) (A), and amiR-MpFER3 + _{pro}MpMRI:MpMRI^{R240C} lines #6 (B) and #8 (C), which both partially suppressed the bursting rhizoid phenotype (Fig. 6).

(D and E) Relative expression of MpFER (D) and MpMRI (E) against the geometric mean of the reference genes MpACT1, MpACT7, and MpAPT3 in WT, amiR-MpFER3-2, and 5 lines (#5 to #9) co-transformed with the amiR-MpFER3-2 and _{pro}MpMRI:MpMRI^{R240C} constructs. Expression levels of three biological replicates were assessed by droplet digital PCR (ddPCR). The y-axis corresponds to the log2-ratio between the test and the

geometric mean of the reference genes. Shown are means $\pm$ SEMs of three biological replicates. *P < 0.01, one-way ANOVA.

(F) Representative images of the antheridial receptacle of WT and *proMpEF1:MpFER-Cit* plants. Scale bar, 2 mm.

Figure S7. Structure Assessment and Quality Estimations of the MpFER and MpLRE 3-dimensional Models. Related to Figure 6.

QMEAN score barplot indicating quality estimates of the predicted models, across their aminoacidic sequence. Below the barplots, the corresponding secondary structures are displayed for both target and template sequences, with alpha-helices represented by light purple boxes and beta-sheets by green arrows. All plots were adapted from the model report produced by the SWISS-MODEL workspace.

(A) MpFER^{ECD}, for which AtFER was used as a template (PDB 6a5b.1A).

(B) MpLRE1, for which AtLLG2 was used as a template (PDB 6a5d.1A).

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Ueli Grossniklaus (grossnik@botinst.uzh.ch).

Materials Availability,

Requests for other strains and plasmids should be directed to the Lead Contact.

Data and Code Availability.

This study did not generate datasets or code.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

M. polymorpha subsp. *ruderalis* plants were grown on sterile culture on half-strength Gamborg's B5 basal medium (PhytoTechnology Laboratories), supplemented with 1% phytoagar in Petri dishes sealed with air-permeable tape. The plants were kept under fluorescent light and long-day conditions (16 h light at 22°C, 8 h dark at 20 °C) in Percival growth cabinets (models AR-41L3 and AR-41/L2). Alternatively, to induce sexual reproduction, plants were cultivated in jiffy pots filled with a 1:1 mixture of soil ("Einheitserde D73 + Bims", Universalerde) and sand or in culture boxes in sterile culture on half-strength Gamborg's B5 basal medium (Duchefa Eco2 Box, #E1654), under fluorescent light supplemented with far-red light (GreenPower LED module HF far-red, #929000464503 and #929000632103 Philips).

Wild-type (WT) plants in all experiments were *Arabidopsis thaliana* L. (Heyn) accession Ler-0. The *Atfer-2* mutant was obtained from the Signal Collection at the Salk Institute. Plants were grown in soil (ED73; Universalerde), covered with a thin quartz sand layer, under long photoperiods (16 hs light/8 hs dark) at 23°C and 60% relative humidity.

METHOD DETAILS

Phylogenetic analysis

Protein sequences were identified in plant genomes via BLASTp searches in <https://phytozome.jgi.doe.gov/pz/portal.html>. To elucidate the evolutionary relationship across land plant evolutionary history, we focused on *A. thaliana*, *Selaginella*

moellendorffii, *Physcomitrella patens* and *M. polymorpha*, as representative species for angiosperms, lycophytes, mosses and liverworts, respectively. Complete or partial coding protein sequences were aligned using the ClustalW parameters and were conducted in MEGA7. Phylogenetic trees were constructed with Neighbor-joining method, with a bootstrap test of 1000 replicates. The evolutionary distances were computed using the Poisson correction method.

Vector construction

Expression constructs: a BJ36 plasmid [77] containing a tripleVENUS-NLS (trpVNS) fragment was modified by adding a ligation-independent cloning (LiC) adapter site [78]. A promoter fragment of Mp*FER* of 3.2 kb was amplified using primers specified below and cloned into the BJ36 vector via LiC cloning. *pro*Mp*FER* promoter fragment fused to trpVNS was then shuttled via *NotI* restriction sites into the HART01 [77] expression vector. The expression vector was also directly modified to contain the LiC sites, so promoter fragments can now be directly cloned in front of the trpVNS in the HART01 vector via LiC-cloning. This vector was called VHL (trpVNS-containing HART01 vector with LiC sites).

amiRNA constructs: For functional analyses three independent artificial microRNA (amiRNA) constructs were made (Figure S2). The endogenous microRNA precursor Mp*miR160* was used as both backbone and template to design the constructs which generate single species small RNA molecules of 21 nt length, complementary to the target gene transcript [63,79]. Three miR160 backbones containing independent amiR-Mp*FER*/amiR-Mp*FER** duplexes were synthesized by GenScript and fused to the *pro*Mp*EF1* promoter in the BJ36 shuttle vector and shuttled to the HART01 expression vector as described previously [79]. The folding structure and design procedures were

explained in detail in [63,79]. The sequences for the amiRNAs were designed to retain the exact physical properties of the endogenous *MpmiR160* template. The folding patterns were analyzed in the Mfold software [80] and the sequences synthesized (Figure S2).

CRISPR/Cas gRNA: Selection of target sites was done using CasFinder [81]. Complementary oligos were designed and annealed for ligation into gRNA, in the pMpGE_En03 vector, previously digested with BsaI restriction enzyme. Resulting gRNAs were incorporated into binary vector pMpGE011 through gateway recombination.

Over-expression of *MpFER* and *AtFER*: The full-length *MpFER* sequence was amplified from genomic *M. polymorpha* DNA with attB sites and Gateway-cloned via pDONR207. For *A. thaliana* expression, *MpFER* was introduced into the expression vectors pMDC201, which contains a 2X35S promoter fragment and an in-frame C-terminal mGFP6 [82]. For *M. polymorpha* expression, *MpFER* was introduced into the binary vector pMpGWB308, which contains a *MpEF1* promoter and an in-frame C-terminal citrine [83]. Similarly, for expression of *AtFER* in *M. polymorpha*, coding sequence of *AtFER* was introduced into pMpGWB308 binary vector, for the generation of *proMpEF1:AtFER-Cit* construct.

Quantitative real-time PCR (qRT-PCR) and droplet digital PCR (ddPCR)

RNA extraction from *M. polymorpha*: RNA extraction from thallus tissue (ca. 100mg including apical notch) was done using the Rneasy plant mini kit (#74904, Qiagen). To remove contaminating DNA, the TURBO DNAfree™ Kit (AM1907 Ambion) was used according to manufacturer's recommendations. To extract RNA from the gametophores (2 antheridiophores > 5mm diameter were pooled, respectively, for each replicate), the Direct(-zol) RNA MiniPrep (#R2050, ZymoResearch) was used with TRIzol Reagent

