THE EMBO JOURNAL

Acidic cell elongation drives cell differentiation in the Arabidopsis root.

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Review timeline:

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Editors: Ieva Gailite/Deniz Senyilmaz Tiebe

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 23rd March 20 18

Thank you for submitting your manuscript to The EMBO Journal. We have now received two referee reports on your manuscript, which are included below for your information.

As you will see from the reports, both referees express interest in the work and the presented hypothesis. However, both referees point out that the currently presented evidence of the proposed cell size-induced root meristem differentiation is not sufficiently compelling, and further analysis of cell size changes and differentiation marker expression would be required. Additionally, referee #2 finds that further genetic evidence for the role of EXPA1 and AHA2 in the root meristem size regulation should be provided. I agree with the referees that addressing these issues is crucial for consideration here. Therefore, before taking a decision, I would like to give you the opportunity to reply to these comments in a preliminary point-by-point response and to let me know if you can extend the analysis along these lines in a revised version of the manuscript. Please keep in mind that although our standard revision period is three months, we can extend it to six months in case of more extensive revision.

Please feel free to contact me if you have any questions regarding this pre-decision consultation approach. I'm looking forward to your response.

REFEREE REPORTS:

Referee #1:

Pacifici et al have unraveled a developmental module in which cytokinins, via ARR1, induce AH1/AH2 to acidify cell walls and EXPA (direct interaction), to promote cell elongation (most likely through wall loosening). Conversely, they observe that EXPA activity and effects are positively correlated with differentiation. From this, the authors deduce that acidic cell elongation triggers cell differentiation.

Overall the work is carefully done, and the dissection of the cytokinin/AH1/EXPA module is of interest as it bridges the hormonal control of meristem/elongation shift to cell wall modifications.

This is also an interesting case of synergistic interaction, as the activity of EXPA depends on AH1/AH2 wall acidification. However, I am more circumspect on putting the feedback forward (elongation triggering differentiation, rather than differentiation triggering elongation). Not because it is untrue, but rather because everything in biology is about feedback and such loops are widespread (if not everywhere) and usually both signal and targets are modulating one another, with no clear precedence. A large part of this conclusion actually comes down to the exact definition of differentiation, which might be very much root-specific and/or too vague. Also, whereas the title insists on the feedback, most of the data actually help understand the way forward (CK to EXPA, not EXPA to differentiation). How EXPA controls differentiation is and remains unknown. With the exact same data, I would thus consider putting the focus on the identified module (CK -> AH1/EXPA, which in itself is a strong piece of work) and only mention the feedback (which is not properly addressed to me, as it stands - see below).

Specific points in the text:

1/ Main question and conclusion

Line 31: « Whether these changes in size are necessary to initiate cell differentiation or they are only a consequence of the incipient differentiation program is still unclear. » This binary, exclusive, view of development seems oversimplistic : The consensus, in a systems biology approach, is rather that it is a feedback loop (i.e. they are triggering each other)

Line 48: « we set to understand whether this sudden change in size is a prerequisite for the initiation of cell differentiation, or rather an early consequence of it. » The definition of cell differentiation is too vague to ask this question as is: (line 28) « Cell differentiation is a complex process through which cells acquire distinct identities and specialized functions. » For instance, is wall loosening part of cell differentiation? If so, differentiation also precedes cell elongation. I believe the authors are not addressing that question, but instead are investigating whether changing cell size can in turn affect the differentiation 'program'. However, in that scenario, the authors have not analyzed later markers of differentiation to support their claim.

Line 179: « Our results provide clear evidence that a change in cell shape (volume), is not a consequence of, but rather a prerequisite for the initiation of the cell differentiation program. » I disagree with this conclusion (see above : definition of differentiation, feedback loop imply that both trigger the other). The conclusion and title are overspeculative. In fact the authors acknowledge this when they say « It is thus tempting to propose a model (...) a sudden change in volume (elongation) (...) is the functional trigger of differentiation ». The authors should instead say that they integrated wall remodeling (acidification and expansin-dependent loosening) as a target and also as an early signal in cell differentiation. In fact, if the conclusion were true (i.e. cell elongation trigger differentiation), the meristem should fully differentiate in EXP OE lines, but this is not observed.

