The single *Marchantia polymorpha FERONIA* homolog reveals an ancestral role in regulating cellular expansion and integrity

Martin A. Mecchia, Moritz Rövekamp, Alejandro Giraldo-Fonseca, Dario Meier, Philippe Gadient, Hannes Vogler, Daria Limacher, John L. Bowman and Ueli Grossniklaus
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Original submission

First decision letter

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MS TITLE: The single Marchantia polymorpha FERONIA homolog reveals an ancestral role in regulating cellular expansion and integrity

AUTHORS: Martin A Mecchia, Moritz Rovekamp, Alejandro Giraldo-Fonseca, Dario Meier, Philippe Gadient, Hannes Vogler, Daria Limacher, John A Bowman, and Ueli Grossniklaus

I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision.

We are aware that you may be experiencing disruption to the normal running of your lab that make experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

In this paper Mecchia, RÃ¶vekamp et al. characterize some members of the CrRLK signaling pathway as well as their genetic and putative physical interactions in the species Marchantia polymorpha. The study is globally very nicely conducted and is particularly interesting as it allows to question the function of such CrRLK in a species that only has one of these genes, as opposed to Arabidopsis thaliana which has 17. Interestingly, some but not all functions appear conserved and mpFER and atFER are not interchangeable. It would be interesting to test in the future if other members of the Arabidopsis CrRLK are interchangeable with mpFER. Nevertheless, I think this is an important step toward understanding the still relatively mysterious and diverse functions of CrRLK in a species that only has one to understand what happens when there is none.

Comments for the author

Minor comments:
- Unless I missed it, I could not find the description of a complementation of the mpfer CRISPR mutants that have been generated. This may not appear necessary, but since the atFER complementation in Marchantia doesn't work, I wonder if an equivalent mpFER complementation would work. Related to this, I am wondering how the complementation in the amiR-MpFER lines is supposed to work.
Isn't there a risk that the atFER would be silenced as well? How specific is the signal observed in figure 6C?
- It is mentioned that atFER and mpFER don't inter-complement, but only root hair phenotypes, rhizoïds and thallus size are scored. I think other phenotypes (reproduction, cell bursting,…) should be at least mentioned before stating that the interspecific complementation was unsuccessful.
- The isoxaben treatment was performed only on the mpfer-1 and amiR-MpFER3-2 which are weak alleles. Would you expect the result to be the same in a null mutant?
- Why use Ler as a control for fer-2? The fer-2 allele that I know is in a Col background. Maybe specify the precise nature of the fer-2 line used or its ID from salk or other?
- Can you specify at what stage the cell area measurement was done in figure 3G and S3 B,C, and notably relative to the thallus growth measurement in figure S3A? Also in Fig 3G it appears that many cells would have values close to 0 in the case of mpfer1? Is that the case?
- It would be good to show all underlying data points in all the graphs.
- In the discussion it is stated: "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". However this was recently reported for atFer in Malivert et al., 2021 (https://doi.org/10.1371/journal.pbio.3001454). Also on a separate point, the gene At5g24010 (figure 1 and S1) was proposed to be named theseus1/feronia-related1 (tfr1) in this same publication.
- Line 248 typo "cell wall properticss"

Reviewer 2

Advance summary and potential significance to field

In this interesting paper, Mecchia et al investigate the role of the FERONIA homolog MpFER in growth and development of the liverwort Marchantia polymorpha.
In all angiosperms and mosses sequenced thus far, the CrRLK1L subfamily comprises multiple members, making it difficult to distinguish primary versus derived CrRLK1L functions. Here, the authors take advantage of the fact that MpFER is the sole CrRLK1L encoded in the Marchantia genome to create amiRNA knock-down and CRISPR knock-out mutants of MpFER and thereby identify ancestral functions of the protein family.