(#15596026, Ambion) according to the manufacturer's protocol, including on-column DNase digestion and subsequent TURBO DNA-free™ Kit (AM1907 Ambion) treatment. RNA samples were quantified using the Qubit® RNA HS Assay Kit (#Q32852, Life Technologies), a Nanodrop ND-1000 Spectrometer or an Agilent 2100 BioAnalyzer. cDNA synthesis: cDNA was synthesized from 0.5 µg or 1.0 µg of total RNA. Reverse transcription was performed in 25 µl with 20µg/ml Oligo(dT) 12-18 primers and 200 units of SuperScript® II Reverse Transcriptase (#18064-014, Invitrogen) according to the manufacturer's protocol. The resulting cDNA was diluted 1:9 by adding nuclease-free water. To control for genomic DNA contamination, control replicates of all samples were incubated without SuperScript® II reverse transcriptase. Primer tests and quantitative RT-PCR: Primer efficiency and concentration tests were carried out for *MpFER* and suitable reference genes as described previously (Rövekamp et al., 2016, Althoff et al., 2014). Amplification experiments were carried out using a 7500 Applied Biosystem Fast Real-Time PCR System. Reactions were performed in 20 µl volumes containing 10 µl 2X SYBR-green mastermix (SsoAdvanced™ Universal SYBRGreen Supermix), 200 nM (*MpEF1* and *MpFER*) or 250 nM (*MpACTIN*) forward and reverse primers, and 1 µl diluted cDNA. Where possible, three technical and biological replicates were performed for each reaction. The primers used for the qRT-PCR are summarized in Table S1. For ddPCR analysis, individual PCR reactions were performed in a total volume of 25 µl, using 1X ddPCR EvaGreen Supermix, with droplets generated according to manufacturer's recommendations. Reading of the PCR-amplified droplets was carried out by the QX200 Droplet Reader (Bio-Rad) and analysed by the QuantaSoft™ Software (v1.4, Bio-Rad). The counts from ddPCR were normalised through a log2 transformation. Afterwards, relative expression was calculated against the geometric mean of the counts

for all three reference genes (MpACT1, MpACT7, and MpAPT3) [84]: ($\log_2(\text{gene tested} + 1) - \log_2(\text{geom. mean of all references} + 1)$). The primers used for the ddPCR are summarized in Table S1.

Transformation into *M. polymorpha* and *A. thaliana*

Agrobacterium-mediated transformation using regenerating thalli of *M. polymorpha* was done by co-cultivation 15-days old Marchantia fragments with transformed *Agrobacterium tumefaciens* (GV3101) cells [85,86]. After three days of incubation, positive transformants were selected on Gamborg's B5 plates supplemented with 10 $\mu\text{g/ml}$ Hygromycin B (Invitrogen), 0.5 μM Chlorosulfuron or Kanamycin, and 100 $\mu\text{g/ml}$ of Cefotaxime sodium. Isogenic lines were obtained by using plants derived from gemmae of the T1 generation (G1 generation). G1 or subsequent gemmae generations were used for all experiments, as they are derived from single cells [87,88]. Transformation of *A. thaliana* via *A. tumefaciens* (GV3101) was performed by floral dipping according to [89]. Primers used for amplification of promoter fragments of MpFER are listed in Table S2.

Microscopy

Plants were observed in a Lumar.V12 (Zeiss) or a Leica MZFLIII dissection microscope and photographed with an AxioCam HRc (Zeiss) or Leica DFC 420C camera. Clearings were observed using a Leica DMR microscope and photographed with an AxioCam 105 color camera. Fluorescence reporter expression were analyzed using a Leica SP5 confocal microscope. Fiji [90] and GIMP (version 2.8.10) software were used for adjustments of brightness, contrast, channel selection, and image size. Tissue clearing for bright field microscopy: *M. polymorpha* tissues were fixed in Carnoy's solution and incubated at 4°C overnight, followed by rehydration in an ethanol series of

85%, 70%, 50%, and 30%. Samples were incubated for 1 hour at 4°C for each step and then incubated in chloral hydrate solution at 4°C overnight. Samples were mounted in chloral hydrate.

Calcofluor-white staining for cell size measurements: Pieces of fresh thallus tissue located in fully differentiated zones [91] of plants grown on plates were dissected out and put into water. The pieces were transferred to PBS pH 6.1 containing 100 µg/ml Calcofluor-white and vacuum-infiltrated for 1 hour before being mounted on slides. The area of the epidermal cells was measured using Imaris 8.3.1. software. Cells of air pores and the two adjacent cell layers were excluded from the analysis, as well as the spiralling cells surrounding newly developing air pores.

For cell-death staining, seven-days old gemmalings were stained with lactophenol-trypsin (10 mL of lactic acid, 10 mL of glycerol, 10 g of phenol, 10 mg of trypan blue, dissolved in 10 mL of distilled water) and boiled for approximately 1 min in the stain solution and then decolorized in chloral hydrate (2.5 g of chloral hydrate dissolved in 1 mL of distilled water) for at least 30 min. They were mounted in chloral hydrate and imaged in dissecting microscope.

Western blot analysis

M. polymorpha explants were ground in liquid nitrogen and resuspended in 300 µL of Laemmli buffer [92]. After centrifugation at 6,000 rpm for 5 min at 4°C, the supernatant was recovered. Protein accumulation was confirmed by Western blotting, using the anti-GFP antibody (1:3000, Torrey Pines Biolabs). As secondary antibodies, the Agrisera S09602 goat anti-rabbit antibody was used with the ECL-chemistry of FUSION FX - Western Blot & Chemi Imaging (Vilber Lourmat).

Growth rate experiments

Gemmae of wild-type, *Mpfer-1*, and independent transformation lines of amiR-Mp*FER* lines were grown on plates and the thallus area was measured over time. 10 or 30 gemmae of four independent transformation lines were grown on half-strength Gamborg's B5 and scanned on an Epson 2450 Photo scanner at 600dpi. Plant area was measured using color threshold settings and particle analysis in the Fiji software [90].

Expression map

Gene expression of CrRLK1L signaling pathway components was calculated from publicly available RNA-seq data of 39 samples of *M. polymorpha* Tak-1 and Tak.2. Samples were grouped by tissue and analyzed under the same pipeline: Nextera paired-end adapters were trimmed from sequencing reads using the bbdut tool embedded in the BBMap software package [93]. Read ends with quality below 20 were also trimmed and the minimum read length was set to 25. The rest of parameters of bbdut were set as default. Reads were mapped to *M. polymorpha* reference genome (v3.1) [45] using the Tophat software (v2.1.1) [94], designed for RNA-sequencing read mapping. Tophat contains Bowtie (v2.3.2.0) [95] as the aligner software. All parameters were set to default values except for the RAM memory and number of used Cores. Mapped reads were sorted using samtools (v1.3.1) [96] and counted with the software HTSeq-count" (v.0.9.1) [97]. Gene expression values were calculated using the package DESeq2 (Release 3.6) [98] for R software (v3.4.4) [99]. Another R package, ggplot2 (release 2.2.1) [100] was used for producing the figures. A summary of the samples that were used in this study is provided in Table S3.

Three-dimensional (3D) protein structure modeling

Comparative modeling of protein 3D structures was performed using the SWISS-MODEL online-tool [60]. The software was fed with the three protein sequences of MpFER, MpRALF1, and MpLRE1 as input to model, and one PDB file as a 3D template, which contains the published crystal structure of the protein complex formed by AtFER, AtRALF23, and AtLLG2 [35]. Quality control of the model was assessed using the same tool and the results are summarized in Figure S5E. Spatial comparisons of the *M. polymorpha* predicted structures with the crystal structures of *A. thaliana* were performed in PyMol [101].

QUANTIFICATION AND STATISTICAL ANALYSIS

Analyses were performed in InfoStat (<http://www.infostat.com.ar>). Tests are indicated in the corresponding figure legend.