2/ Wall loosening

Although I agree with the authors that EXPA would mediate its effect via wall loosening (the most parsimonious scenario), there is no measurement of wall elasticity here. In other words, the present data confirm a correlation between low pH and EXPA-dependent promotion of cell elongation, but not wall loosening. The authors should thus be more cautious. Here is one example (this issue appears on several occasion in the text):

Line 114: "These data confirm that low apoplastic pH activates the EXPA proteins, and that by loosening the cell wall these proteins change the shape of the cell. »

3/ P-values are quite high (only 1 * ($P<0.05$)). Is this due to small sample size?

4/ Measurements:

The authors mention several times that cells are either bigger or smaller, but what is measured is cell length. Formally, the authors should measure cell volume or at least provide evidence that cells are not becoming flatter or narrower, to justify using cell length as a proxy for cell size. Also, the

absence of cell division should be confirmed to formally justify not analyzing growth (otherwise, smaller cells would emerge from cell division, not reduced cell elongation).

5/ A question: could the author expand on the question of scaling. Check e.g. Gruel 2016 Sci Adv: scaling response seems to be true also in the expa mutant (see e.g. Fig 5E)

Figures:

Fig 1I: a close-up of the TZ zone with enhanced signal intensity should be provided (the root cap is too bright to see the signal there)

Fig 2A: the position of the white arrowhead is difficult to verify from the images (and how it is positioned is not clearly stated in the method section; I guess the Perilli et al 2010 paper would help, but the authors should summarize the method here too)

Fig 2B (and elsewhere) : The number do not correspond to "meristem cell number" (but meristem cell number on a given section)

Fig2: the take home message is rather that EXPA activity is revealed in planta (which is not always easy to show, but has already been done in the past, see e.g. Cho 2000 PNAS, not cited or discussed)

Fig 3D-G: Green pixel number may vary from one sample to another, or from one section to another. The authors could measure the quantity of AH1/2-GFP protein in a native PAGE from root extracts to detect a difference.

Fig 4b: unclear which comparison is made for the statistical tests (i.e. the two expa mutants +/- DEX should also be compared)

Minor point:

1/ Abstract: 1st sentence can be removed.

2/ Line 137: « However, analysis of the aha1-8 and aha2-4 loss-of function mutants revealed no root phenotype (Extended Data Fig. 3b). » You mean no ALTERED phenotype

Referee #2:

The mechanism(s) controlling how cells switch from division to differentiation is of wide interest to biologists. The co-authors have previously performed elegant studies demonstrating the important role that the hormone cytokinin and its signalling components such as ARR1 play controlling the switch from cell division to differentiation in the transition zone (TZ) of the Arabidopsis root. The manuscript reports that the cytokinin-dependent cell differentiation switch is controlled by a novel mechanism involving the pH dependent regulation of a member of the cell wall EXPANSIN enzyme class. If properly experimentally validated, this new mechanism promises to provide a breakthrough in the field's understanding of plant cell differentiation and Expansin function.

The authors initially report that expression of several EXPANSIN genes (EXPA1, 10, 14 & 15) are controlled by cytokinin. Unlike the other EXPA genes, EXPA1 appears to be a direct target for regulation by ARR1 (based on multiple lines of in/direct evidence). One mutant line (lacking EXPA1) exhibits a larger root meristem and longer overall length. While this is a promising observation, the manuscript surprisingly only shows data for this one expa1 allele. No independent genetic evidence is provided linking this root trait with the EXPA1 gene, such as independent expa1 mutant alleles and/or EXPA1 complementation data. This additional genetic evidence is ESSENTIAL, otherwise the current data can only be considered preliminary. Next, the authors report that a transcriptional EXPA1 reporter is expressed in several root tissues, including TZ epidermal cells. Again, no effort was made to test the functional relevance of their observation (such

as tissue-specific EXPA1 expression in expa1 to assess the ability to rescue of the mutant's root phenotype).