The authors generated transgenic Marchantia expressing MpFERpro:3xVenus-NLS to show that MpFER is broadly expressed in most gametophytic and sporophytic tissues, including reproductive tissues. Marchantia mutants exhibited defective gametophyte development, producing significantly

smaller thalli with reduced epidermal cell size. In the more severely affected CRISPR knock-out mutants viability staining demonstrated that multiple epidermal cells had lost cellular integrity and overall, the epidermis of mpfer KO mutants exhibited reduced apparent stiffness as measured with cellular force microscopy. Overexpression of MpFER resulted in severe loss of epidermal integrity, with significant tearing of the epidermis around air pores. Reproductive development in male gametophytes of mutants was impaired, with amiR-lines producing fewer antheridiophores and individual antheridia generating less spermatogenous tissue. Finally, the authors showed that while AtFER and MpFER have significant sequence similarity reciprocal functional complementation was unsuccessful, presumably due to difference in the surfaces where respective FER proteins interact with their RALF ligands and LRE/LLG co-receptors.

While there is no new mechanistic insight into (Mp)FER function, overall, the presented results are solid and support the conclusion that MpFER controls cell expansion and maintenance of cellular integrity, similar to AtFER and related family members in Arabidopsis.

Comments for the author

I do not have any major reservations, but some conclusions could be further strengthened:
1. The authors performed a phylogenetic analysis of the predicted malectin-like domain of Mp4g15890, the sole CrRLK1L of M. polymorpha, and CrRLK1Ls of Physcomitrium patens, Selaginella moellendorfii and Arabidopsis thaliana and also searched for homologues in the genomes of various Chlorophyte and expression data from several Charophytes. As no evidence for CrRLK1Ls was found in these algae, the authors "propose MpFER to be basal and orthologous to all other land plant CrRLK1Ls, and that the CrRLK1L family arose as plants conquered land"(lines 164ff). This is a rather strong statement: to my knowledge, only a single liverwort has been sequenced to date and given that Physcomitrium in the moss sister clade has 5 CrRLK1Ls, it seems quite possible that other CrRLK1Ls were lost during Marchantia evolution. The conclusion that MpFER is basal and orthologous would be strengthened by also evaluating CrRLK1Ls in the recently sequenced hornworts Anthoceros agrestis and A. punctatus (Li et al., 2020, Nat. Plants 6, 259–272) and searching the genome of Chara braunii (Nishiyama et al 2018, Cell 174:448-464.e24). Furthermore, it would be helpful to know the precise MpFER region that was used for phylogenetic analysis (aa #-#).
2. The authors stress that "CrRLK1Ls have a conserved role in reproduction in addition to their role in cell expansion and integrity during vegetative development" (lines 277f). I think a more careful analysis of the particular defects in reproductive development would strengthen this claim. While there appear to be fewer antheridiophores and less spermatogenous tissue, no attempt was made to identify the cause of these defects. The authors show that cell expansion is impaired in thalli of mpfer mutants. Is this true for spermatogenous cells as well? Or are there also defects in mitosis (both in thalli and in reproductive tissues)? If these defects arise solely/primarily from defects in cell expansion, should one really distinguish between cell expansion in vegetative and reproductive tissues? This seems qualitatively different from the role of FER function in Arabidopsis reproduction where it facilitates the communication between female and male gametophytes during pollen reception.
3. AtFER is known to be an important modulator of Arabidopsis immune signaling, apparently functioning as a scaffold to facilitate immune receptor complex formation. It may be beyond the scope of this work to investigate whether Marchantia mpfer mutants are more susceptible to bacterial pathogens (Gimenez-Ibanez et al, 2019, Curr Biol 29: 2270-2281.e4), but the authors should at least discuss that a role in immune signaling has not been ruled out as an 'ancestral' function.

Other points:
- Cellular force microscopy: is the apparent stiffness really "cell wall" (line 148) stiffness, of just cell stiffness (i.e. with an unknown contribution of turgor – which might be different in mpfer mutants)?
- Figure 3:

Figure 3B legend: "Base complementary between mature": Base complementarity between/and or of/with…)
Figure 3C legend: what is Tak1?
Figure 3C-F: the description of panels in the text does not match the labels in the figure.