References

1. Showalter AM. (1993). Structure and function of plant cell wall proteins. *Plant Cell* 5, 9–23.
2. Franck CM, Westermann J, Boisson-Dernier A. (2018). Plant malectin-like receptor kinases: from cell wall integrity to immunity and beyond. *Annu Rev Plant Biol* 69, 301–328.
3. Shiu SH, Bleecker AB. (2001). Plant receptor-like kinase gene family: diversity, function, and signaling. *Sci STKE* 2001, re22.
4. Steinwand BJ, Kieber JJ. (2010). The role of receptor-like kinases in regulating cell wall function. *Plant Physiol.* 153, 479–84.
5. Wolf S, Hématy K, Hofte H. (2012). Growth control and cell wall signaling in plants. *Annu Rev Plant Biol* 63, 381–407.
6. Escobar-Restrepo J-M, Huck N, Kessler S, Gagliardini V, Gheyselinck J, et al. (2007). The FERONIA receptor-like kinase mediates male-female interactions during pollen tube reception. *Science* 317, 656–660.

- 942 7. Hématy K, Sado PE, Van Tuinen A, Rochange S, Desnos T, et al. (2007). A receptor-like kinase
943 mediates the response of *Arabidopsis* cells to the inhibition of cellulose synthesis. *Curr Biol.* 17,
944 922–931.
- 945 8. Boisson-Dernier A, Roy S, Kritsas K, Grobei MA, Jaciubek M, Schroeder, J.I., Grossniklaus, U. (2009).
946 Disruption of the pollen-expressed *FERONIA* homologs *ANXUR1* and *ANXUR2* triggers pollen tube
947 discharge. *Development* 136, 3279–3288.
- 948 9. Guo H, Li L, Ye H, Yu X, Algreen A, Yin Y. (2009). Three related receptor-like kinases are required for
949 optimal cell elongation in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 106, 7648–7653.
- 950 10. Guo H, Ye H, Li L, Yin Y. (2009). A family of receptor-like kinases are regulated by *BES1* and involved
951 in plant growth in *Arabidopsis thaliana*. *Plant Signal Behav* 4, 784–786.
- 952 11. Kessler SA, Shimosato-Asano H, Keinath NF, Wuest SE, Ingram G, et al. (2010). Conserved molecular
953 components for pollen tube reception and fungal invasion. *Science* 330, 968–971.
- 954 12. Bai L, Ma X, Zhang G, Song S, Zhou Y, et al. (2014). A receptor-like kinase mediates ammonium
955 homeostasis and is important for the polar growth of root hairs in *Arabidopsis*. *Plant Cell* 26, 1497–
956 511.
- 957 13. Schoenaers S, Balcerowicz D, Breen G, Hill K, Zdanio M, Mouille G, Holman TJ, Oh J, Wilson MH, et
958 al., (2018). The auxin-regulated CrRLK1L kinase ERULUS controls cell wall composition during root
959 hair tip growth. *Curr. Biol.* 28, 722–732.
- 960 14. Miyazaki S, Murata T, Sakurai-Ozato N, Kubo M, Demura T, et al. (2009). *ANXUR1* and 2, sister
961 genes to *FERONIA/SIRENE*, are male factors for coordinated fertilization. *Curr Biol.* 19, 1327–1331.
- 962 15. Keinath NF, Kierszniowska S, Lorek J, Bourdais G, Kessler SA, Shimosato-Asano H, Grossniklaus U,
963 Schulze WX, Robatzek S, Panstruga RJ (2010). PAMP (pathogen-associated molecular pattern)-
964 induced changes in plasma membrane compartmentalization reveal novel components of plant
965 immunity. *J Biol Chem* 285, 39140–39149.
- 966 16. Duan Q, Kita D, Li C, Cheung AY, Wu HM. (2010). *FERONIA* receptor-like kinase regulates RHO
967 GTPase signaling of root hair development. *Proc Natl Acad Sci USA* 107, 17821–17826.
- 968 17. Galindo-Trigo S, Blanco-Touriñán N, DeFalco TA, Wells ES, Gray JE, Zipfel C, Smith LM. (2020).
969 CrRLK1L receptor-like kinases HERK1 and ANJEA are female determinants of pollen tube reception.
970 *EMBO Rep* 21, e48466.
- 971 18. Zhu L, Chu LC, Liang Y, Zhang XQ, Chen LQ, Ye D. (2018). The *Arabidopsis* CrRLK1L protein kinases
972 BUPS1 and BUPS2 are required for normal growth of pollen tubes in the pistil. *Plant J* 95, 474–486.
- 973 19. Li C, Yeh F-L, Cheung AY, Duan Q, KitaD, et al. (2015). Glycosylphosphatidylinositol-anchored
974 proteins as chaperones and co-receptors for *FERONIA* receptor kinase signaling in *Arabidopsis*. *eLife*
975 4, e06587.
- 976 20. Richter J, Ploderer M, Mongelard G, Gutierrez L, Hauser MT. (2017). Role of CrRLK1L cell wall
977 sensors HERCULES1 and 2, THESEUS1, and *FERONIA* in growth adaptation triggered by heavy metals
978 and trace elements. *Front Plant Sci* 8, 1554.

- 979 21. Richter J, Watson JM, Stasnik P, Borowska M, Neuhold J, Berger M, Stolt-Bergner P, Schoft V,
980 Hauser MT. (2018). Multiplex mutagenesis of four clustered *CrRLK1L* with CRISPR/Cas9 exposes
981 their growth regulatory roles in response to metal ions. *Sci Rep* 8, 12182.
- 982 22. Stegmann M, Monaghan J, Smakowska-Luzan E, Rovenich H, Lehner A, et al. (2017). The receptor
983 kinase FER is a RALF-regulated scaffold controlling plant immune signaling. *Science* 355, 287–289.
- 984 23. Ngo QA, Vogler H, Lituiev DS, Nestorova A, Grossniklaus U. (2014). A calcium dialog mediated by
985 the *FERONIA* signal transduction pathway controls plant sperm delivery. *Dev Cell* 29, 491–500.
- 986 24. Yu F, Qian L, Nibau C, Duan Q, Kita D, et al. (2012). *FERONIA* receptor kinase pathway suppresses
987 abscisic acid signaling in *Arabidopsis* by activating ABI2 phosphatase. *Proc Natl Acad Sci USA* 109,
988 14693–14698.
- 989 25. Mao D, Yu F, Li J, Van de Poel B, Tan D, et al. (2015). *FERONIA* receptor kinase interacts with S-
990 adenosylmethionine synthetase and suppresses S-adenosylmethionine production and ethylene
991 biosynthesis in *Arabidopsis*. *Plant Cell Env.* 38, 2566–2574.
- 992 26. Deslauriers SD, Larsen PB. (2010). *FERONIA* is a keymodulator of brassinosteroid and ethylene
993 responsiveness in *Arabidopsis* hypocotyls. *Mol Plant* 3, 626–640.
- 994 27. Shih H-W, Miller ND, Dai C, Spalding EP, Monshausen GB. (2014). The receptor-like kinase *FERONIA*
995 is required for mechanical signal transduction in *Arabidopsis* seedlings. *Curr Biol.* 24, 1887–1892.
- 996 28. Denness L, McKenna JF, Segonzac C, Wormit A, Madhou P, et al. (2011). Cell wall damage-induced
997 lignin biosynthesis is regulated by a reactive oxygen species- and jasmonic acid-dependent process
998 in *Arabidopsis*. *Plant Physiol* 156, 1367–1374.
- 999 29. Okuda S, Tsutsui H, Shiina K, Sprunck S, Takeuchi H, Yui R, Kasahara RD, Hamamura Y, Mizukami A,
1000 Susaki D, Kawano N, Sakakibara T, Namiki S, Itoh K, Otsuka K, Matsuzaki M, Nozaki H, Kuroiwa T,
1001 Nakano A, Kanaoka MM, Dresselhaus T, Sasaki N, Higashiyama T. (2009). Defensin-like polypeptide
1002 LUREs are pollen tube attractants secreted from synergid cells. *Nature* 458, 357–361.
- 1003 30. Huck N, Moore JM, Federer M, Grossniklaus U. (2003). The *Arabidopsis* mutant *feronia* disrupts the
1004 female gametophytic control of pollen tube reception. *Development* 130, 2149–2159.
- 1005 31. Rotman N, Rozier F, Boavida L, Dumas C, Berger F, Faure J-E. (2003). Female control of male gamete
1006 delivery during fertilization in *Arabidopsis thaliana*. *Curr Biol.* 13, 432–436.
- 1007 32. Ge Z, Bergonci T, Zhao Y, Zou Y, Du S, et al. (2017). *Arabidopsis* pollen tube integrity and sperm
1008 release are regulated by RALF-mediated signaling. *Science* 358, 1596–1600.
- 1009 33. Haruta M, Sabat G, Stecker K, Minkoff BB, Sussman MR. (2014). A peptide hormone and its receptor
1010 protein kinase regulate plant cell expansion. *Science* 343, 408–411.
- 1011 34. Dünser K, Gupta S, Herger A, Feraru MI, Ringli C, Kleine-Vehn J. (2019). Extracellular matrix sensing
1012 by *FERONIA* and Leucine-Rich Repeat Extensins controls vacuolar expansion during cellular
1013 elongation in *Arabidopsis thaliana*. *EMBO J* 38, pii: e100353.
- 1014 35. Xiao Y, Stegmann M, Han Z, DeFalco TA, Parys K, Xu L, Belkadir Y, Zipfel C, Chai J. (2019).
1015 Mechanisms of RALF peptide perception by a heterotypic receptor complex. *Nature* 572, 270–274.