Next, the authors report that over expressing any of the 4 EXPA genes, then exposing transgenic roots to low pH media, triggers root growth arrest. They conclude that this is causing a "change in the shape of the cell thus inducing cell differentiation of meristematic cell." However, no further data is provided to back this statement up, such as observing dynamic changes in markers for root meristem and differentiation zone identities. Instead, the authors study the role of 2 genes (AHA1 an AHA2) encoding components of the PM proton ATPase which, like EXPA1, are positively regulated by ARR1. Like EXPA1, ubiquitous (inducible) over expression of AHA2 resulted in reduced root meristem size and a switch to cell expansion at low pH media. Given the role of AHA in apoplastic pH regulation, did the authors test whether growing transgenic UBI10::AHA2:GR roots at more neutral/alkaline pH could rescue this root growth phenotype? Intriguingly, the authors report that the UBI10::AHA2:GR root growth effect is EXPA1 dependent. This leads the authors to propose that cytokinin controls root cell differentiation via regulation of AHA and EXPA1 downstream targets. Whilst a plausible and fascinating mechanism, much more additional work is required to validate their model, as highlighted above.

1st Revision - authors' response 24th May 2018

Referee #1:

- Pacifici et al have unraveled a developmental module in which cytokinins,via ARR1, induce AH1/AH2 to acidify cell walls and EXPA (direct interaction), to promote cell elongation (most likely through wall loosening). Conversely, they observe that EXPA activity and effects are positively correlated with differentiation. From this, the authors deduce that acidic cell elongation triggers cell differentiation.
Overall the work is carefully done, and the dissection of the cytokinin/AH1/EXPA module is of interest as it bridges the hormonal control of meristem/elongation shift to cell wall modifications. This is also and interesting case of synergistic interaction, as the activity of EXPA depends on AH1/AH2 wall acidification. However, I am more circumspect on putting the feedback forward (elongation triggering differentiation, rather than differentiation triggering elongation). Not because it is untrue, but rather because everything in biology is about feedback and such loops are widespread (if not everywhere) and usually both signal and targets are modulating one another, with no clear precedence. A large part of this conclusion actually comes down to the exact definition of differentiation, which might be very much root-specific and/or too vague. Also, whereas the title insists on the feedback, most of the data actually help understand the way forward (CK to EXPA, not EXPA to differentiation). How EXPA controls differentiation is and remains unknown. With the exact same data, I would thus consider putting the focus on the identified module (CK ->AH1/EXPA, which in itself is a strong piece of work) and only mention the feedback (which is not properly addressed to me, as it stands - see below).
-
We thank the referee for his/her appreciation of our work and for the thoughtful comments. We agree with the view that *everything in biology is about feedback and such loops are widespread (if not everywhere) and usually both signal and targets are modulating one another, with no clear precedence*.
We consequently rephrased the text to soften our conclusions. Throughout the text we now propose that the *CK ->AH1/EXPA* module "*drives cell differentiation"* rather than "*initiates cell differentiation"*

- Line 31: « Whether these changes in size are necessary to initiate cell differentiation or they are only a consequence of the incipient differentiation program is still unclear. » This binary, exclusive, view of development seems oversimplistic: The consensus, in a systems biology approach, is rather that it is a feedback loop (i.e. they are triggering each other)

We agree with the referee and we rephrased sentences along these lines: "*The significance of these changes in size and whether they can affect cell differentiation is still unclear*".

- *Line 48* now line 50 *: « we set to understand whether this sudden change in size is a prerequisite for the initiation of cell differentiation, or rather an early consequence of it. » The definition of cell differentiation is too vague to ask this question as is : (line 28) « Cell differentiation is a complex process through which cells acquire distinct identities and specialized functions. » For instance, is wall loosening part of cell differentiation? If so, differentiation also precedes cell elongation. I believe the authors are not addressing that question, but instead are investigating whether changing cell size can in turn affect the differentiation 'program'.*
In agreement with this and the previous comments, we rephrased the text, which now reads: "*Here, we set to understand the significance of these changes in size and whether they can affect the cell differentiation program".*

- *However, in that scenario, the authors have not analysed later markers of differentiation to support their claim.*
- We disagree with this point. We analysed the development of root hairs and tracheids that mark, respectively, the completion of epidermis and vascular cell differentiation. These developmental markers are widely used in the field of root development (See Mahonen et al., Nature 2014, Wang et al., PNAS, 2018 as an example).
We now extended this analysis also to *UBQ10-EXPA1* (Fig EV. 2G,H)