Figure 3D: Mpfer-1 transcripts should be sequenced to determine why there are mpfer phenotypes when transcript levels are normal (or the line should be complemented with MpFERpro:MpFER).
Figure 3F: how old were gemmalings? Based on comparison with Fig S3A -14d?
Statistical analysis: which post hoc tests were used after ANOVA to determine which samples was statistically significantly different (Fig 3C,D)?
Figure 4:
Figure 4C/D: the sizes of the thalli shown in the images do not match the sizes presented in Fig 4D (either wrong scale bar or different age).
Figure 4D: I suspect there may have been a conversion error (25 mm2 = 0.25 cm2 not 0.025 cm2?)
Figure 4G: 7-day old (not days old)
Fiigure 4H: y-axis label: stiffness, not stiffmes Statistical analysis: Fig. 4H – which 'multiple comparison test' was used after Kruskal Wallis test? Fig 4D,F – was KW or ANOVA used, followed by which post hoc test?
Figures 3/4:
Lines 239f: "The thallus area of the newly generated, amorphic knock-out mutants was more strongly reduced compared to Mpfer-1 [59], confirming that, like the amiR-MpFER lines, Mpfer-1 is a hypomorphic mutant (Fig. 4C,D)". These data are difficult to compare as Fig 3 and Fig 4 present thallus sizes at different developmental stages (10d in Fig. 4 and 14d (based on Fig S3A) in Fig. 3) and the area units do not match (mm2 in Fig 3 and Fig S3, cm2 in Fig 4).
Figure 5:
Figure 5A:" Means with same letter do not differ significant different with P > 0.05". While technically true, this is a bit confusing as there are no means with the same letters.
Figure 6:
Figure 6A: isn't fer-2 in the Arabodopsis col background (not Ler)?
Figure 7:
Lines 365f:" higher 365 protein levels correlated with more severe phenotype (Fig. 7A,C)." This conclusion is rather vague (what are the phenotypes?) and does not really match the data presented in panels A and C. Lines #5, 6, and 9 appear to have the strongest phenotypes (reduced growth), but no protein levels are shown for these lines. Lines #7, 10, 11 have strong overexpression, but no phenotypes are shown. Lines #12 and 13 have very similar thallus sizes, but very different protein expression levels.
Figure 7D: air pores and chambers should be indicated with arrows for readers less familiar with Marchantia morphology.
While all images of rhizoids clearly show that there are fewer/shorter rhizoids in the mpfer mutants, none of the images support the statement that rhizoids collapse/burst.

First revision

Author response to reviewers' comments

Detailed Point-by-Point Response to the Reviewers' Comments

First, we would like to thank the reviewers for their positive evaluation of our manuscript and their insightful and constructive criticisms. We have addressed all the points they raised in the revised version of our manuscript. Below, please find our point-by-point response to the reviewers' comments.

Reviewer 1
Advance summary and potential significance to field...
In this paper Mecchia, Rövekamp et al. characterize some members of the CrRLK signaling pathway as well as their genetic and putative physical interactions in the species Marchantia polymorpha. The study is globally very nicely conducted, and is particularly interesting as it allows to question the function of such CrRLK in a species that only has one of these genes, as opposed to Arabidopsis thaliana which has 17. Interestingly, some but not all functions appear conserved and mpFER and atFER are not interchangeable. It would be interesting to test in the future if other members of the Arabidopsis CrRLK are interchangeable with mpFER. Nevertheless, I think this is an important step

toward understanding the still relatively mysterious and diverse functions of CrRLK in a species that only has one to understand what happens when there is none.
We thank Reviewer 1 for the positive assessment of our work. Indeed, it could be interesting to do test whether MpFER can complement mutants affecting other members of the CrRLK1L family in A. thaliana. However, we agree with Reviewer 1 that this would go beyond the scope of the current study. Moreover, given that our modelling results indicate that complex formation with heterologous subunits is likely impaired, the prospects for successful complementation are rather low.

Comments for the author...
Minor comments:
- Unless I missed it, I could not find the description of a complementation of the mpfer CRISPR mutants that have been generated. This may not appear necessary, but since the atFER complementation in Marchantia doesn't work, I wonder if an equivalent mpFER complementation would work. Related to this, I am wondering how the complementation in the amiR-MpFER lines is supposed to work. Isn't there a risk that the atFER would be silenced as well? How specific is the signal observed in figure 6C?
We made several attempts to complement the Mpfer mutant with MpFER but the mutant turned out to be recalcitrant to transformation. However, we recovered several independent amorphic Mpfer alleles with the same phenotype and this phenotype is consistent with those of several different amiR-MpFER lines and a hypomorphic T-DNA insertion. Thus, we agree with Reviewer 1, that a complementation experiment is not necessary.
To address the potential silencing of AtFER by amiR-MpFER3 we performed an alignment shown in Fig. 3B and described it on page 13/14 of the revised manuscript. The lack of extensive complementarity clearly indicates that AtFER is not getting silenced by amiR-MpFER3.
The proMpFER promoter is expressed in almost all plant cells very similar to the proMpEF1 promoter, which we used to express AtFER-Cit. The Citrine signal was observed at the plasma membrane, indicating a correct subcellular localization of AtFER-Cit.

- It is mentioned that atFER and mpFER don't inter-complement, but only root hair phenotypes, rhizoïds and thallus size are scored. I think other phenotypes (reproduction, cell bursting,…) should be at least mentioned before stating that the interspecific complementation was unsuccessful.
We agree with Reviewer 1 and have qualified this statement saying that "MpFER and AtFER cannot substitute each other with respect to their function in cell expansion and tip growth" on page 14 of the revised manuscript.

- The isoxaben treatment was performed only on the mpfer-1 and amiR-MpFER3-2, which are weak alleles. Would you expect the result to be the same in a null mutant?
We repeated this assay and added the amorphic Mpfer mutant whose growth is inhibited by isoxaben and shows increased cell death. The new results are shown in Fig. 5F,G of the revised manuscript.

- Why use Ler as a control for fer-2? The fer-2 allele that I know is in a Col background. Maybe specify the precise nature of the fer-2 line used or its ID from salk or other?
We thank Reviewer 1 for pointing this out. This was a mistake on our side as we use the Atfer-1 allele originally isolated in our group, which is in the Ler accession. We have corrected this in the text and figure.

- Can you specify at what stage the cell area measurement was done in figure 3G and S3 B,C, and notably relative to the thallus growth measurement in figure S3A? Also in Fig 3G it appears that many cells would have values close to 0 in the case of mpfer1? Is that the case?
The thalli were about 3 weeks old but, importantly, only areas with fully expanded cells were used for the measurements, which is now indicated in the legend of Fig. 3F. We added the data points into the violin plots, showing that some values were indeed close to 0.

- It would be good to show all underlying data points in all the graphs.
We have added data points into the violin plots as suggested, except for the qRT-PCR data where this does not seem necessary.

- In the discussion it is stated: "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". However this was recently reported for atFer in Malivert et al., 2021 (https://doi.org/10.1371/journal.pbio.3001454). Also, on a separate point, the gene At5g24010 (figure 1 and S1) was proposed to be named theseus1/feronia-related1 (tfr1) in this same publication.
We thank Reviewer 1 for pointing this out and refer to this point and cite the paper in the revised version of the manuscript. We also changed At5g24010 to AtTFR1.

- Line 248 typo “cell wall properticss”
Thank you. We corrected this typo in the revised manuscript.

Reviewer 2
Advance summary and potential significance to field...
In this interesting paper, Mecchia et al investigate the role of the FERONIA homolog MpFER in growth and development of the liverwort Marchantia polymorpha. In all angiosperms and mosses sequenced thus far, the CrRLK1L subfamily comprises multiple members, making it difficult to distinguish primary versus derived CrRLK1L functions. Here, the authors take advantage of the fact that MpFER is the sole CrRLK1L encoded in the Marchantia genome to create amiRNA knock-down and CRISPR knock-out mutants of MpFER and thereby identify ancestral functions of the protein family.
The authors generated transgenic Marchantia expressing MpFERpro:3xVenus-NLS to show that MpFER is broadly expressed in most gametophytic and sporophytic tissues, including reproductive tissues. Marchantia mutants exhibited defective gametophyte development, producing significantly smaller thalli with reduced epidermal cell size. In the more severely affected CRISPR knock-out mutants, viability staining demonstrated that multiple epidermal cells had lost cellular integrity and overall, the epidermis of mpfer KO mutants exhibited reduced apparent stiffness as measured with cellular force microscopy. Overexpression of MpFER resulted in severe loss of epidermal integrity, with significant tearing of the epidermis around air pores. Reproductive development in male gametophytes of mutants was impaired, with amiR-lines producing fewer antheridiophores and individual antheridia generating less spermatogenous tissue. Finally, the authors showed that while AtFER and MpFER have significant sequence similarity, reciprocal functional complementation was unsuccessful, presumably due to difference in the surfaces where respective FER proteins interact with their RALF ligands and LRE/LLG co-receptors.
While there is no new mechanistic insight into (Mp)FER function, overall, the presented results are solid and support the conclusion that MpFER controls cell expansion and maintenance of cellular integrity, similar to AtFER and related family members in Arabidopsis.
We thank Reviewer 2 for the overall positive evaluation of our work and the constructive points raised in the comments below.

Comments for the author...
I do not have any major reservations, but some conclusions could be further strengthened:
1. The authors performed a phylogenetic analysis of the predicted malectin-like domain of Mp4g15890, the sole CrRLK1L of M. polymorpha, and CrRLK1Ls of Physcomitrium patens, Selaginella moellendorfii and Arabidopsis thaliana and also searched for homologues in the genomes of various Chlorophyte and expression data from several Charophytes. As no evidence for CrRLK1Ls was found in these algae, the authors “propose MpFER to be basal and orthologous to all other land plant CrRLK1Ls, and that the CrRLK1L family arose as plants conquered land”(lines 164ff). This is a rather strong statement: to my knowledge, only a single liverwort has been sequenced to date and given that Physcomitrium in the moss sister clade has 5 CrRLK1Ls, it seems quite possible that other CrRLK1Ls were lost during Marchantia evolution. The conclusion that MpFER is basal and orthologous would be strengthened by also evaluating CrRLK1Ls in the recently sequenced hornworts Anthoceros agrestis and A. punctatus (Li et al., 2020, Nat. Plants 6, 259–272) and searching the genome of Chara braunii (Nishiyama et al, 2018, Cell 174:448-464.e24). Furthermore, it would be helpful to know the precise MpFER region that was used for phylogenetic analysis (aa #-#).
We thank Reviewer 2 for this helpful comment. Accordingly, we repeated the phylogenetic analysis by adding more basal plants, including 3 genomes from 3 different Anthoceros species/accessions (revised Fig. 1B). Only one CrRLK1L member was found in each of the Anthoceros genomes,

similarly to what we found in M. polymorpha. It is thus unlikely that M. polymorpha lost some members during evolution strengthening our initial conclusion that a single CrRLK1L represents the ancestral state as stated on page 7 of the revised manuscript. We also added information on the MpFER region (aa 76-431) used for the phylogenetic analysis to the figure legend.
We also analysed the Chara brunii genome. The closest protein we could identified, however, does not cluster together with the CrRLK1Ls. Moreover, 3D structure prediction indicates that this protein does not have a malectin-like domain.
2. The authors stress that "CrRLK1Ls have a conserved role in reproduction in addition to their role in cell expansion and integrity during vegetative development" (lines 277f). I think a more careful analysis of the particular defects in reproductive development would strengthen this claim. While there appear to be fewer antheridiophores and less spermatogenous tissue, no attempt was made to identify the cause of these defects. The authors show that cell expansion is impaired in thalli of mpfer mutants. Is this true for spermatogenous cells as well? Or are there also defects in mitosis (both in thalli and in reproductive tissues)? If these defects arise solely/primarily from defects in cell expansion, should one really distinguish between cell expansion in vegetative and reproductive tissues? This seems qualitatively different from the role of FER function in Arabidopsis reproduction where it facilitates the communication between female and male gametophytes during pollen reception.
We agree with Reviewer 2 that this is an interesting point worth further investigation. However, it is very difficult to get Mpfer plants that produce reproductive structures, and we were unable to induce more plants to perform additional analyses. As stated in the manuscript, some aspects of the reproductive phenotype could also result from the reduced size of Mpfer plants. Thus, in order to be able to draw clear conclusion, it would thus be better to use a conditional system in which MpFER function could be eliminated or reduced only during reproductive development, which would clearly go beyond the scope of the current study. Since even in the healthiest plants we obtained, several aspects of reproduction are affected (number of antheridiophores, size of spash platform, size of spermatogenous tissue, fertility of spermatocytes), the conclusion that MpFER plays a role in reproductive development seems justified. However, to accommodate this comment, we state that the reduction in spermatogenous tissue could be either due to reduced cell expansion or cell proliferation on page 12 of the revised manuscript.

3. AtFER is known to be an important modulator of Arabidopsis immune signaling, apparently functioning as a scaffold to facilitate immune receptor complex formation. It may be beyond the scope of this work to investigate whether Marchantia mpfer mutants are more susceptible to bacterial pathogens (Gimenez-Ibanez et al, 2019, Curr Biol 29: 2270-2281.e4), but the authors should at least discuss that a role in immune signaling has not been ruled out as an 'ancestral' function.
Indeed, investigating the role of MpFER in immune signaling will be an interesting point to address in the future. We mention that it is an open question whether the role of CrRLK1Ls in immunity is also ancestral function in the discussion on page 18/19 of the revised manuscript.

Other points:
- Cellular force microscopy: is the apparent stiffness really "cell wall" (line 148) stiffness, of just cell stiffness (i.e. with an unknown contribution of turgor – which might be different in mpfer mutants)?
The apparent stiffness is indeed dependent on both turgor and cell wall stiffness. However, since the apparent stiffness is reduced in the Mpfer-2 mutants compared to the wild type, it is unlikely a higher turgor but rather a lower cell wall stiffness that leads to cell rupture. This is explained in more detail on page 11 of the revised manuscript.

-Figure 3: Figure 3B legend: "Base complementary between mature": Base complementarity between/and or of/with…)
We have corrected this in the revised text.

Figure 3C legend: what is Tak1?
Tak1 is the name of the male wild-type accession used in our experiments. We state this now clearly in the methods section and removed it from the figure legends.

Figure 3C-F: the description of panels in the text does not match the labels in the figure.
We apologize for this mistake; we corrected the text accordingly.

Figure 3D: Mpfer-1 transcripts should be sequenced to determine why there are mpfer phenotypes when transcript levels are normal (or the line should be complemented with MpFERpro:MpFER).
As suggested by Reviewer 2, we sequenced the MpFER transcript in the Mpfer-1 mutant, but no mutation was found. Thus, we performed a modified 3'RACE-PCR which ultimately showed that the transcript terminates about 1.3 kb in the T-DNA insertion and is not polyadenylated. As mentioned on page 9/10 of the revised manuscript, we suggest that the lack of a poly(A)-tail leads to inefficient translation and thus to reduced MpFER protein levels.

Figure 3F: how old were gemmalings? Based on comparison with Fig S3A -14d?
For the qRT-PCR, 14-day old gemmalings were used. This is now indicated in the figure legend.

Statistical analysis: which post hoc tests were used after ANOVA to determine which samples was statistically significantly different (Fig 3C,D)?
It was a Duncan test. In the revised manuscript, we added information on the statistical tests used for analysis in all the figure legends.

Figure 4: Figure 4C/D: the sizes of the thalli shown in the images do not match the sizes presented in Fig 4D (either wrong scale bar or different age).
We apologize for the mistake and corrected the figure accordingly.

Figure 4D: I suspect there may have been a conversion error (25 mm2 = 0.25 cm2, not 0.025 cm2?)
We thank Reviewer 2 for pointing this out. We have corrected the figure accordingly.

Figure 4G: 7-day old (not days old)
We corrected this in the revised manuscript.

Fiigure 4H: y-axis label: stiffness, not stiffmes
We corrected this in the revised manuscript.

Statistical analysis: Fig. 4H – which 'multiple comparison test' was used after Kruskal Wallis test? Fig 4D,F – was KW or ANOVA used, followed by which post hoc test?
In the revised manuscript, we added information on the statistical tests used for analysis in all the figure legends.

Figures 3/4: Lines 239f: "The thallus area of the newly generated, amorphic knock-out mutants was more strongly reduced compared to Mpfer-1 [59], confirming that, like the amiR-MpFER lines, Mpfer-1 is a hypomorphic mutant (Fig. 4C,D)". These data are difficult to compare as Fig 3 and Fig 4 present thallus sizes at different developmental stages (10d in Fig. 4 and 14d (based on Fig S3A) in Fig. 3) and the area units do not match (mm2 in Fig 3 and Fig S3, cm2 in Fig 4).
We apologize for the mistakes in the legend of Fig 4. In the revised manuscript, all plants shown are 14 days old.

Figure 5: Figure 5A:" Means with same letter do not differ significant different with $P < 0.05$".
While technically true, this is a bit confusing as there are no means with the same letters.
Indeed! We corrected the figure legend and have made sure to include information on the statistical tests and P-values applied in all the figure legends.

Figure 6: Figure 6A: isn't fer-2 in the Arabodopsis col background (not Ler)?
As already explained in the response to the comments of Reviewer 1, this was a mistake: we used the fer-1 allele which is in the Ler background.

Figure 7: Lines 365f:" higher 365 protein levels correlated with more severe phenotype (Fig. 7A,C)." This conclusion is rather vague (what are the phenotypes?) and does not really match the data presented in panels A and C. Lines #5, 6, and 9 appear to have the strongest phenotypes (reduced growth), but no protein levels are shown for these lines. Lines #7, 10, 11 have strong overexpression, but no phenotypes are shown. Lines #12 and 13 have very similar thallus sizes, but very different protein expression levels.
Yes, we agree that this was not very clear and modified Figure 7. In the revised manuscript, we show the effects of different levels of MpFER protein only in the Supplemental Information (Fig.

S6F,G). Line #12 has low MpFER levels and its phenotype resembles that of a wild-type plant, while line #13 has has higher expression and strong developmental defects.

Figure 7D: air pores and chambers should be indicated with arrows for readers less familiar with Marchantia morphology.
We used colours to mark one air chamber (blue area) and one air pore (red area) in Figure 7B.

While all images of rhizoids clearly show that there are fewer/shorter rhizoids in the mpfer mutants, none of the images support the statement that rhizoids collapse/burst.
We added a new figure (Fig S3D) showing a time course that illustrate the collapse of a rhizoid in the Mpfer-1 mutant.

Second decision letter

MS ID#: DEVELOP/2022/200580

MS TITLE: The single Marchantia polymorpha FERONIA homolog reveals an ancestral role in regulating cellular expansion and integrity

AUTHORS: Martin A Mecchia, Moritz Rovekamp, Alejandro Giraldo-Fonseca, Dario Meier, Philippe Gadient, Hannes Vogler, Daria Limacher, John A Bowman, and Ueli Grossniklaus
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

I find the manuscript is improved compared to the previous version.

Comments for the author

The authors have properly answered all my concernes.

Reviewer 2

Advance summary and potential significance to field

The authors have thoroughly addressed (almost) all of my previous comments (see below)

Comments for the author

I still think the values of thallus area shown in Fig. 4D do not match the images in C: if the scale bar is 5 mm (0.5 cm), then - unless I am mistaken - the WT thallus area should be well over 30 mm2 (and other genotypes correspondingly larger as well).

Author response to reviewers' comments

We thank Reviewer 2 for pointing this out. After reviewing the data, we realized that this was a mistake on our side. While 14-day old gemmalings are shown in Fig. 4C, the measurements for Fig. 4D were done on 7-day old gemmalings, explaining this discrepancy. We have corrected the mistake in the revised figure legend.