- 1016 36. Mecchia MA, Santos-Fernandez G, Duss NN, Somoza SC, Boisson-Dernier A, et al. (2017). RALF4/19
1017 peptides interact with LRX proteins to control pollen tube growth in *Arabidopsis*. *Science* 358,
1018 1600–1603.
- 1019 37. Capron A, Gourgues M, Neiva LS, Faure J-E, Berger F, Pagnussat G, Krishnan A, Alvarez-Mejia C,
1020 Vielle-Calzada JP, Lee YR, Liu B, Sundaresan V. (2008). Maternal control of male-gamete delivery in
1021 *Arabidopsis* involves a putative GPI-anchored protein encoded by the *LORELEI* gene. *Plant Cell* 20,
1022 3038–3049.
- 1023 38. Boisson-Dernier A, Kessler SA, Grossniklaus U. (2011). The walls have ears: the role of plant
1024 *CrRLK1*Ls in sensing and transducing extracellular signals. *J Exp Bot* 62.
- 1025 39. Hématy K, Höfte H. (2008). Novel receptor kinases involved in growth regulation. *Curr Opin Plant*
1026 *Biol.* 11, 321–328.
- 1027 40. Cheung AY, Wu HM. (2011). THESEUS 1, FERONIA and relatives: a family of cell wall-sensing
1028 receptor kinases? *Curr Opin Plant Biol* 14, 632–641.
- 1029 41. Nissen KS, Willats WGT, Malinovsky FG. (2016). Understanding *CrRLK1*L function: cell walls and
1030 growth control. *Trends Plant Sci* 21, 516–527.
- 1031 42. Feng W, Kita D, Peaucelle A, Cartwright HN, Doan V, Duan Q, Liu MC, Maman J, Steinhorst L,
1032 Schmitz-Thom I, Yvon R, Kudla J, Wu HM, Cheung AY, Dinneny JR. (2018). The FERONIA receptor
1033 kinase maintains cell-wall integrity during salt stress through Ca^{2+} signaling. *Curr Biol.* 28, 666–675.
- 1034 43. Lindner H, Müller LM, Boisson-Dernier A, Grossniklaus U. (2012). *CrRLK1*L receptor-like kinases: not
1035 just another brick in the wall. *Curr Opin Plant Biol* 15, 656–669.
- 1036 44. Galindo-Trigo S, Gray JE, Smith LM. (2016). Conserved roles of *CrRLK1*L receptor-like kinases in cell
1037 expansion and reproduction from algae to angiosperms. *Front Plant Sci* 7, 1269.
- 1038 45. Bowman JL, Kohchi T, Yamato KT, Jenkins J, Shu S, Ishizaki K, et al. (2017). Insights into land plant
1039 evolution garnered from the *Marchantia polymorpha* genome. *Cell* 171, 287-304.e15.
- 1040 46. Rövekamp M, Bowman JL, Grossniklaus U. (2014). Back to the rhizoids of plant sexual development.
1041 Abstr. 23rd Intl. Congr. Sex. Plant Reprod. Porto Port.,
1042 [https://www.fc.up.pt/sprporto2014/doc/23rd%20International%20congress%20on%20sexual%20p](https://www.fc.up.pt/sprporto2014/doc/23rd%20International%20congress%20on%20sexual%20plant%20reproduction%20ABSBook.pdf)
1043 [lant%20reproduction%20ABSBook.pdf](https://www.fc.up.pt/sprporto2014/doc/23rd%20International%20congress%20on%20sexual%20plant%20reproduction%20ABSBook.pdf).
- 1044 47. Bowman JL, Floyd SK, Sakakibara K (2007). Green genes-comparative genomics of the green branch
1045 of life. *Cell* 129, 229–234.
- 1046 48. Nishiyama T, Fujita T, Shin-I T, Seki M, Nishide H, Uchiyama I, Kamiya A, Carninci P, Hayashizaki Y,
1047 Shinozaki K, et al. (2003). Comparative genomics of *Physcomitrella patens* gametophytic
1048 transcriptome and *Arabidopsis thaliana*: implication for land plant evolution. *Proc Natl Acad Sci USA*
1049 100, 8007–8012.
- 1050 49. Qiu YL, Li L, Wang B, Chen Z, Knoop V, Groth-Malonek M, Dombrovskaya O, Lee J, Kent L, Rest J, et al.
1051 (2006). The deepest divergences in land plants inferred from phylogenomic evidence. *Proc Natl*
1052 *Acad Sci USA* 103, 15511–15516.