- *Line 179* now line 195 *: « Our results provide clear evidence that a change in cell shape (volume), is not a consequence of, but rather a prerequisite for the initiation of the cell differentiation program. » I disagree with this conclusion (see above : definition of differentiation, feedback loop imply that both trigger the other). The conclusion and title are over speculative. In fact the authors acknowledge this when they say « It is thus tempting to propose a model (...) a sudden change in volume (elongation) (...) is the functional trigger of differentiation ». The authors should instead say that they integrated wall remodeling (acidification and expansin-dependent loosening) as a target and also as an early signal in cell differentiation. In fact, if the conclusion were true (i.e. cell elongation trigger differentiation), the meristem should fully differentiate in EXP OE lines, but this is not observed.*
- We agree with the first part of this comment, and we rephrased the text as follows: "*Our results provide clear evidence that cell wall remodelling due to acidification and expansin-dependent loosening brings about a change in cell shape (dimentions) that has an important role in controlling the cell differentiation program."*
However, as of the EXP OE lines, we have now provided additional clear evidence, based on QC- and stem cell-specific markers, that in these lines the stem cell niche is fully differentiated (Fig2 B).
We therefore modified only slightly the title of the manuscript, which now reads: "*Acidic cell elongation drives cell differentiation in the Arabidopsis root*" (before it was: "*Acidic cell elongation induces cell differentiation in the Arabidopsis root*")

- *Although I agree with the authors that EXPA would mediate its effect via wall loosening (the most parsimonious scenario), there is no measurement of wall elasticity here. In other words, the present data confirm a correlation between low pH and EXPA-dependent promotion of cell elongation, but not wall loosening. The authors should thus be more cautious. Here is one example (this issue appears on several occasion in the text): Line 114: "These data confirm that low apoplastic pH activates the EXPA proteins, and that by loosening the cell wall these proteins change the shape of the cell.*
We agree with this comment and we rephrased the relevant sentences in the text along this line: "*These data confirm that low apoplastic pH activates the EXPA proteins, and that probably by loosening the cell wall these proteins change the shape of the cell ...*" line 128

- *P-values are quite high (only 1 * ($P<0.05$)). Is this due to small sample size?*

We agree with the reviewer and we now added the exact P value for each experiment. In the previous version we indicated P<0.05 as it is the maximum P value obtained for statistical significance. Now we specify the exact P value as follow: **P < 0.01 and *** P < 0.001. The number of samples analyzed and experimental replicates is reported in the figure legends and in the methods section.

- *The authors mention several times that cells are either bigger or smaller, but what is measured is cell length. Formally, the authors should measure cell volume or at least provide evidence that cells are not becoming flatter or narrower, to justify using cell length as a proxy for cell size.*
The root cells belonging to the same tissue are piled in a cell file starting from the stem cell that generates that particular tissue. At the TZ, where cell differentiation starts, cells undergo a shootward unidirectional elongation process that changes the volume of the cells. Cells do not become flatter or narrower (as suggested by the reviewer) because otherwise this would result in dramatic changes in the shape of the entire root, which we do not observe. This is why we consider cell length as an indication of cell size. Nevertheless, for the sake of completeness we now measured also the area of the cells (See Fig 1I, Fig2E, Fig 4 D).

- *Also, the absence of cell division should be confirmed to formally justify not analyzing growth (otherwise, smaller cells would emerge from cell division, not reduced cell elongation).*
- Indeed, the expression of the differentiation marker RCH2::GFP and the lack of expression of the division marker CYCB1;1:GUS indicate that these cells are not dividing. We now specifyed this point more clearly in the text (line 73): "*This analysis revealed that distal meristematic cell that in wild-type roots express the RCH2 marker line now express the CYCB1;1:GUS cell cycle reporter causing a shootward shift of the TZ in expa1 as is the case of cytokinin signaling mutants"*

- *A question: could the author expand on the question of scaling. Check e.g. Gruel 2016 Sci Adv: scaling response seems to be true also in the expa mutant (see e.g. Fig 5E)*
- We thank the referee for this interesting suggestion but we believe this is out of the focus of this work.
Nevertheless, we now mention this paper in the manuscript as, in line with our observations, it points to the epidermis as an important tissue in controlling organ size. (line 92):

- *Fig 1I* now Fig 1 J*: a close-up of the TZ zone with enhanced signal intensity should be provided (the root cap is too bright to see the signal there)*
- We agree, and we included the requested close-up.

- *Fig 2A: the position of the white arrowhead is difficult to verify from the images (and how it is positioned is not clearly stated in the method section; I guess the Perilli et al 2010 paper would help, but the authors should summarize the method here too)*
- We thank the referee for the suggestion. We now added this information in the methods section (line 225).

