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Tightly controlled WRKY23 expression mediates Arabidopsis embryo development

Wim Grunewald, Ive De Smet, Bert De Rybel, Helene S. Robert, Brigitte van de Cotte,Viola Willemsen, Godelieve Gheysen, Dolf Weijers, Jiri Friml and Tom Beeckman

Corresponding author: Wim Grunewald and Ive De Smet, Ghent University

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(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.)

Editor: Barbara Pauly

1st Editorial Decision 18 April 2013

Thank you very much for the submission of your research manuscript to our editorial office. We have now received the full set of reviews on your manuscript.

You will see that while referee 2 is more negative, reviewers 1 and 3 appreciate the potential interest of your study. However, all referees also raise partially overlapping concerns about the conclusiveness of the current data set. For example, both referees 1 and 3 feel that the data on the altered auxin distribution need to be strengthened and referee 3 also points out that the evidence for the auxin-independency of the SHR-SCR pathway on WRKY23 expression would need to be improved. Importantly, reviewers 1 and 2 state that stronger proof for the hierarchy of the WRKY23/Monopteros (referee 1 and 2) and the WRKY23/SHR-SCR pathway (referee 2) would need to be provided. In addition, referee 2 also feels that it would benefit the manuscript if at least some of the findings could be confirmed in WRKY23 mutant cells. Referee 3 also recommends more directly comparing the effect of the SHR-SCR pathway on WRKY23 expression in embryos and seedlings. Finally, reviewer 1 suggests to more carefully present the data and discuss their implications.

While it is clear that a significant amount of work is needed before we can consider publication of

your study in EMBO reports, I would like to give you the opportunity to revise your manuscript, with the understanding that the main concerns of the reviewers should be addressed, especially with regard to the claim that WRKY23 acts downstream of Monopteros and the SHR-SCR pathway. Acceptance of the manuscript will depend on a positive outcome of a second round of review and I should also remind you that it is EMBO reports policy to allow a single round of revision only and that therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. If you feel that this period is insufficient for a successful submission of your revised manuscript I can potentially extend this period slightly. Also, the length of the revised manuscript should not exceed roughly 29,000 characters (including spaces and references). If you feel that the additional data requested by the reviewers would make the manuscript too long you may consider including some peripheral data in the form of Supplementary information. However, materials and methods essential for the repetition of the key experiments should be described in the main body of the text and may not be displayed as supplemental information only.

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We also welcome the submission of cover suggestions or motifs that might be used by our Graphics Illustrator in designing a cover.

I look forward to seeing a revised form of your manuscript when it is ready. Should you in the meantime have any questions, please do not hesitate to contact me.

REFEREE REPORTS:

Referee #1:

In their study, Grunewald et al. investigate the embryonic function of the WRKY23 transcription factor. The authors convincingly show that knock-downs of the gene lead to impaired specification of the embryonic root pole and disproportional expression of the auxin response marker DR5. If there is an inappropriate distribution of auxin in the embryos, it is probably not because of quercetin-mediated auxin-transport defects, because there are no such defects in quercetin deficient mutants. WRKY23 transcripts are strongly reduced in shr and scr mutants and partial suppression of mp by WRKY23 transactivation suggests that it functions downstream of MP.
The displayed data are novel and of interest to a large readership. This paper would be most valuable, if the listed corrections and amendments could be considered. Even then, however, the data will leave room for interpretation. This does not diminish their importance, but they should definitely be presented in a factual format, not by spoonfeeding the reader with preferred conclusions. The subheadings and figure legend headings should therefore be neutral, not carrying interpretations. For example, the exclusion of flavonols from list of possible mechanism is based on circumstantial evidence, so is implicating auxin distribution in it. There are more examples where more cautious language would have benefited the presentation without taking anything away from the significance of the data.