- 1053 50. Wickett NJ, Mirarab S, Nguyen N, Warnow T, Carpenter E, Matasci N, Ayyampalayam S, Barker MS,
1054 Burleigh JG, Gitzendanner MA, et al. (2014). Phylotranscriptomic analysis of the origin and early
1055 diversification of land plants. *Proc Natl Acad Sci USA* 111, E4859–E4868.
- 1056 51. Mishler BD, Churchill SP (1984). A cladistic approach to the phylogeny of the “bryophytes.”
1057 *Brittonia* 36, 406–424.
- 1058 52. Kenrick P, Crane PR (1997). The origin and early evolution of plants on land. *Nature* 389, 33–39.
- 1059 53. Floyd SK, Bowman JL. (2007). The ancestral developmental tool kit of land plants. *Int J Plant Sci* 168,
1060 1–35.
- 1061 54. Bowman JL, Araki T, Arteaga-Vazquez MA, Berger F, Dolan L, Haseloff J, Ishizaki K, Kyoizuka J, Lin SS,
1062 Nagasaki H, Nakagami H, Nakajima K, Nakamura Y, Ohashi-Ito K, Sawa S, Shimamura M, Solano R,
1063 Tsukaya H, Ueda T, Watanabe Y, Yamato KT, Zachgo S, Kohchi T (2016). The naming of names:
1064 guidelines for gene nomenclature in *Marchantia*. *Plant Cell Physiol* 57, 257–261.
- 1065 55. Ishizaki K (2017). Evolution of land plants: insights from molecular studies on basal lineages. *Biosci*
1066 *Biotechnol Biochem* 81, 73–80.
- 1067 56. Honkanen S, Jones VAS, Morieri G, Champion C, Hetherington AJ, Kelly S, Proust H, Saint-Marcoux
1068 D, Prescott H, Dolan L. (2016). The mechanism forming the cell surface of tip-growing rooting cells
1069 is conserved among land plants. *Curr Biol*. 26, 3238–3244.
- 1070 57. Westermann J, Streubel S, Franck CM, Lentz R, Dolan L, Boisson-Dernier A. (2019). An evolutionarily
1071 conserved receptor-like kinases signaling module controls cell wall integrity during tip growth. *Curr*
1072 *Biol*. 29, 4153.
- 1073 58. Kessler SA, Lindner H, Jones DS, Grossniklaus U. (2014). Functional analysis of related *CrRLK1L*
1074 receptor-like kinases in pollen tube reception. *EMBO Rep* 16, 107–115.
- 1075 59. Hirano N, Marukawa Y, Abe J, Hashiba S, Ichikawa M, Tanabe Y, (2015). A receptor-like kinase,
1076 related to cell wall sensor of higher plants, is required for sexual reproduction in the unicellular
1077 Charophycean alga, *Closterium peracerosum–strigosum– littorale* Complex. *Plant Cell Physiol* 56,
1078 1456–1462.
- 1079 60. Schwede T, Kopp J, Guex N, Peitsch MC (2003). SWISS-MODEL: an automated protein homology-
1080 modeling server. *Nucleic Acids Res.* 31, 3381–3385.
- 1081 61. Liu L, Zheng C, Kuang B, Wei L, Yan L, Wang T (2016). Receptor-like kinase RUPO interacts with
1082 potassium transporters to regulate pollen tube growth and integrity in rice. *PLoS Genet* 12,
1083 e1006085.
- 1084 62. Schulze-Muth P, Irmeler S, Schröder G, Schröder J (1996). Novel type of receptor-like protein kinase
1085 from a higher plant (*Catharanthus roseus*): cDNA, gene, intramolecular autophosphorylation, and
1086 identification of a threonine important for auto- and substrate phosphorylation. *J Biol Chem* 271,
1087 26684–26689.
- 1088 63. Flores-Sandoval E, Dierschke T, Fisher TJ, Bowman JL (2016). Efficient and inducible use of artificial
1089 microRNAs in *Marchantia polymorpha*. *Plant Cell Physiol* 57, 281–290.

- 1090 64. Althoff F, Kopischke S, Zobell O, Ide K, Ishizaki K, Kohchi T, Zachgo S. (2014). Comparison of the
1091 MpEF1 α and CaMV35 promoters for application in *Marchantia polymorpha* overexpression studies.
1092 Transgenic Res. 23, 235–244.
- 1093 65. Gonneau M, Desprez T, Martin M, Doblas VG, Bacete L, Miart F, Sormani R, Hématy K, Renou J,
1094 Landrein B et al. (2018). Receptor kinase THESEUS1 is a RAPID ALKALINIZATION FACTOR34 receptor
1095 in *Arabidopsis*. Curr Biol. 28, 2452–2458.
- 1096 66. Gachomo EW, Jno Baptiste L, Kefela T, Saidel WM, Kotchoni SO (2014). The *Arabidopsis* CURVY1
1097 (CVY1) gene encoding a novel receptor-like protein kinase regulates cell morphogenesis, flowering
1098 time and seed production. BMC Plant Biol 14, 221.
- 1099 67. Merz D, Richter J, Gonneau M, Sanchez-Rodriguez C, Eder T, Sormani R, Martin M, Hématy K, Höfte
1100 H, Hauser MT (2017). T-DNA alleles of the receptor kinase THESEUS1 with opposing effects on cell
1101 wall integrity signaling. J Exp Bot 68, 4583–4593.
- 1102 68. Chiyoda S, Ishizaki K, Kataoka H, Yamato KT, Kohchi T (2008). Direct transformation of the liverwort
1103 *Marchantia polymorpha* L. by particle bombardment using immature thalli developing from spores.
1104 Plant Cell Rep 27, 1467–1473.
- 1105 69. Srivastava R, Liu JX, Guo H, Yin Y, Howell SH (2009). Regulation and processing of a plant peptide
1106 hormone, AtRALF23, in *Arabidopsis*. Plant J 59, 930–939.
- 1107 70. Boisson-Dernier A, Franck CM, Lituiev DS, Grossniklaus U. (2015). Receptor-like cytoplasmic kinase
1108 MARIS functions downstream of CrRLK1L-dependent signaling during tip growth. Proc Natl Acad Sci
1109 USA 112, 12211–12216.
- 1110 71. Liao H-Z, Zhu M-M, Cui H-H, Du X-Y, Tang Y, et al. (2016). MARIS plays important roles in
1111 *Arabidopsis* pollen tube and root hair growth. J Integr Plant Biol 58, 927–940.
- 1112 72. Bowen EJ (1935). A note on the conduction of water in *Fimbraria bleumeana*. Ann. Bot. 49, 844–
1113 848.
- 1114 73. Fujikura U, Elsaesser L, Breuninger H, Sánchez-Rodríguez C, Ivakov A, Laux T, Findlay K, Persson S,
1115 Lenhard M. (2014). Atkinesin-13A modulates cell-wall synthesis and cell expansion in *Arabidopsis*
1116 *thaliana* via the THESEUS1 pathway. PLoS Genet 10, e1004627.
- 1117 74. Pierik R, Tholen D, Poorter H, Visser EJ, Voesenek LA (2006). The Janus face of ethylene: growth
1118 inhibition and stimulation. Trends Plant Sci 11, 176–183.
- 1119 75. Wolf S, Höfte H. (2014). Growth control: a saga of cell walls, ROS, and peptide receptors. Plant Cell
1120 26, 1848–1856.
- 1121 76. Humplík JF, Bergougnoux V, Van Volkenburgh E (2017). To stimulate or inhibit? That Is the question
1122 for the function of abscisic acid. Trends Plant Sci 22, 830–841.
- 1123 77. Flores-Sandoval E, Eklund DM, Bowman, JL (2015). A simple auxin transcriptional response system
1124 regulates multiple morphogenetic processes in the liverwort *Marchantia polymorpha*. PLoS Genet
1125 11, e1005207.

- 1126 78. De Rybel B, van den Berg W, Lokerse AS., Liao C-Y, van Mourik H, Moller B, Llavata-Peris CI, Weijers
1127 D (2011). A versatile set of ligation-independent cloning vectors for functional studies in plants.
1128 Plant Physiol. 156, 1292–1299.
- 1129 79. Rövekamp M, Bowman JL, Grossniklaus U (2016). *Marchantia* MpRKD regulates the gametophyte-
1130 sporophyte transition by keeping egg cells quiescent in the absence of fertilization. Curr. Biol. 26,
1131 1782–1789.
- 1132 80. Zuker M. (2003). Mfold web server for nucleic acid folding and hybridization prediction. Nucleic
1133 Acids Res 31, 3406–3415.
- 1134 81. Aach J, Mali P, Church GM (2014). CasFinder: flexible algorithm for identifying specific Cas9 targets
1135 in genomes. BioRxiv Doi 101101005074.
- 1136 82. Curtis MD, Grossniklaus U (2003). A Gateway cloning vector set for high-throughput functional
1137 analysis of genes *in planta*. Plant Physiol. 133, 462–469.
- 1138 83. Ishizaki K, Nishihama R, Ueda M, Inoue K, Ishida S, Nishimura Y, Shikanai T, Kohchi T (2015).
1139 Development of Gateway binary vector series with four different selection markers for the
1140 liverwort *Marchantia polymorpha*. PLoS ONE 10, e0138876.
- 1141 84. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. (2002).
1142 Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple
1143 internal control genes. Genome Biol 3, RESEARCH0034.
- 1144 85. Kubota A, Ishizaki K, Hosaka M, Kohchi T (2013). Efficient *Agrobacterium*-mediated transformation
1145 of the liverwort *Marchantia polymorpha* using regenerating thalli. Biosci Biotechnol Biochem 77,
1146 167–172.
- 1147 86. Ishizaki K, Chiyoda S, Yamato KT, Kohchi, T (2008). *Agrobacterium*-mediated transformation of the
1148 haploid liverwort *Marchantia polymorpha* L., an emerging model for plant biology. Plant Cell
1149 Physiol. 49, 1084–1091.
- 1150 87. Barnes CR, Land WJG. (1907). Bryological papers. I. The origin of air chambers. Bot Gaz 44, 197–213.
- 1151 88. Hughes SJ (1971). On conidia of fungi, and gemmae of algae, bryophytes, and pteridophytes. Can J
1152 Bot 49, 1319–1339.
- 1153 89. Bent A. (2006). *Agrobacterium* protocols. Totowa NJ Humana Press Volume 1.
- 1154 90. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, et al. (2012).
1155 Fiji: An open-source platform for biological-image analysis. Nat. Methods 9, 676–682.
- 1156 91. Solly JE, Cuniffe NJ Harrison CJ (2017). Regional growth rate differences specified by apical notch
1157 activities regulate liverwort thallus shape. Curr. Biol. 27, 16–26.
- 1158 92. Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of
1159 bacteriophage T4. Nature 227, 680–685.
- 1160 93. Bushnell B (2014). BBMap: a fast, accurate, splice-aware aligner (No. LBNL-7065E). Lawrence
1161 Berkeley Natl. LabLBNL Berkeley, CA, USA.

1162 94. Trapnell C, Pachter L, Salzberg SL (2009). TopHat: discovering splice junctions with RNA-Seq.
1163 Bioinformatics 25, 1105–1111.

1164 95. Langdon WB (2015). Performance of genetic programming optimised Bowtie2 on genome
1165 comparison and analytic testing (GCAT) benchmarks. BioData Min. 8, 1.

1166 96. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al (2009). The sequence
1167 alignment/map format and SAMtools. Bioinformatics 25, 2078–2079.

1168 97. Anders S, Pyl PT, Huber W. (2015). HTSeq—a Python framework to work with high-throughput
1169 sequencing data. Bioinformatics 31, 166–169.

1170 98. Love MI, Huber W, Anders S (2014). Moderated estimation of fold change and dispersion for RNA-
1171 seq data with DESeq2. Genome Biol. 15, 550.

1172 99. Team RC (2013). R: A language and environment for statistical computing.

1173 100. Wickham H (2016). ggplot2: elegant graphics for data analysis. *Springer*.

1174 101. DeLano WL (2002). Pymol: An open-source molecular graphics tool. CCP4 Newsl. Protein
1175 Crystallogr. 40, 82–92.

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Figure 1. CrRLK1Ls Are Conserved among Land Plants

(A) Representation of AtFER and MpFER proteins. Malectin-like domains are represented in blue and kinase domains in green. SP, signal peptide; TM, transmembrane domain (yellow). Amino acids important for regulation and activity are marked in red, violet, and light blue as described in Figure 1E.

(B) A rooted neighbor-joining tree of the amino acid sequence of the predicted malectin-like domain. CrRLK1L members from *Marchantia polymorpha* (Mp), *Physcomitrella patens* (Pp), *Selaginella moellendorffii* (Smo), and *Arabidopsis thaliana* (At) were used. The numbers indicate the bootstrap values (%) from 1000 replications. The given scale represents a substitution frequency of 0.1 amino acids per site.

1190 (C) Cartoon representation of the predicted three-dimensional structure of the MpFER
 1191 malectin-like domain, showing predicted alpha-helix and beta-sheet structures.
 1192 (D) Structural superposition of the malectin-like domains of AtFER (blue) and MpFER
 1193 (green).
 1194 (E) Alignment of the cytoplasmic domains of MpFER, AtERU, AtFER, and CrRLK. Kinase
 1195 domains are in green, putative phosphorylation sites in light blue (Ser) and violet (Thr),
 1196 and the conserved catalytic Lys in red.
 1197 See also Figure S1
 1198

Figure 2. MpFER Is Broadly Expressed in most Tissues throughout the *M. polymorpha* Life Cycle

Expression of *proMpFER:trpVNS* in different organs of male and female *M. polymorpha* plants. Fluorescence is visualized as either green (epifluorescence microscope) or yellow (confocal microscope) color, depending on the panel.

(A to D) Bright field (left) and epifluorescence (right) images of a thallus, (A) rhizoid (C), gemmae cups (C), and an archegoniophore (D). Red arrows indicate meristematic zones, the yellow arrow gemmae. Scale bars, 0.5 mm.

(E and F) Confocal images of archegonia before (E) and 2 days after fertilization (F). Scale bar, 50 μm.

(G) Bright field (left) and epifluorescence (right) images of an antheridiophore. Scale bar, 1 mm.

(H) Confocal image of antheridia. Scale bar, 100 μm.

(I) Confocal image of sporophyte and spores. Scale bar, 25 μm.

Figure 3. MpFER Controls Cell Size during Gametophyte Development

(A) Representation of the mature *MpFER* mRNA. Coding sequence are in green, locations of the amiRNA target sites in blue.

(B) Base complementarity between mature amiR-*MpFER1*, amiR-*MpFER2*, and amiR-*MpFER3* with the *MpFER* transcript.

(C) Relative expression level of *MpFER* in thallus tissue from wild-type (WT; Tak1) and two independent insertion lines of amiR-*MpFER2* and amiR-*MpFER3*, as measured by qRT-PCR. *MpEF1* was used as internal control. Shown are means $\pm$ standard errors of the means (SEM) of three biological replicates. * $P < 0.01$, one-way analysis of variance (ANOVA).

(D) Relative expression level of *MpFER* in WT and *Mpfer-1* thallus tissue, as measured by qRT-PCR. *MpEF1* was used as internal control. Shown are means $\pm$ SEM of three biological replicates. * $P < 0.01$, one-way ANOVA.

1230 (E) Representative pictures of 10-day old gemmalings of WT, *Mpfer-1*, and amiR-
 1231 *MpFER3-2* lines. Scale bar, 1 mm.

1232 (F) Thallus area of WT, *Mpfer-1*, and amiR-*MpFER3-2* lines. n = 30, outliers are indicated
 1233 as black dots.

1234 (G) Violin plot of cell size in WT, *Mpfer-1*, and amiR-*MpFER3-2* lines. Significant
 1235 differences are indicated according to the non-parametric Kruskal-Wallis test and linear
 1236 regression models (**p<0.001).

1237 See also Figures S2 and S3

1238

1239

Figure 4. The Integrity of Thalli from *Mpfer* Knock-out Lines Is Severely Affected

(A) Representation of the mature *MpFER* mRNA with the coding sequence in green, and the location of the gRNA target sites in red.

(B) Sequences of gRNA target sites in the confirmed *Mpfer* knock-out mutants. Deletions and insertions are highlighted in red.

(C) Representative pictures of 10-day old gemmalings of WT, *Mpfer-1*, and two independent *Mpfer* knock-out mutants. Scale bar, 0.5 cm.

(D) Thallus area of 10-day old gemmalings of WT, *Mpfer-1*, and two independent *Mpfer* knock-out mutants. n = 30.

(E) Representative pictures of 3-day old gemmalings growing in upside-down plates of WT, *Mpfer-1*, and two independent *Mpfer* knock-out mutants. Scale bar, 500 μ m.

(F) Rhizoid number in 3-day old gemmalings of WT, *Mpfer-1*, and two independent *Mpfer* knock-out mutants. n = 30.

(G) Trypan blue staining of 7-days old gemmalings of WT, *Mpfer-1*, and two independent *Mpfer* knock-out mutants. Scale bar, 1 mm.

Figure 5. Conservation of CrRLK1L Function in Land Plants

(A) Number of antheridiophores produced per plant for wild-type (WT, n=9) and two independent amiR-MpFER3 lines (amiR-MpFER3-2, n = 9; amiR-MpFER3-3, n = 12). Shown are means $\pm$ SEM of two biological replicates. Means with same letter do not differ significant different with $P > 0.05$, one-way ANOVA, Duncan test.

(B) Antheridiophore splash platform size distribution of WT (n = 32), amiR-MpFER2-3 (n = 34), and amiR-MpFER3-3 (n = 11) lines. For all antheridia with a stalk >8 mm, length and width of the platform were recorded.

(C) qRT-PCR of *MpFER* levels in antheridiophores in a WT and amiR-MpFER3-3 line. *MpEF1* was used as internal control. Shown are means $\pm$ SEM of three biological replicates. * $P < 0.01$, ANOVA.

(D and E) Mature antheridia of a WT (D) and amiR-Mp*FER3*-3 line (E). Bright field images (left) and in cross-section (right). The spermatogenous areas are indicated in red. Scale bar, 100 μ m.

(F) Thallus area of 15-day old gemmalings of WT, *Mpfer-1* mutant, and an amiR-Mp*FER3*-2 line growing on media containing different isoxaben concentrations (nM). n = 30, outliers are indicated as black dots. Shown are means $\pm$ SEM of two biological replicates. Means designated by the same letter do not significantly differ at $P > 0.05$, one-way ANOVA, Duncan test.

See also Figure S3

Figure 6. Interspecific Complementation of *A. thaliana* and *M. polymorpha* Plants with Reduced Levels of *FER* Activity

(A) Roots of independent *A. thaliana* lines expressing 35S:MpFER-GFP in Ler wild-type (WT) and *Atfer-2* plants. Scale bar, 200 μ m.

(B) 10-day old gemmalings of WT and amiR-MpFER3-2 *M. polymorpha* plants with and without expression of *AtFER-Cit*. Scale bar, 0.5 mm.

(C) Citrine expression in 7-day old gemmalings of WT and amiR-MpFER3-2 plants with and without expression of *AtFER-Cit*. Scale bar, 100 μ m.

(D) Cartoon representation of the structural conservation of the CrRLK1L^{ECD}/LRE/RALF complex by comparing AtFER/MpFER and AtLLG2/MpLRE1. Blue and green represent spatially highly conserved regions of CrRLK1L and LRE homologs, respectively. Red indicates structural regions with low spatial conservation, lilac the C-terminal part of AtRALF23, and black arrows the interaction regions of the three proteins.

(E) Complementation of amiR-MpFER lines with a dominant-active version of MpMRI under its own promoter (*proMpMRI:MRI^{R240C}*). Scale bar, 1 mm.

See also Figures S4-S7

Figure 7: Overexpression of MpFER Affects Morphological Integrity

(A) Representative pictures of 10-day old gemmalings of wild-type (WT, Tak1) and different lines overexpressing MpFER (*proMpEF1:MpFER-Cit*). Scale bar, 1 mm.

(B) Citrine expression at the plasma membrane in WT and *proMpEF1:MpFER-Cit* lines #5 and #9 as observed under confocal microscopy. Scale bar, 20 μ m.

(C) Western blot analysis of *proMpEF1:MpFER-Cit* lines using an anti-GFP antibody. *proMpEF1:Cit* and WT lines were used as positive and negative controls, respectively. The Ponceau membrane staining of the most intense band at 55 kDa (presumably Rubisco) was used as a loading control.

(D) Scanning electron microscopical images of thalli from 20-day old plants of WT and *proMpEF1:MpFER-Cit* lines #5 and #9. Scale bar, 500 μ m.

(E) Representative pictures of 3-day old gemmalings growing in upside-down plates of WT and *proMpEF1:MpFER-Cit* lines #5 and #9. Scale bar, 1 mm.

(F) Number of rhizoids in 3-day old gemmalings of WT and *proMpEF1:MpFER-Cit* lines #5 and #9. n = 30.

See also Figure S6

Figure S1. The CrRLK1L Family Appeared together with Land Plants. Related to Figure 1.

(A) Percentage of identity of the malectin-like domain, the complete ECD, and the full protein of MpFER with AtFER, AtANX1, AtANX2, and AtTHE1.

(B) Percentage of identity of the ECD and full protein of CpRLK1 with MpFER, AtFER, and Mapoly001s0111.

(C) A rooted neighbor-joining tree of the amino acid sequence of the predicted malectin-like domain was generated using ClustalW. CrRLK1L members from *Marchantia polymorpha* (Mp), *Physcomitrella patens* (Pp), *Selaginella moellendorffii* (Smoe), and *Arabidopsis thaliana* (At) were used. Algal CpRLK1 and Mapoly001s0111 were also included. The numbers indicate the bootstrap values (%) from 1000 replications. The given scale represents a substitution frequency of 0.1 amino acids per site.

1333 (D) Qmean value for prediction of MpFER or CpRLK1 3D structures of the ECD based
 1334 on the AtFER ECD. Green numbers indicate a good modelling fit, red numbers indicates
 1335 a bad modelling fit.
 1336 (E) Structural superposition of the ECD of AtFER (blue) and CpRLK1 (purple).
 1337

A *MpmiR160* Mplg26670
GCACCTCCTCTCTCCGACTGCAGCCCGTTTCGAGATCCGAGGACTTGCTCGACGCGACTAATTGGGGAGGCCAGACTG
CACTT**GGCTGGCTCCCTGTATGCCA**ACTGAGGAGCTCCTCAGAGACCTTGACAGGCTCCGTAGCTGGCATT**CAGGGG**
CCATGCAGGAGGAAGTCGCTACCTCCCGCAAGGTGCGACTAGCTTTCTGTCTTGGGTGCACACCTCACTGATGTTTGA
TAGATTACTTA

amiR-Mp**FER1**
TGCAAGCTTGCACCTCCTCTCTCCGACTGCAGCCCGTTTCGAGATCCGAGGACTTGCTCGACGCGACTAATTGGGGAG
GCCAGACTGCACT**TGACGTAGGCAAACTTGT**CGAACTGAGGAGCTCCTCAGAGACCTTGACAGGCTCCGTAGCT**CGAC**
ATGTTTGTCTACCTCAGGAGGAAGTCGCTACCTCCCGCAAGGTGCGACTAGCTTTCTGTCTTGGGTGCACACCTCACT
GATGTTTGATAGATTACTTAG**GATCCATA**

amiR-Mp**FER2**
TGCAAGCTTGCACCTCCTCTCTCCGACTGCAGCCCGTTTCGAGATCCGAGGACTTGCTCGACGCGACTAATTGGGGAG
GCCAGACTGCACT**TCGATGT**CGAAAT**CCAGGTG**ACTGAGGAGCTCCTCAGAGACCTTGACAGGCTCCGTAGCT**CACCT**
GCATTTTTGACAACGAGGAGGAAGTCGCTACCTCCCGCAAGGTGCGACTAGCTTTCTGTCTTGGGTGCACACCTCACT
GATGTTTGATAGATTACTTAG**GATCCATA**

amiR-Mp**FER3**
TGCAAGCTTGCACCTCCTCTCTCCGACTGCAGCCCGTTTCGAGATCCGAGGACTTGCTCGACGCGACTAATTGGGGAG
GCCAGACTGCACT**TCGAACTTCTCTCAGAGACT**CACTGAGGAGCTCCTCAGAGACCTTGACAGGCTCCGTAGCT**GAGTC**
TGTGAGGGAGTTGGGAGGAGGAAGTCGCTACCTCCCGCAAGGTGCGACTAGCTTTCTGTCTTGGGTGCACACCTCACT
GATGTTTGATAGATTACTTAG**GATCCATA**

Figure S2. Design of amiR-MpFER** Precursors. Related to Figure 3.**

(A) *MpmiR160* (Mapoly0002s0211, Mp1g26670) and amiR-Mp**FER1**, amiR-Mp**FER2**, and amiR-Mp**FER3** sequences. miRNA sequences are in red and miRNA* in blue. Cloning sequences from amiR-Mp**FER** constructs are in bold.

1344 (B) Drawing of the minimum free energy structure of *MpmiR160* and *amiR-MpFER3*
 1345 constructs predicted by the RNAfold web server ([http://rna.tbi.univie.ac.at/cgi-](http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi)
 1346 [bin/RNAWebSuite/RNAfold.cgi](http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi)). Red arrows indicate location and orientation of the
 1347 mature miRNA in the precursor. The structures are colored by base-pairing probabilities;
 1348 for unpaired regions the color denotes the probability of being unpaired.
 1349

Figure S3. Reduction in Thallus and Cell Area in Plants with Reduced *MpFER* Levels. Related to Figures 3 and 5.

(A) Thallus area of wild-type (WT), *Mpfer-1*, and *amiR-MpFER3-2* plants at different days after putting gemmae on plates. $n = 30$, outliers are indicated as black dots. Areas were estimated using ImageJ software.

(B) Representative images from cell surface areas measured in WT, *amiR-MpFER2-1*, and *amiR-MpFER3-2* plants. Scale bar, 50 μm .

(C) Difference in cell size between WT, *amiR-MpFER2-1*, and *amiR-MpFER3-2* plants is significant ($p < 0.001$) based on the nonparametric Kruskal-Wallis test and a linear regression model with a highly significant interaction ($p < 0.001$).

(D) Induction of antheridiophores in WT and two independent *amiR-MpFER3* lines. Three plants were grown in each sterile plastic box under far-red light induction.

Figure S4. MpRALF Peptides Belong to the AtRALF1-clade of the RALF Family. Related to Figure 6.

(A) A rooted neighbor-joining tree of the amino acid sequence of the predicted mature RALF peptides was generated using ClustalW. RALF members from *M. polymorpha* and *A. thaliana* were used. Red arrows indicate RALFs that are known ligands of CrRLK1Ls. The numbers indicate the bootstrap values (%) from 1000 replications. The given scale represents a substitution frequency of 0.1 amino acids per site.

(B) Amino acid sequence comparison of AtRALF1 and MpRALF1-3. Predicted signal peptides are in black, predicted mature peptides in light blue, conserved Cys in red, and predicted S1P recognition sites in violet.

(C) Processing pathway AtRALF1 by the S1P protease.

(D) A rooted neighbor-joining tree of the amino acid sequence of the S1P orthologs was generated using ClustalW. S1P members from *M. polymorpha*, *P. patens*, and *A. thaliana* were used. The numbers indicate the bootstrap values (%) from 1000 replications. The given scale represents a substitution frequency of 0.1 amino acids per site.

Figure S5. Two LORELEI-like Proteins Are Encoded in the *M. polymorpha* Genome.
Related to Figure 6.

(A) A rooted neighbor-joining tree of the amino acid sequence of LRE orthologs was generated using ClustalW. LRE members from *M. polymorpha*, *P. patens*, and *A. thaliana* were used. The numbers indicate the bootstrap values (%) from 1000 replications. The given scale represents a substitution frequency of 0.1 amino acids per site.

(B) Amino acid sequence of AtLRE, MpLRE1, and MpLRE2. Conserved Cys are in light blue, the ND motif in red, and the GPI-anchoring site in green.

(C) Cartoon representation of the predicted 3-dimensional structure of MpLRE1, showing predicted alpha-helices.

(D) Structural superposition of AtLGG2 (blue) and MpLRE1 (green).

(E) Cartoon representation of the predicted 3-dimensional structure of the MpFER/MpLRE1/MpRALF1 complex, showing predicted alpha-helices and beta-sheets.

(F) Heatmap depicting relative gene expression based on RNAseq data (row-Z-score of vs normalized counts) of MpFER and the *M. polymorpha* orthologs of AtMRI, AtRALF1, and AtLRE across different tissues. Vegetative and reproductive tissues are grouped by green and orange, respectively. Averaged expression values are represented with colors of increasing red and blue intensity indicating upregulation and downregulation of gene expression, respectively.

Figure S6. Expression of MpMRI^{R240C} Suppresses the Bursted Rhizoid Phenotype of amiR-MpFER3 Lines but Leads to Aberrant Epidermis Development. Related to Figures 6 and 7.

(A to C) Epidemical pictures of thalli from the wild-type (WT, Tak-1) (A), and amiR-Mp*FER3* + *pro*MpMRI:MpMRI^{R240C} lines #6 (B) and #8 (C), which both partially suppressed the bursting rhizoid phenotype (Fig. 6).

(D and E) Relative expression of Mp*FER* (D) and MpMRI (E) against the geometric mean of the reference genes Mp*ACT1*, Mp*ACT7*, and Mp*APT3* in WT, amiR-Mp*FER3-2*, and 5 lines (#5 to #9) co-transformed with the amiR-Mp*FER3-2* and *pro*MpMRI:MpMRI^{R240C} constructs. Expression levels of three biological replicates were assessed by droplet digital PCR (ddPCR). The y-axis corresponds to the log2-ratio between the test and the geometric mean of the reference genes. Shown are means ± SEMs of three biological replicates. *P < 0.01, one-way ANOVA.

(F) Representative images of the antheridial receptacle of WT and *pro*Mp*EF1*:Mp*FER-Cit* plants. Scale bar, 2 mm.

Figure S7. Structure Assessment and Quality Estimations of the MpFER and MpLRE 3-dimensional Models. Related to Figure 6.

QMEAN score barplot indicating quality estimates of the predicted models, across their aminoacidic sequence. Below the barplots, the corresponding secondary structures are displayed for both target and template sequences, with alpha-helices represented by light purple boxes and beta-sheets by green arrows. All plots were adapted from the model report produced by the SWISS-MODEL workspace.

(A) MpFER^{ECD}, for which AtFER was used as a template (PDB 6a5b.1A).

(B) MpLRE1, for which AtLLG2 was used as a template (PDB 6a5d.1A).