- *Fig 2B (and elsewhere) : The number do not correspond to "meristem cell number" (but meristem cell number on a given section)*
- We thank the referee for noting this, we express root meristem size as the number of cortex cells extending from the quiescent center to the first elongated cortex cell (excluded). We now explicitly describe this methodology in the methods section (line 225).

- *Fig2: the take home message is rather that EXPA activity is revealed in planta (which is not always easy to show, but has already been done in the past, see e.g. Cho 2000 PNAS, not cited or discussed)*
- We thank the referee for this comment. We changed the text accordingly:"Activated EXPA1 protein induces cell differentiation revealing their function in vivo"), and refer to the Cho paper in the text (line 55).

- *Fig 3D-G: Green pixel number may vary from one sample to another, or from one section to another. The authors could measure the quantity of AH1/2-GFP protein in a native PAGE from root extracts to detect a difference.*
- We disagree with this comment: the pictures shown are only representative of the many that we analysed to draw our conclusions. Furthermore, q-PCR analysis clearly shows a difference in *AHA1* and *AHA2* mRNA levels upon cytokinin treatment and in the *arr1* mutant background.

- *Fig 4b: unclear which comparison is made for the statistical tests (i.e. the two expa mutants +/- DEX should also be compared)*
- We thank the referee for noticing it. We now clarified in the figure legend the way the comparison was made (i.e. everything versus the *UBQ10::AHA2:GR* untreated plants). We also realized that there was a mistake in the way the columns were labeled (i.e. pUBQ10::AHA2:GR,expa1 was inverted with pUBQ10::AHA2:GR Dex 10 mm).

Referee #2:

- *The mechanism(s) controlling how cells switch from division to differentiation is of wide interest to biologists. The co-authors have previously performed elegant studies demonstrating the important role that the hormone cytokinin and its signalling components such as ARR1 play controlling the switch from cell division to differentiation in the transition zone (TZ) of the Arabidopsis root. The manuscript reports that the cytokinin-dependent cell differentiation switch is controlled by a novel mechanism involving the pH dependent regulation of a member of the cell wall EXPANSIN enzyme class. If properly experimentally validated, this new mechanism promises to provide a breakthrough in the field's understanding of plant cell differentiation and Expansin function.*
We thank the referee for his/her appreciation and enthusiasm.

- *The authors initially report that expression of several EXPANSIN genes (EXPA1, 10, 14 & 15) are controlled by cytokinin. Unlike the other EXPA genes, EXPA1 appears to be a direct target for regulation by ARR1 (based on multiple lines of in/direct evidence). One mutant line (lacking EXPA1) exhibits a larger root meristem and longer overall length. While this is a promising observation, the manuscript surprisingly only shows data for this one expa1 allele. No independent genetic evidence is provided linking this root trait with the EXPA1 gene, such as independent expa1 mutant alleles and/or EXPA1 complementation data. This additional genetic evidence is ESSENTIAL, otherwise the current data can only be considered preliminary.*
We agree with the referee that it is important to provide *independent expa1 mutant alleles and/or EXPA1 complementation data.*
We now provided expa1 complementation data (line 85, Fig EV 1G). Providing an additional allele via the CRISPR/Cas9 system would have been too time-consuming. We would like to point out that different mutant combinations of EXPAs show exactly the same phenotype of the single *expa1* mutant. Thus, we believe that this observation together with the complementation analysis strongly supports the notion that EXPAs are involved in controlling cell differentiation in the root.

- *Next, the authors report that a transcriptional EXPA1 reporter is expressed in several root tissues, including TZ epidermal cells. Again, no effort was made to test the functional relevance of their observation (such as tissue-specific EXPA1 expression in expa1 to assess the ability to rescue of the mutant's root phenotype).*
The *EXPA1* expression pattern is already quite specific at the epidermal TZ and in the columella. Although we agree with the referee that EXPA1 tissues specific complementation analysis would be interesting, these experiments are very time-consuming and we believe that they would not add crucial information to support our hypothesis.

- Next, the authors report that over expressing any of the 4 EXPA genes, then exposing transgenic roots to low pH media, triggers root growth arrest. They conclude that this is causing a "change in the shape of the cell thus inducing cell differentiation of meristematic cell." However, no further data is provided to back this statement up, such as observing dynamic changes in markers for root meristem and differentiation zone identities.
We thank the referee for this suggestion. We have now provided additional clear evidence, based on QC- and stem cell-specific markers, that in these lines the stem cell niche is differentiated (line 123, Fig 2B).