Specific points:
- In the abstract, using the term "complement" for a partial suppression in a typical epistasis

argument is misleading.
- n values for embryonic observations inserted in text have to be provided
- the penetrance of the embryo defects are central for the interpretation of the results. They have to be visible parts of the manuscript itself, e.g. as a column diagram in Fig.1. more details may then be provided as supplemental tables.
- DR5 expansion has never been considered a sufficient reason to conclude altered auxin distribution, unless additional experiments support distribution over altered auxin responsiveness. Also the term "is correlated" needs to be clarified. If it meant to indicate more than the mere fact shown in the figure, then one would have to show that such anticipated auxin distributions, by themselves, lead to the indicated defects. After all, this is just the inversion of the argument used in the following paragraph to demontrate that quercitin is not involved.
- On top of this, the wording "follows the installation of the root stem cell niche" is misunderstandable, so as to treat it as a mere marker.
- SHR/SCR pathway should probably be SHR-SCR pathway
- The partial suppression of mp by WRKY23 is a central claim and needs to be documented more clearly. The text should not amalgate numbers from two different genotypes/experiments. Importantly, the figure has to define transparent criteria by which suppressed mutant individuals can be unequivocally distinguished from normal looking (3/4) embryos on one side and from unsuppressed mutants on the other.
- Certainly, it would be interesting to know, whether the suppression also restores fertility. In this case, the true breeding line could simply be analyzed in greatest detail.
- The figure legends need to be redone. Right now they combine a concluding heading with a narrative presentation from the authors' perspective. The reader would instead simply like to know what is in those figures. Is this a whole-mount in situ hybridization? From where is the quantified RNA? On how many biological replica is it based? These types of information would be helpful to have in the legends.

Referee #2:

The Authors report data suggesting that WRKY23 may function downstream of MP, BDL, SHR, and SCR in embryonic root formation. By the Authors' own admission, some of these data are an expansion of previously published data; the novel aspects in this study are thus limited. While the data may be relevant for the specific field, the study seems to lack broad biological significance and thus appears of limited general interest. Even for a more specialized venue, however, the study seems preliminary--and thus represents insufficient advance--and the evidence presented cannot be so unambiguously interpreted, as instead the Authors claim. For example, genetic and molecular evidence is lacking that supports the conclusion that WRKY23 functions downstream of SHR/SCR in the same pathway. Furthermore, normalization of mp defects by WRKY23 overexpression could result from function of WRKY23 in a pathway parallel to that in which MP functions (e.g., bypass suppression of unc-54 by hypermorphic mutations in myo-3), so strong evidence--genetic or molecular--is also lacking in support of the conclusion that WRKY23 functions downstream of MP in the same pathway. It's also unclear why the Authors have not used wrky23 mutants for their study; a quick search reveals the existence of a line (SALK_033414) with a T-DNA insertion in exon 2. I appreciate the effort toward quantification of defects (Table S1 and S2--though quantification should be equally applied to all other experiments/images), but "polarity defects", "hypophysis defects", and "patterning defects" are too vague descriptors to be meaningful, and those tables could use images to illustrate the phenotype classes (as the Authors have done in Fig. 4I-K, for example). Moreover, a minimum of two transgenic lines should be analyzed and their effects reported separately. On the positive side, the manuscript is quite easy to read, though there are here and there some easy-to-fix mistakes (e.g., "plants were grinded").

Referee #3:

This is an interesting manuscript that describes a role for the transcription factor WRKY23 in defining the apical-basal boundary in the Arabidopsis embryo and establishing the embryonic root pole. WRKY23 is proposed to function as an intermediate molecule incorporating MP/BDL-

mediated auxin signaling and the SHR/SCR mediated developmental pathway during establishment of the root pole in Arabidopsis embryogenesis.

Major points:
* Do we know that SCR & SHR are really auxin-independent or do we just not have any evidence that they're auxin dependent?

* In a similar vein, the GUS images in Fig 3F-G show that there is no change in the auxin signalling domain in shr-2, but do not address the strength of auxin signalling in this domain. Since WRKY23 is auxin regulated, a reduction in auxin signalling might reduce its expression. To conclusively show that the change in WRKY23 is not due to changes in auxin signalling, the authors should quantify auxin signalling levels (eg, by showing no change in the expression level of other known auxin responsive genes).

* Since WRKY23 plays a role in proper specification of the root pole and the knock-down mutant shows QC defect, the changes observed in DR5 expression (in Fig 2) may be due to changes in cell identity in these mutants. It would therefore be informative to show that tissue specific markers, such as SHR, SCR, and pCO2, are unchanged in the WRKY23 RNAi background.

* In Fig 3, the qPCR data is from seedlings while the in situ expression data is from embryos. I understand that it would be challenging to perform a qPCR on embryos, but it would be helpful if the authors showed that the expression in seedlings corresponds to that in embryos, thus making these two sets of data comparable.

* There appears to be a higher level of WRKY23 expression in both Fig 3C & E. If this difference is, in fact, not statistically significant, this should be made clear in the text. If Fig 3C is not representative, perhaps another image could be used. As it stands, the figure and the text seem to be at odds with one another.

* Based on their previous paper, both Knock-down and Overexpressor of WRKY23 show meristem defect in seedling stage, and they emphasized the importance of it dosage controls of the root stem cell niche. Is this also applicable to embryo stage? Is it possible that SHR modulates its expression in a constant level instead of just repressing its
expression? At least in discussion part, i think they should give answers to my questions.

Minor points:
* The phrase "WRKY23 expression follows [...] the root stem cell niche" may be confusing, since 'follows' can also suggest temporal succession. I suggest the authors use "overlaps with" instead, unless there is a reason not to do so.

* Figure 3 and 4 are referenced in a confusing order in the text. I would find it more clear if Fig 4A,B,D,E were moved into Fig 3, though I understand that the authors & editor may disagree here.

* The authors should indicate what age the seedlings were in the qRT-PCR experiments.

1st Revision - authors' response 31 August 2013

We thank the reviewers and editor for their constructive comments. We have addressed the majority of their concerns with new experiments.

Below is a point-to-point reply to the referees' comments.

Referee #1:

- *should definitely be presented in a factual format, not by spoonfeeding the reader with preferred conclusions. The subheadings and figure legend headings should therefore be*

neutral, not carrying interpretations. For example, the exclusion of flavonols from list of possible mechanism is based on circumstantial evidence, so is implicating auxin distribution in it. There are more examples where more cautious language would have benefited the presentation without taking anything away from the significance of the data.

REPLY: The mixture of results and discussion requires an interpretation of our results throughout the text that might have provoked the impression of spoon-feeding the reader. We therefore have rewritten the text, using more cautious language.

- *In the abstract, using the term "complement" for a partial suppression in a typical epistasis argument is misleading.*

REPLY: text has been amended as follows "can partially rescue the root-forming inability of *mp* embryos"

- *n values for embryonic observations inserted in text have to be provided*

REPLY: In the original manuscript, n values were added in the form of Table S1. To maintain the readability of the manuscript, we have added only key n values for several embryo observations in the text.

- *the penetrance of the embryo defects are central for the interpretation of the results. They have to be visible parts of the manuscript itself, e.g. as a column diagram in Fig.1. more details may then be provided as supplemental tables.*

REPLY: a column diagram was added to Figure 1.

- *DR5 expansion has never been considered a sufficient reason to conclude altered auxin distribution, unless additional experiments support distribution over altered auxin responsiveness. Also the term "is correlated" needs to be clarified. If it meant to indicate more than the mere fact shown in the figure, then one would have to show that such anticipated auxin distributions, by themselves, lead to the indicated defects. After all, this is just the inversion of the argument used in the following paragraph to demontrate that quercitin is not involved.*

REPLY: We agree with the reviewer's comment and we have rephrased this ("the auxin response maximum"). We also added data on PIN1 localisation in WRKY23 embryos to support that auxin distribution is affected as well. Text added: "These results suggest that defects seen in loss of WRKY23 function embryos might be due to defects in auxin distribution. This is further supported by analyses of PIN FORMED1 (PIN1) protein localisation in 35S::WRKY23-SRDX embryos (33%, n = 18), which deviated from the wild type (n = 7) (Supplementary Figure S2 online). For example, ectopic PIN1 under the QC could explain the *DR5* expression pattern, namely more diffuse and into the suspensor. Therefore, auxin flow and distribution are regulated by and/or downstream of WRKY23."

- *On top of this, the wording "follows the installation of the root stem cell niche" is misunderstandable, so as to treat it as a mere marker.*

REPLY: Rephrased as "*WRKY23* expression overlaps with the root stem cell niche", as suggested by referee 2.

- *SHR/SCR pathway should probably be SHR-SCR pathway*

REPLY: The text has been modified.

- *The partial suppression of mp by WRKY23 is a central claim and needs to be documented more clearly. The text should not amalgate numbers from two different genotypes/experiments. Importantly, the figure has to define transparent criteria by which suppressed mutant individuals can be unequivocally distinguished from normal looking (3/4) embryos on one side and from unsuppressed mutants on the other.*

REPLY: We have corrected this and distinguished between the two different genotypes. In addition, we clarified the criteria that distinguish class I, II and III: "Following a Mendelian segregation, mp^{B4149} heterozygous plants produced 76.2% (*mp*/+ *UAS::WRKY23* x *mp*/+; n= 437) or 73.5% (*mp*/+ *RPS5A::GAL4* x *mp*/+; n =336) wild-type looking embryos (class I) and 23.3% (*mp*/+ *UAS::WRKY23* x *mp*/+) or 26.5% (*mp*/+ *RPS5A::GAL4* x *mp*/+) mutant embryos (class II, lacking a proper root and hypocotyl and often displaying 1 cotyledon). Interestingly, although the ¼ segregation was maintained in RPS5A>>WRKY23 x mp^{B4149} embryos, we could morphologically divide the mutant embryos into two classes: characteristic homozygous mp^{B4149} embryos lacking a root and hypocotyl (class II, 8.5%, n=282) and embryos that are less affected in their basal parts (class III, seeming restoration of the root, 17.0%, n=282) (Fig. 4I-L)."

- *Certainly, it would be interesting to know, whether the suppression also restores fertility. In this case, the true breeding line could simply be analyzed in greatest detail.*

REPLY: Given the time available for revision, we have not been able to generate full homozygous lines to check this. In addition, while this might indeed allow further analyses of the restoration phenotype(e), we feel that this would not alter the general message of the manuscript.

- *The figure legends need to be redone. Right now they combine a concluding heading with a narrative presentation from the authors' perspective. The reader would instead simply like to know what is in those figures. Is this a whole-mount in situ hybridization? From where is the quantified RNA? On how many biological replica is it based? These types of information would be helpful to have in the legends.*

REPLY: We have rephrased the legends. The relevant n-values have been added to the text. Information on biological repeats has been added to the Materials and Methods section.

Referee #2:

- *genetic and molecular evidence is lacking that supports the conclusion that WRKY23 functions downstream of SHR/SCR in the same pathway.*

REPLY: Given that the SHR-SCR pathway acts as a negative regulator on WRKY23, one would need to down regulate *WRKY23* in the *shr or scr* background. However the effect of the *shr* or *scr* mutation on root tip organization is so similar to the *WRKY23* knock down effect, hampering reliably analyses using a genetic approach. However, we have added additional qPCR data (Figure S4) and a comparison of WRKY23 and SHR expression (with and without auxin) (Figure S5). In addition, we have further tested the auxin-independency of the SHR-SCR effect on WRKY23 (Supplementary Fig S4 online). Taken together, we feel this convincingly positions SHR-SCR upstream of WRKY23.

- *normalization of mp defects by WRKY23 overexpression could result from function of WRKY23 in a pathway parallel to that in which MP functions (e.g., bypass suppression of unc-54 by hypermorphic mutations in myo-3), so strong evidence--genetic or molecular--is also lacking in support of the conclusion that WRKY23 functions downstream of MP in the same pathway.*

REPLY: We have now better described the mp rescue experiment (Fig 4) to further clarify that this provides strong evidence for WRKY23 acting downstream of MP. In addition, we present molecular

evidence (using qPCR and GUS) to demonstrate altered WRKY23 expression in mp and bdl. We therefore are convinced that the genetic evidence presented is conclusive.

- *It's also unclear why the Authors have not used wrky23 mutants for their study; a quick search reveals the existence of a line (SALK_033414) with a T-DNA insertion in exon 2.*

REPLY: The observed phenotypes have been found in several independent lines using several approaches to perturb WRKY23 levels or activity (e.g. SRDX, RNAi, amiRNA), providing convincing results for a role of WRKY23 in embryo development. We feel that testing an additional T-DNA knock out line (which was previously not available) would not contribute a lot to the manuscript. However, as suggested by referee 2 we obtained the newly identified T-DNA line Salkseq_8394 from NASC and have initiated its characterization. However, in the timeframe for the revision we have not been able to properly characterize this line (given the difficulties with genotyping and background). SALK_033414 was the parental line (in an intergenic region between AT4G15430 and AT4G15440) for SALKseq_8395, and was identified following a reindexing of the T-DNA collections with a new next-generation sequencing approach. Although we observed a high percentage of aborted ovules in progeny likely heterozygous for *wrky23* and wildtype for the parental locus, we have not yet been able to convincingly test this line in the time frame for this revision (e.g. complementation of the T-DNA line). We therefore prefer to not include the results, although they are promising.

- *I appreciate the effort toward quantification of defects (Table S1 and S2--though quantification should be equally applied to all other experiments/images), but "polarity defects", "hypophysis defects", and "patterning defects" are too vague descriptors to be meaningful, and those tables could use images to illustrate the phenotype classes (as the Authors have done in Fig. 4I-K, for example).*

REPLY: We have defined this better in the text, referring to representative pictues.

- *Moreover, a minimum of two transgenic lines should be analyzed and their effects reported separately.*

REPLY: We have not only analyzed several transgenic lines [added in the text “Therefore, we evaluated our transgenic lines (at least 2 independent lines for each construct)…”], but – importantly – used 4 different constructs / approaches to downregulate WRKY23 levels (WRKY23::WRKY23RNAi, WRKY23::WRKY23amiRNA, 35S::WRKY23-SRDX, WRKY23::WRKY23-SRDX) and , each of which resulted in embryonic defects. We have now also added additional pictures from independent lines to Supplementary Figure S1.

- *On the positive side, the manuscript is quite easy to read, though there are here and there some easy-to-fix mistakes (e.g., "plants were grinded").*

REPLY: We thank the reviewer for this positive comment, and have further improved the manuscript by making several edits.

Referee #3:

- *Do we know that SCR & SHR are really auxin-independent or do we just not have any evidence that they're auxin dependent?*

REPLY: Based on qPCR analyses we could demonstrate that WRKY23 is strongly upregulated by auxin, while SHR and SCR are not affected. We have added a new supplemental figure and

implemented this in the text as follows "In addition, the SHR–SCR-mediated effect on *WRKY23* is not mediated by auxin, as neither *SHR* nor *SCR* are auxin inducible (Supplementary Fig S6 online)."

- *the GUS images in Fig 3F-G show that there is no change in the auxin signalling domain in shr-2, but do not address the strength of auxin signalling in this domain. Since WRKY23 is auxin regulated, a reduction in auxin signalling might reduce its expression. To conclusively show that the change in WRKY23 is not due to changes in auxin signalling, the authors should quantify auxin signalling levels (eg, by showing no change in the expression level of other known auxin responsive genes).*

REPLY: We agree with this comment, and have tested auxin responsive genes in *shr-2* mutant root tips. While *WRKY23* is strongly upregulated, the other genes (BDL, MP, IAA19, IAA28) are not as strongly affected. However, there is an up regulation of auxin responsive genes in *shr-2*, which does not allow us to fully exclude that the effect of SHR is through auxin response. We have added these data as Supplementary Fig S4. We have added "While no difference could be observed in the DR5-visualised auxin response between *shr* and wild-type roots (Fig. 3J-K), the strong up regulation of *WRKY23* in *shr-2* (~10-fold) was associated with a mild increase in the expression level a selection of auxin-responsive genes (~3-5-fold) (Supplementary Fig S4 online)". We also added experiments on *WRKY23* expression in the root tip upon auxin treatment and implemented this as follows "However, while auxin strongly up regulated *WRKY23* in a wildtype root tip, this auxin-mediated up regulation of *WRKY23* occurred outside the inner tissues where *SHR* is expressed (Supplementary Fig S5 online). In conclusion, we added "Based on these results, we cannot fully exclude that the repressing action of the SHR−SCR pathway on *WRKY23* expression is an indirect effect of affected auxin signalling; but, our data support that this is likely not the case."

- *Since WRKY23 plays a role in proper specification of the root pole and the knock-down mutant shows QC defect, the changes observed in DR5 expression (in Fig 2) may be due to changes in cell identity in these mutants. It would therefore be informative to show that tissue specific markers, such as SHR, SCR, and pCO2, are unchanged in the WRKY23 RNAi background.*

REPLY: We agree with the reviewer that this could be the case, and this change of cell identity is already reflected in the loss of a QC marker in the WRKY23 RNAi lines. But as is often the case, cause and consequence are difficult to distinguish in this respect. We therefore feel that testing further markers will not provide useful insights.

- *In Fig 3, the qPCR data is from seedlings while the in situ expression data is from embryos. I understand that it would be challenging to perform a qPCR on embryos, but it would be helpful if the authors showed that the expression in seedlings corresponds to that in embryos, thus making these two sets of data comparable.*

REPLY: Indeed, for obvious technical reasons, we used Arabidopsis root tips to validate parts of our network. We have added new pictures on the *WRKY23* expression pattern in mature tissues, highlighting the appropriateness and reproducibility of our approach. We attempted qPCR analyses on embryos, but, while the fold changes were somewhat indicative, it was difficult to support this by statistics because of a low level of reproducibility using embryos that are at not exactly the same developmental stage.

- *There appears to be a higher level of WRKY23 expression in both Fig 3C & E. If this difference is, in fact, not statistically significant, this should be made clear in the text. If Fig 3C is not representative, perhaps another image could be used. As it stands, the figure and the text seem to be at odds with one another.*

REPLY: We have rephrased this as the difference is not significant, which is often an issue with selecting pictures from mRNA in situ. The observations are further supported through the qPCR data presented in 3E and a new representative figure for *plt1plt2* (Fig 3F).

- *Based on their previous paper, both Knock-down and Overexpressor of WRKY23 show meristem defect in seedling stage, and they emphasized the importance of it dosage controls of the root stem cell niche. Is this also applicable to embryo stage? Is it possible that SHR modulates its expression in a constant level instead of just repressing its expression? At least in discussion part, i think they should give answers to my questions.*

REPLY: We have added this to the discussion "Since dosage control of *WRKY23* is important for root stem cell niche maintenance [10] this is likely also the case in the embryo and it is possible that SHR modulates *WRKY23* expression in a constant level instead of just repressing its expression."

- *The phrase "WRKY23 expression follows [...] the root stem cell niche" may be confusing, since 'follows' can also suggest temporal succession. I suggest the authors use "overlaps with" instead, unless there is a reason not to do so.*

REPLY: The title has been altered as suggested into "*WRKY23* expression overlaps with with the root stem cell niche"

- *Figure 3 and 4 are referenced in a confusing order in the text. I would find it more clear if Fig 4A,B,D,E were moved into Fig 3, though I understand that the authors & editor may disagree here.*

REPLY: We have altered the order of the figures to improve readability.

- *The authors should indicate what age the seedlings were in the qRT-PCR experiments.*

REPLY: Details were added to Figure legend 3 and 4.

2nd Editorial Decision 30 September 2013

Many thanks for submitting your revised manuscript to our editorial office. It was sent to the three original reviewers and we have now received their evaluations.

As you will see, while referees 1 and 3 now support publication of the study in our journal, referee 2 still raises concerns about the strength of the evidence that WRKY23 acts downstream of SHR/SCR and MP/BDL. Upon further discussions of this criticism with reviewer #1, we decided that we would not require you to perform the additional experiments requested by referee 2. However, referee 1 agrees that some parts of the text (subheadings, figure legends, discussion) should be worded more carefully to better reflect the actual data and that alternative interpretations should be discussed as well.

I would therefore kindly ask you to change the text of the manuscript accordingly before we can accept it for publication here.

Please submit the final version of your manuscript through our website. Alternatively, you can also send us the amended text via email and we will upload it into our system ourselves. Formally, your manuscript would need to be accepted by October 18th (6 months after the initial decision on it), but since the remaining changes are only textual, I do not foresee any problems meeting this deadline.

Thank you very much for your cooperation and I look forward to receiving the revised manuscript as soon as it is ready, as we do not want to unnecessarily delay its publication, which, in the end, the reviewers support.

REFEREE REPORTS:

Referee #1:

The revised manuscript by Grunewald et al has addressed the main points spotted in the initial submission. This refers to all experimental and documentation issues. The style remains strongly interpretation-oriented also throughout the result section. This is not uncommon, yet a lot of readers and journals object to it.

Referee #2:

The Authors did not address--in either manuscript or response to the reviewers' comments--the limited novelty and general interest. However, I do appreciate the Authors' efforts to address my other concerns.

Based on published data, I respectfully disagree with the Authors when they claim that root tip defects of shr or scr are too similar to those of WRKY23 knockdowns to allow genetic analysis. I still maintain that none of the presented data supports the conclusion that WRKY23 functions downstream of SHR/SCR in the same pathway (or that SHR/SCR functions upstream of WRKY23 in the same pathway). However, in the revised manuscript the Authors simply claim that SHR and SCR are negative regulators or WRKY23 expression, and the data they present in support of this conclusion are very convincing.

Far less convincing is the conclusion drawn from the results of the rescue experiment of mp defects by WRKY23 overepression. I appreciate the more detailed description of the experiment, but the description of the experiment was never a point of contention. Work in animals has shown that results such as those obtained by the Authors are consistent with the possibility that WRKY23 function downstream of MP in the same pathway--as the Authors contend; however, that work has also shown that the very same results are also consistent with the possibility that WRK23 function in a pathway parallel to that in which MP functions. The only experimental test to discriminate the two mechanisms seems to be double-mutant analysis. I am aware that--should the phenotype of the double-mutant suggest that WRKY23 and MP function in the same pathway--the similarity between defects of mp and those of WRKY23 knockdowns would prevent determining the direction of the epistatic relationship. But that could be easily resolved by comparing phenotypes of WRKY23 overexpression in mp background to those of MP overexpression in WRKY23 knockdowns. Thus, in the absence of unambiguous experimental evidence that can discriminate between epistasis and bypass suppression, I believe the Authors should at least be more cautious and present alternative--and equally likely, after all--scenarios.

Referee #3:

I have no further comments. The paper appears suitable for publication.

2nd Revision - authors' response 04 October 2013

Thank you for the positive decision on our manuscript.
We have now made the suggested edits and textual additions to our manuscript (attached as a final version and a version with track changes). We hope that our more careful

wording (both in the main text, the subtitles and the figure legends) and the discussion of alternative hypotheses are sufficient to accept the manuscript for publication.

3rd Editorial Decision 04 October 2013

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, this File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: emboreports@embo.org]. If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