- Instead, the authors study the role of 2 genes (AHA1 an AHA2) encoding components of the PM proton ATPase, which, like EXPA1, are positively regulated by ARR1. Like EXPA1, ubiquitous (inducible) over expression of AHA2 resulted in reduced root meristem size and a switch to cell expansion at low pH media. Given the role of AHA in apoplastic pH regulation, did the authors test whether growing transgenic UBI10::AHA2:GR roots at more neutral/alkaline pH could rescue this root growth phenotype?
Possibly, the referee misunderstood our observation. We show that overexpression of AHA2 resulted in reduced root meristem size in standard media not in *low-pH media as in the case of EXPA1*. In fact, it is the change in pH induced by the deregulation of AHA2 that causes the observed changes in meristem size.
In any case, since EXPANSINs are active at acidic pH and their activity at alkaline pH is not predictable, we believe that using neutral/alkaline media would not be so informative for the focus of the study.

2nd Editorial Decision 6th June 2018

Thank you for submitting a revised version of your manuscript. It has now been seen by one of the original referees whose comments are shown below. As you will see, the referee #1 finds that all criticisms have been sufficiently addressed and recommends the manuscript for publication. However, before we can officially accept the manuscript there are a few editorial issues concerning text and figures that I need you to address.

• Please address the remaining concerns of the referee #1.

Thank you again for giving us the chance to consider your manuscript for The EMBO Journal, I look forward to your revision.

2nd Revision - authors' response 12th June 2018

Thank you very much for considering our manuscript entitled “Acidic cell elongation drives cell differentiation in the Arabidopsis root” for publication in the EMBO Journal. We address the remaining concerns of the referee #1 as follow:

- The authors have addressed most of my concerns, I only have one remaining comment:« Here, we show that changes in cell size drive cell differentiation, » The authors cannot conclusively say that a geometrical cue drive differentiation: the authors would need to change cell volume without changing the biochemistry or mechanics of the cell and see whether this is sufficient to induce differentiation. I realize that is close to impossible to do, so I just want to add a word of caution here. The authors show that cells need to reach a given size before differentiation (in the root) but this is quite different from showing that cell size drives differentiation. And this is true, only when considering a root-specific definition of differentiation: some may say that CLV3-expressing cells in the shoot apical meristem are more differentiated than undifferentiated: they don't divide too much, they have a specific genetic program and even stiffer cell walls than their neighbours. The same goes for stomata, where differentiation actually involves a compartmentalization of a cell, rather than an increase in size. I acknowledge that the new data showing full differentiation of the

QC when EXP is overexpressed is consistent with their claim, but scenarios, e.g. that EXP induces/releases biochemical signals in the cell wall that induce differentiation (not only volume increase). The authors should go through the manuscript and check for this (over) interpretation (at least to me). The new title is actually OK, as the "acidic elongation" wording does not imply that geometry alone induces differentiation. Apart from that, I it does not exclude other think this is a strong piece of work and definitely worth publication in EMBO J.

We changed the sentence as follow: "Here, we show that changes in cell size are essential for the initial steps of cell differentiation, and that..." (line 21 Pag 1).

- A small point in the abstract: «These findings provide an elegant example on the importance of mechanical events during organogenesis. » That conclusion is too general and not informative. I would rather insist on the identification of a growth module that builds on a synergy between cytokinin-dependent pH modification and wall remodeling to drive differentiation through the mechanical control of cell walls.

We changed the conclusions as follow: ". These findings identify a growth module that builds on a synergy between cytokinin-dependent pH modification and wall remodelling to drive differentiation

Accepted 13th June 2018

Thank you for submitting the revised version of your manuscript. I have now looked at everything and all looks fine. Therefore I am very pleased to accept your manuscript for publication at The EMBO Journal.

Congratulations on the very nice work!

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Sabrina Sabatini
Journal Submitted to: EMBO J
Manuscript Number: EMBOJ-2018-99134R

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	NA
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NO
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	YES
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	YES
Is there an estimate of variation within each group of data?	YES
Is the variance similar between the groups that are being statistically compared?	YES

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

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http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	NA
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA