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SUPERMAN regulates floral whorl boundaries through control of auxin biosynthesis

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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 20 July 2017

Thank you for submitting your manuscript for consideration by the EMBO Journal. It has now been seen by three referees whose comments are shown below.

As you will see, the referees appreciate your work. However, they also think that a lot more insight is needed to make your manuscript a strong candidate for publication here. I won't list the specific issues the referees note, as all reports are very clear and constructive, and the lack of insight seems straightforward to address given the referees' comments.

Should you be able to address the criticisms of all referees in full, we could consider a revised manuscript. I should remind you that it is EMBO Journal 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 in this revised version and on strong support from all referees. I do realize that addressing all the referees' criticisms will require a lot of additional time and effort. I would therefore understand if you wish to publish the manuscript rapidly elsewhere, in which case please let us know so we can withdraw it from our system.

If you decide to thoroughly revise the manuscript for the EMBO Journal, please include a detailed point-by-point response to the referees' comments. Please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: http://www.embo.org/embo-press

We generally allow three months as standard revision time, but I can extend the revision to six months. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However,

we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Referee #1:

Floral organ identity is a complex interplay of several feedback pathways. Here the authors assessed the function of SUPERMAN in floral organogenesis and meristem determinacy. The work is of very high quality and I certainly envision interest for the broad readership of EMBO Journal.
Initially, this work confirms previous assumptions on increased floral meristem size in sup mutants, using molecular markers, such as pCLV3::GFP-ER and pSTM::STM-VENUS. They moreover confirm, using a functional pSUP::SUP-3xVENUS-N7 construct, that SUP proteins reside in the boundary, but non-cell autonomously impact on CLV3 and WUS. Notably, CUC2 is upregulated in sup mutants, hinting at a possible link to the phytohormone auxin. Auxin signalling reporters suggest high levels of auxin. Consistently, sup mutant phenotype is partially suppressed by the application of an "anti-auxin" PCIB. The authors could use the very same assay to test whether the misexpression of CUC2 is indeed due to elevated auxin signalling. The authors conclude that the auxin biosynthesis is upregulated, presumably leading to higher auxin levels. It would be very nice to actually measure auxin levels in flowers of wild type and sup mutants to confirm this. The authors used ChIP to illustrate SUP binding to YUC1 and YUC4 auxin biosynthesis genes. It would be nice if the authors were commenting on potential binding sites. Notably, the binding site resides in the coding region. Full length reporters show distinct expression compared to promoter reporters, suggesting that either protein stability or expression regulation is distinct. The authors state that this "data confirmed that the SUP binding regions of YUC1/4 suffice for their negative regulation". This is an overstatement and I ask the authors to modulate the discussion on this aspect or to provide further data. The increase of auxin biosynthesis in the SUP expression domain partially phenocopies sup mutants. Moreover, the sup yuc double mutant partially suppresses the sup mutant phenotype. This analysis nicely confirms the assumptions of the authors. Finally, the authors used yeast two hybrid and bimolecular fluorescent complementation assay to suggest that SUP functions in a repressor complex with CLF and TFL2. Accordingly, the study finishes with a highly interesting finding, but in its current form this aspect is a bit preliminary. Both methods have a high false positive rate. Therefore, I would like the authors to use additional, more sensitive methods to show the interaction and genetic studies to confirm the claims. Alternatively, the authors could also remove this data from the manuscript or strongly balance the discussion on this matter (e.g. shifting the data into the supplements and just mention it as a possible scenario).
As the authors indicate that auxin biosynthesis in the boundary is imposing changes in the floral meristem, it is likely that auxin transport regulation is important for this regulation. However, the manuscript falls short on this mechanistic aspect. Some anti-PIN immunolocalisation could bridge this gap.

In summary, the main part of the manuscript (Fig 1-4) is very convincing and I have only some suggestions to possibly further improve this part. The missing link to auxin transport would be crucial for further mechanistic insights. On the other hand, data in Fig 5 seems a bit preliminary to me.

Minor issues:
Most microscopy images are not quantified (this is particularly apparent in the supplemental Figures). It would be nice to measure the fluorescent intensity or spatial distribution of the markers. In the same line, the frequency of phenotypes are not defined. This is certainly important for the rescue experiments (see for example Figure S6 or S7)

Referee #2:

In this well-structured manuscript, Xu et al. explain and give evidence on how SUPERMAN controls correct flower development. Although the phenotypic consequences of sup mutation has been known for years, the regulatory mechanism was not understood. Here, the authors show how SUPERMAN is necessary to repress auxin biosynthesis genes to maintain well defined borders between structural elements of the floral meristem (FM). Although not all aspects of auxin distribution and transport in FMs have been investigated here, the link of SUP to auxin biosynthesis and, furthermore, the explanation of how SUP is repressing some of its targets is new and provides valuable mechanistic insights about the process of FM development.

Questions:
Page12: The authors explain what phenotypes would be expected of yuc1/yuc4 and yuc4 in sup background, but have they actually studied these genotypes?
Strong phenotypes of yuc4 or yuc1/yuc4 in contrast to lack of flower phenotype in a single yuc1 mutant, suggest very subtle or very specific role of yuc1. Still, yuc1 mutation reverts sup mutant phenotype. It would be beneficial for the manuscript to see the spatial distribution of auxin response (e.g. DR5 reporter) in the FM of sup yuc1 background which shows reverted phenotype.
Along the same lines, can sup yuc1 mutant phenotype be reverted to sup phenotype by external application of auxin? How does DR5 reporter behave in these backgrounds?
Can authors also provide in the supplementary data an image of sup DR5 line treated with PCIB showing changes in distribution of auxin response in the FM.

Minor comments: The authors could be more specific with naming their controls. The ap1 cal1 P35S::AP1-GR background is hardly the wildtype (even if the sample is sup mutant in addition). Along these lines, a short explanation of this system could help the reading of this manuscript (page 6).
Second half of page 7 should be shorter. As the authors state, the non-cell autonomous function of SUP was shown before, the new finding is limited to the immobility of SUP-GFP.
Page 9: Can the authors show that IAM has no effect on flower development (i.e. IAM treatment on Wt plants as a second control)?
Page 12: Can the author comment on why GUS reporter were chosen to visualize the YUC1/4 expression patterns?
The interaction of SUP with CLF and TFL2 is not discussed. A short explanation of the function of CLF and TFL2 in the PcG complex and discussion on how SUP might change this would be helpful.
Can authors provide SD bars for all samples in Fig 2J and 4J. Also, can authors add wt control in with its corresponding SD values (Fig4J).

General:
Figure 5H is mentioned in the text when the authors actually refer to 4H
Page 12: relatively can be deleted, as it is implied by ... higher than... (YUC1 expression was relatively higher than that of YUC4, which is consistent with previous reports)

Referee #3:

This manuscript by Xu and colleagues explores the mechanism by which the SUPERMAN (SUP) gene acts in the establishment of the whorl 3 / whorl 4 boundary of the flower. It provides data to demonstrate that the sup phenotype is linked to a non-cell autonomous action on the flower stem cell niche and that this effect is mediated by auxin. It further shows that SUP acts on auxin by regulating 2 YUCCA genes involved in auxin biosynthesis, a regulation that involves repression by recruitment of PcG proteins CLF and TFL2 by SUP to the promoter. The data presented are interesting and the molecular mechanism identified potentially of high interest to understand how a boundary gene functions. They is some overlap with the accompanying manuscript but the two papers are complementary enough to justify two manuscripts.
However, there are a number of major issues with the logic of the manuscript and the type of data presented:
1- At the moment the manuscript (that is very lengthy) is made of two parts, one on a demonstration that SUP acts on stem cells (largely redundant with the accompanying manuscript) and another one

on how SUP might act through regulation of biosynthesis of auxin. It is not really clear how the two are really connected notably because data on auxin concerns mostly the boundary itself where new organs are supposed to come from (which is not really demonstrated). How is this linked to an enlargement of the stem cell niche (and FM)? There is a need for clarification here as I am not convinced the two are really connected and the authors themselves state at the end of the manuscript that "Future studies may indicate whether the reduction of auxin in FMs contributes to a larger meristem size". This probably requires to study in more detail the spatial characteristics of the auxin action (using GUS for the YUCCA reporters preclude from having a clear idea of this).
2- The paper uses a genomic approach (microarray) to identify targets of SUP and hardly anything is said on the results obtained besides the fact that PIN3,4 and YUC1,4 have been identified (plus a few others). A detailed description of the targets is required (and a description of the statistical procedure used for the analysis that is missing in the manuscript) with a full analysis of the GO enrichments in target lists. From the manuscript, it is impossible to say whether the analysis was pointing to auxin (backed up with statistics). The authors do not cite a paper in JXB from Nibau et al 2010 in which a link is made with auxin and CK. This could be enough to justify looking into auxin, not to mention the well known role of auxin on floral organ initiation and development.
3- The regulation of target genes by recruitment of PcG is very interesting but it would be much more pertinent to provide data showing that it is a general mechanism of SUP action. The authors have all the genetic material and looking at histone methylation at the genome level is now largely accessible.

Other important concerns that needs to be addressed:
- "However, STM expression domain appears larger in sup than in the wild type (Figure S2)": this claim requires a quantification.
- P8 "The ectopic CUC2 expression in sup did not totally overlap with the SUP expression region, indicating the change of CUC2 expression is an indirect effect of the sup mutation. " : I do not understand this. If a protein acts non-cell autonomously, overlap in expression does not say anything on how direct the regulation is. The authors need to revise their reasoning.
- P9 when using PCIB: the authors should use DR5 and DII-VENUS to analyze the effect of the treatment and understand how the rescue occurs.
- Fig 2H-J and corresponding text P9: the control is said to be the untreated wild-type. A wild-type control treated with IAM is needed.
- P10 when identifying the auxin-related targets: the authors discard PIN3/4 because their results are not fully consistent but transport regulation makes a lot of sense for obtaining a spatio-temporal specificity in an auxin-mediated regulation. The authors actually discuss it at the end of the discussion without even mentioning again that they identified the two putative targets. A better justification for not analyzing these two genes is required and they need to be mentioned in the discussion.
- P12: the authors compare YUC translational and translational to demonstrate a need for the coding region of the YUC genes. This experiment does not support their conclusion at all. The GUS activity from a translational fusion is regulated by post-transcriptional mechanisms. The authors need another approach to approach the problem (mutation of some of the sequences ?) and this is an important part of the analysis.
- CLF and PcG proteins have been involved in regulation of the stem cell niche activity in the flower (direct regulation of AG). Mentioning this would add an interesting dimension to the discussion.

More minor comments:
- Ref 22 needs to be completed
- P7 when discussing non-cell autonomy: the authors seems to suggest that if the SUP protein was moving that would argue against non-cell autonomy. This needs to be rephrased.
- P10 "biotic and abiotic stress": how does this relate to what is shown on Fig 3A?

Additional correspondence 28 July 2017

Thank you very much for your very helpful handing of our manuscript. I discussed with the co-authors on all the comments from reviewers, and we found that most of them are very constructive and helpful to validate our story. We have made a revision plan. It includes two rounds of genetic

crossing of transgenic lines, which takes ~5 months. We plan to resubmit the manuscript in Jan 2018, if everything works fine. Thus, please extend our revision time to six months..

Additional correspondence 28 July 2017

Many thanks for your message and for outlining the revision time to me. We will extend the revision time in our system accordingly.

1st Revision - authors' response 12 February 2018

Response to Reviewers
We would like to thank the reviewers for their helpful comments on our manuscript. To fully address them, we have conducted, over the past 6 months, all the experiments the reviewers suggested. We also added, as requested, new information and discussion points throughout the manuscript.
In brief, we have measured by LC/MS auxin levels in WT and *sup-5* floral buds at early developmental stages, confirming the negative regulation of auxin biosynthesis by SUP. To further investigate the rescue of the *sup-5* mutant phenotype in response to the auxin inhibitor PCIB, we have analyzed the activity of the auxin reporter *DR5* after the treatment and found it to be reduced. Furthermore, we confirmed that *yuc4* can partially rescue *sup-5* and that the *yuc1 yuc4* double mutant is epistatic to *sup-5*. Using GUS reporters, we detected ectopic expression of *YUC1/4* in *sup* mutant flowers. We further quantified the expression levels of YUC1/4 GUS reporters with or without coding region, confirming that the region covered by the repressive H3K27me3 mark is involved in transcriptional repression. We also validated the physical interaction between SUP and CLF *in vivo*. As requested, we have added text to discuss the function of the interaction of SUP with CLF/TFL2. We also incorporated data from chemical treatments to show that, in addition to auxin biosynthesis, polar auxin transport is important for the defects of *sup-5* mutant flowers.
A detailed list of changes and a point-by-point response to the reviewers' comments are given below.

Reviewers' comments:

Referee #1

Floral organ identity is a complex interplay of several feedback pathways. Here the authors assessed the function of SUPERMAN in floral organogenesis and meristem determinacy. The work is of very high quality and I certainly envision interest for the broad readership of EMBO Journal.

Initially, this work confirms previous assumptions on increased floral meristem size in sup mutants, using molecular markers, such as pCLV3::GFP-ER and pSTM::STM-VENUS. They moreover confirm, using a functional pSUP::SUP-3xVENUS-N7 construct, that SUP proteins reside in the boundary, but non-cell autonomously impact on CLV3 and WUS. Notably, CUC2 is upregulated in sup mutants, hinting at a possible link to the phytohormone auxin. Auxin signalling reporters suggest high levels of auxin.
1. *Consistently, sup mutant phenotype is partially suppressed by the application of an "anti-auxin" PCIB. The authors could use the very same assay to test whether the misexpression of CUC2 is indeed due to elevated auxin signaling*.
Response: As requested, we have conducted this experiment and provide photos of the *DR5* and *CUC2* reporters in *sup-5* flowers after PCIB treatment in Supplemental Figure S6. While the DR5 reporter showed the expected reduction after the treatment (Figure S6C-D), *CUC2* expression was not reversed to a more WT-like expression pattern but instead ectopically induced at 4-6 hours after the PCIB treatment (Figure S6A-B). This indicates that *CUC2* expression is not directly proportional to auxin levels, as previously proposed by Heisler et al. (2005) Curr. Biol. **15**, 1899-911. We are now specifically discussing this result in the manuscript.

2. *The authors conclude that the auxin biosynthesis is upregulated, presumably leading to higher auxin levels. It would be very nice to actually measure auxin levels in flowers of wild type and sup mutants to confirm this.*

Response: In collaboration with H. Sakakibara's group at RIKEN (Japan), we have measured IAA levels in wild-type and *sup* mutant flowers at an early developmental stage. In agreement with our observation that *YUC1/4* are up-regulated in *sup*, we found that IAA levels are significantly higher in *sup* when compared to wild-type flowers. The new results are shown in Figure 3D and a description of the methods used was added to the Materials and Methods section.

3. *The authors used ChIP to illustrate SUP binding to YUC1 and YUC4 auxin biosynthesis genes. It would be nice if the authors were commenting on potential binding sites. Notably, the binding site resides in the coding region. Full length reporters show distinct expression compared to promoter reporters, suggesting that either protein stability or expression regulation is distinct. The authors state that this "data confirmed that the SUP binding regions of YUC1/4 suffice for their negative regulation". This is an overstatement and I ask the authors to modulate the discussion on this aspect or to provide further data.*

Response: We fully agree with the reviewer and tested the transcription levels of the *GUS* gene in the GUS reporter lines (Supplemental Figure S11F). We have confirmed that the transcriptional level is correlated well with the GUS staining, indicating that the coding region covered by high levels of H3K27me3 has a negative effect for transcription. SUP was reported to bind to DNA regions with the AGT core motif, by its single Cys_2–His_2 zinc finger domain, and two basic regions located on either side (Dathan N. et al., (2002) Nucleic Acids Res., **30**(22):p4945–4951). SUP binds to wide regions of *YUC1/4* genome, and thus it is difficult to predict potential binding sites as the high frequent presence of the AGT motif. Notably, a recent genome-wide analysis identified both CLF and TFL2 are essential for H3K27me3 mark spreading (Wang et al., (2016) PLoS Genet, **12**(1): p. e1005771). We added the statement in the discussion on the possibility of SUP involving in the spreading of H3K27me3 mark at *YUC1/4*.

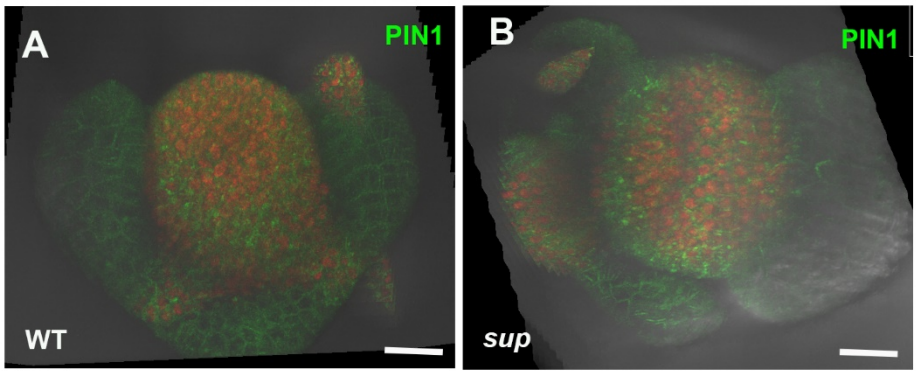
4. *The increase of auxin biosynthesis in the SUP expression domain partially phenocopies sup mutants. Moreover, the sup yuc double mutant partially suppresses the sup mutant phenotype. This analysis nicely confirms the assumptions of the authors. Finally, the authors used yeast two hybrid and bimolecular fluorescent complementation assay to suggest that SUP functions in a repressor complex with CLF and TFL2. Accordingly, the study finishes with a highly interesting finding, but in its current form this aspect is a bit preliminary. Both methods have a high false positive rate. Therefore, I would like the authors to use additional, more sensitive methods to show the interaction and genetic studies to confirm the claims. Alternatively, the authors could also remove this data from the manuscript or strongly balance the discussion on this matter (e.g. shifting the data into the supplements and just mention it as a possible scenario).*

Response: To address this comment, we have confirmed the interaction between SUP and CLF *in vivo* by co-IP assay. To this end, we generated a functional *pCLF::HA-CLF* transgene and introgressed it into the *ap1 cal p35S::AP1-GR* background with or without *pSUP::SUP-GFP*. Using an GFP antiserum, we found that we could pull-down HA-CLF in stage 4 floral buds of *ap1 cal p35S::AP1-GR pSUP::SUP-GFP pCLF::HA-CLF*, but not of *ap1 cal p35S::AP1-GR pCLF::HA-CLF* plants. We have replaced the BiFC data with the new results from the Co-IP assays in Figure 5C and moved the BiFC data to Supplemental Figure S15B.

5. *As the authors indicate that auxin biosynthesis in the boundary is imposing changes in the floral meristem, it is likely that auxin transport regulation is important for this regulation. However, the manuscript falls short on this mechanistic aspect. Some anti-PIN immunolocalisation could bridge this gap. In summary, the main part of the manuscript (Fig 1-4) is very convincing and I have only some suggestions to possibly further improve this part. The missing link to auxin transport would be crucial for further mechanistic insights. On the other hand, data in Fig 5 seems a bit preliminary to me*

Response: We fully agree with the reviewer that polar auxin transport could also be important for the SUP's function in FM regulation. The overlapping but different expression patterns between *YUC1/4* (in Supplemental Figures S11 and newly added S12) and *DR5* signal (in Figure 2) hinted at polar auxin transport being involved in the *sup* morphology. To test this, we determined the expression pattern of the auxin efflux carrier PIN3, but did not detect any obvious differences in expression between WT and *sup* flowers (Supplemental Figure S14). We also examined *pPIN1::PIN1-GFP* in *sup-5* and WT flowers, but did not obtained conclusive data for PIN1 expression in this case (shown below). In addition, we tested the effects of the polar auxin transport inhibitor N-1-naphthylphthalamic acid (NPA) on *sup* flowers (Supplemental Figure S7). The application of the NPA at floral stages 4-6 can partially rescue both the carpel morphology and stamen number defects of *sup* mutants. These results suggest that the derepression of *YUC1/4* genes in *sup* requires auxin transport to bring about morphological effect, although the WT-level of auxin

polar transporter may be enough to establish the change of auxin gradient and maxima after *sup* mutation. We now discuss this point in the manuscript.



Minor issues:
1. *Most microscopy images are not quantified (this is particularly apparent in the supplemental Figures). It would be nice to measure the fluorescent intensity or spatial distribution of the markers.*
Response: We have quantified the expression domain of *STM* at stage 5 and updated the data in the legend for Supplemental Figure S2A-B. We also have carefully analyzed the spatial distribution of representative markers in WT, *sup-5,* and *sup-5 yuc1* flowers, with or without PCIB treatment in the figures, which are summarized in Supplemental Figure S16. We also have replaced *pSTM::STM-VENUS* with *pSTM::CFP-N7* and added clearer floral images of late stage in Supplemental Figure S2, to show that the termination of stem cell activity is delayed and stem cell population is increased at stage 6 in *sup-5*.

2. In the same line, the frequency of phenotypes are not defined. This is certainly important for the rescue experiments (see for example Figure S6 or S7).
Response: As requested, we have added quantitative information for transgenic and mutant lines. For *pSUP::SUP-GR sup* and *p35S::SUP-GR* lines, all flowers (more than 100 flowers from 20 individual plants) we examined showed the reported phenotypes after DEX treatment. We have removed the data of some transgenic lines (p*35S::SUP-GR p35S::PI*, p*35S::SUP-GR p35S::AP3*, and p*35S::SUP-GR ag-1*), since they seemed not essential for the manuscript and the penetrance of phenotypes was more variable in these cases.

Referee #2:

In this well-structured manuscript, Xu et al. explain and give evidence on how SUPERMAN controls correct flower development. Although the phenotypic consequences of sup mutation has been known for years, the regulatory mechanism was not understood. Here, the authors show how SUPERMAN is necessary to repress auxin biosynthesis genes to maintain well defined borders between structural elements of the floral meristem (FM). Although not all aspects of auxin distribution and transport in FMs have been investigated here, the link of SUP to auxin biosynthesis and, furthermore, the explanation of how SUP is repressing some of its targets is new and provides valuable mechanistic insights about the process of FM development.

Questions:
1. *Page12: The authors explain what phenotypes would be expected of yuc1/yuc4 and yuc4 in sup background, but have they actually studied these genotypes? Strong phenotypes of yuc4 or yuc1/yuc4 in contrast to lack of flower phenotype in a single yuc1 mutant, suggest very subtle or very specific role of yuc1. Still, yuc1 mutation reverts sup mutant phenotype.*
Response: While revising the manuscript, we analyzed *yuc1 sup* and *yuc4 sup* double or *yuc1 yuc4 sup* triple mutants and replaced the original Figure 4G-I with the new data (Figure 4G-H). Both *yuc1* and *yuc4* can partially rescue *sup-5*, while *yuc1 yuc4* appears to be epistatic to *sup-5*. We confirmed

that the single mutant of *yuc1* have no obvious morphological defect, while *yuc4* flowers form floral organs with reduced size. Consistent with the mutant phenotypes, our GUS reporter analysis (Supplemental Figure S11A-D) show that *YUC4* has a broader expression pattern including in the petals, while the expression of *YUC1* is more tissue-specific in young floral buds (Cheng *et al*. (2006) *Genes Dev*. **20**, 1790-9). However, in contrast to the previous report, we did not observe any obvious reduction in floral organ numbers in the single *yuc4* mutant (SALK_047083). As expected, both *yuc1* and *yuc4* can partially rescue stamen and carpel numbers in *sup*, and *yuc1* rescues *sup* to a much greater extent than *yuc4*. Quantitative data of these analyses are shown in Figure 4H.

2. *It would be beneficial for the manuscript to see the spatial distribution of auxin response (e.g. DR5 reporter) in the FM of sup yuc1 background which shows reverted phenotype. Along the same lines, can sup yuc1 mutant phenotype be reverted to sup phenotype by external application of auxin? How does DR5 reporter behave in these backgrounds?*

Response: To address this comment, we have analyzed *DR5* reporter activity in *sup-5 yuc1* flowers. In agreement with the partial rescue of the *sup* determinacy phenotype by *yuc1*, we observed a strong reduction of *DR5* signal in *sup yuc1* flowers relative to the wild type. The new data are shown in Supplemental Figure S13.

The 2,4-dichlorophenoxy acetic acid (2,4-D) and the naphthalene-1-acetic acid (NAA) are two most frequently used synthetic auxins. 2,4-D is poorly transported by auxin efflux carriers, while NAA enters cells predominantly by diffusion and its accumulation level is controlled by efflux carriers (Delbarre et al., (1996) Planta, 198, pp. 532-541). Therefore, we treated the plants with optimal concentration of NAA. The application of NAA (100 µM) could not rescue *sup-5 yuc1* to *sup-5*-like based on morphology and *DR5* reporter. We also noticed the same NAA treatment on *sup-5* can partially rescue the carpel morphology but not the stamen number (please see below). We hypothesized that the change of localized biosynthesis is responsible for the *sup* morphology but it can not be achieved by exogenous auxin application. Eventually we have decided not to include this negative data in the manuscript, since it still contains various different interpretation and may confuse readers. However, we are happy to add them back in if the reviewer feels that they should be presented.

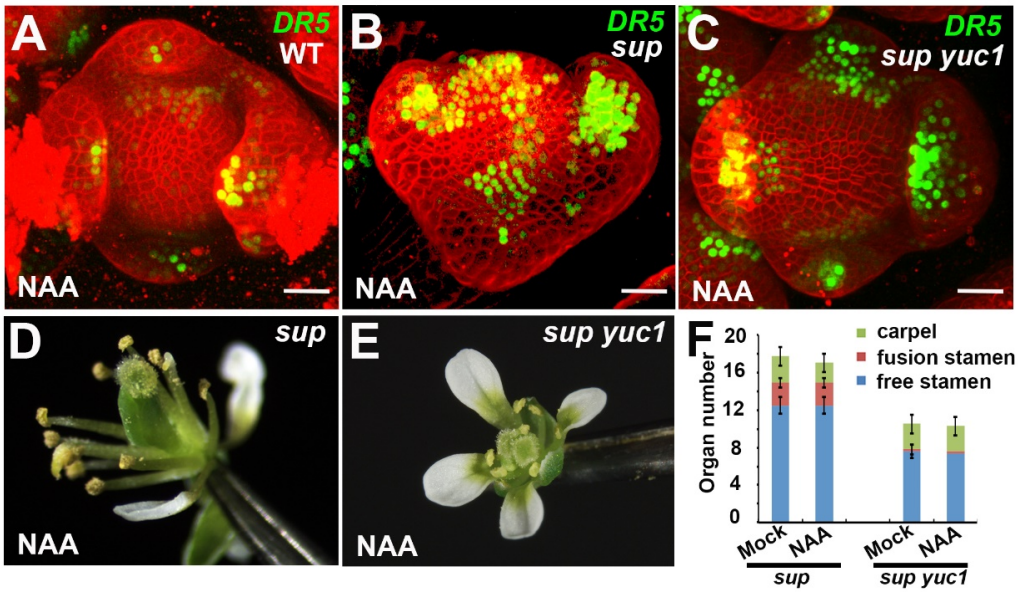


Figure Sx. The application of NAA cannot rescue *sup-5 yuc1* to *sup-5*-like phenotype
(A-C) The pattern of *pDR5rev::3xVENUS-N7* in WT (A), sup-5 (B) and *sup-5 yuc1* (C) 4-6 hours after the NAA application.
(D) The NAA application at stage 4 can rescue the carpel of sup-5 only.
(E) The NAA application cannot rescue *sup-5 yuc1* to *sup-5*-like phenotype.
(F) The statistics analysis of floral organ number to show there are no significant ($p>0.05$) between the mock and NAA treated *sup-5 yuc1*.
Scale bars, 20 µm in (A-C).

3. *Can authors also provide in the supplementary data an image of sup DR5 line treated with PCIB showing changes in distribution of auxin response in the FM.*
Response: As requested, we now show images of *DR5* in *sup-5* flowers after PCIB treatment in Supplemental Figure S6. We found that the *DR5* signal is greatly reduced in *sup* 4-6 hours after the PCIB treatment, which is consistent with the observed rescue of *sup-5* through PCIB.

Minor comments:
1. *The authors could be more specific with naming their controls. The ap1 cal1 P35S::AP1-GR background is hardly the wildtype (even if the sample is sup mutant in addition). Along these lines, a short explanation of this system could help the reading of this manuscript (page 6).*
Response: As requested, we added a brief explanation of the *ap1 cal p35S::AP1-GR* system and made numerous changes throughout the manuscript to be more specific about naming controls.

2. *Second half of page 7 should be shorter. As the authors state, the non-cell autonomous function of SUP was shown before, the new finding is limited to the immobility of SUP-GFP.*
Response: We have shortened the paragraph, as requested.

3. *Page 9: Can the authors show that IAM has no effect on flower development (i.e. IAM treatment on Wt plants as a second control)?*
Response: As requested, we have treated wild-type plants with IAM and show the results in Figure 2J.

4. Page 12: Can the author comment on why GUS reporter were chosen to visualize the YUC1/4 expression patterns?
Response: We initially tried *in situ* hybridization analysis of *YUC1/4* mRNA expression in inflorescences and realized that the signal is quite low in young floral buds. Since in situ hybridization is not suitable for widely-expressed genes at low levels, we chose the more sensitive GUS reporter to visualize the change of *YUC1/4* expression in *sup*. We now state this specifically in the text.

5. *The interaction of SUP with CLF and TFL2 is not discussed. A short explanation of the function of CLF and TFL2 in the PcG complex and discussion on how SUP might change this would be helpful.*

Response: We have added this to the discussion, as requested. Notably, a recent genome-wide analysis identified both CLF and TFL2 are essential for H3K27me3 mark spreading (Wang et al. (2016) *PLoS Genet.*, **12**, e1005771). Based on this study, we now discuss the possibility that SUP may also be involved in spreading H3K27me3 mark at the *YUC1/4* loci.

6. *Can authors provide SD bars for all samples in Fig 2J and 4J. Also, can authors add wt control in with its corresponding SD values (Fig4J).*
Response: We have done this, as requested. For Figure 4, we have replaced the original Figure 4G-I with new data (Figure 4G-H) to quantitatively show that both *yuc1* and *yuc4* can partially rescue *sup-5*. A wild-type flower is displayed at Figure 4G.
General:
1. *Figure 5H is mentioned in the text when the authors actually refer to 4H*
Response: We have corrected this.
2. *Page 12: relatively can be deleted, as it is implied by ... higher than... (YUC1 expression was relatively higher than that of YUC4, which is consistent with previous reports)*
Response: We have deleted this, as requested.

Referee #3:

This manuscript by Xu and colleagues explores the mechanism by which the SUPERMAN (SUP) gene acts in the establishment of the whorl 3 / whorl 4 boundary of the flower. It provides data to demonstrate that the sup phenotype is linked to a non-cell autonomous action on the flower stem cell niche and that this effect is mediated by auxin. It further shows that SUP acts on auxin by regulating 2 YUCCA genes involved in auxin biosynthesis, a regulation that involves repression by recruitment of PcG proteins CLF and TFL2 by SUP to the promoter. The data presented are interesting and the

molecular mechanism identified potentially of high interest to understand how a boundary gene functions. There is some overlap with the accompanying manuscript but the two papers are complementary enough to justify two manuscripts.
However, there are a number of major issues with the logic of the manuscript and the type of data presented:

1. *At the moment the manuscript (that is very lengthy) is made of two parts, one on a demonstration that SUP acts on stem cells (largely redundant with the accompanying manuscript) and another one on how SUP might act through regulation of biosynthesis of auxin. It is not really clear how the two are really connected notably because data on auxin concerns mostly the boundary itself where new organs are supposed to come from (which is not really demonstrated). How is this linked to an enlargement of the stem cell niche (and FM)? There is a need for clarification here as I am not convinced the two are really connected and the authors themselves state at the end of the manuscript that "Future studies may indicate whether the reduction of auxin in FMs contributes to a larger meristem size". This probably requires to study in more detail the spatial characteristics of the auxin action (using GUS for the YUCCA reporters preclude from having a clear idea of this).*
Response: We have shortened the manuscript by removing the redundant information throughout the text.
We agree with the reviewer that we do not yet fully understand how auxin regulates floral meristems.
However, in the revised manuscript, we provided data from several different experiments that support the notion that ectopic auxin biosynthesis in the *SUP* expression domain is responsible for the enlarged floral meristem size in *sup* mutant flowers: (1) stem cell number is increased in the FMs of *sup* mutants, which is in agreement with the increased number of reproductive floral organs; (2) *DR5* activity is up-regulated in the *SUP* expression domain; (3) Auxin levels are increased in *sup* mutant floral buds relative to the wild type; (4) *YUC1/YUC4* are de-repressed in *sup* mutant flowers; (5) *YUC1/4* genes are required for the *sup* mutant phenotypes; (6) The IAM-treated *pSUP::IAAH* transgenic flower mimic *sup* mutants. (7) We newly added information that auxin transport may also play an important role; treatments with the auxin-transport inhibitor NPA can rescue the *sup* mutant phenotype. To specifically address the comment, we have added this discussion to the text.

2. *The paper uses a genomic approach (microarray) to identify targets of SUP and hardly anything is said on the results obtained besides the fact that PIN3,4 and YUC1,4 have been identified (plus a few others). A detailed description of the targets is required (and a description of the statistical procedure used for the analysis that is missing in the manuscript) with a full analysis of the GO enrichments in target lists. From the manuscript, it is impossible to say whether the analysis was pointing to auxin (backed up with statistics). The authors do not cite a paper in JXB from Nibau et al 2010 in which a link is made with auxin and CK. This could be enough to justify looking into auxin, not to mention the well known role of auxin on floral organ initiation and development.*
Response: We have re-analyzed the microarray data. Genes showing a 2-fold change in expression within a 95% confidence interval were considered to be differentially expressed and are presented in Supplementary Table 3. We have analyzed Gene Ontology term enrichments using the agriGO software version 1.2 (http://bioinfo.cau.edu.cn/agriGO/). Enriched GO terms were further refined by REVIGO (http://revigo.irb.hr/) to reduce redundancy and to visualize results. While there is no significant enrichment for up-regulated genes, for down-regulated genes, 8 GO terms were significantly enriched, which were then classified into 3 of superclusters based on their relatedness by REVIGO, including “hormone metabolism” and “response to endogenous stimulus” (Figure 3A). The previous *p35S::SUP* study [24] as well as our reporter assays suggest that SUP may function in the auxin signaling pathway. Thus, the supercluster of “hormone metabolism”, which contains 3 hormone-related GO biological processes: hormone metabolism, P_{FDR}=0.00039; regulation of hormone level, P_{FDR}=0.0011; auxin synthesis, P_{FDR}=0.0042 (Figure 3A), was further inspected. There are total 17 genes in the category of “hormone metabolism”. Among these, 12 genes belong to “regulation of hormone level”, and interestingly, 8 of these 12 genes are listed under “auxin synthesis” as well (Table S1). Among these 8 genes, three participated in auxin biosynthesis: *YUC* flavin monooxygenases *YUC1/4* and a TRP-a-transferase *TRYPTOPHAN AMINOTRANSFERASE RELATED 2* (*TAR2*).
We have updated the Material and Methods, Figure 3A and Table S1, and Results section to reflect the new analysis. The JXB paper by Nibau et al. is also cited in this context, as requested.

3. *The regulation of target genes by recruitment of PcG is very interesting but it would be much more pertinent to provide data showing that it is a general mechanism of SUP action. The authors have all the genetic material and looking at histone methylation at the genome level is now largely accessible.*
Response: We agree that this would be useful (and in fact, we intend to do this in the future) but feel that this would go beyond the scope of the present manuscript where we decided to specifically focus on the interaction of SUP with the auxin biosynthetic pathway.

Other important concerns that needs to be addressed:
1. *"However, STM expression domain appears larger in sup than in the wild type (Figure S2)": this claim requires a quantification.*
Response: As requested, we have quantified the expression domain of *STM* at stage 5 and updated the data in the legend for Supplemental Figure S2A-B. We also added new images to Supplemental Figure S2 C-D to show that *STM* promoter activity is maintained beyond stage 10 in *sup*, consistent with the delayed termination of stem cell activity.

2. *P8 "The ectopic CUC2 expression in sup did not totally overlap with the SUP expression region, indicating the change of CUC2 expression is an indirect effect of the sup mutation. " : I do not understand this. If a protein acts non-cell autonomously, overlap in expression does not say anything on how direct the regulation is. The authors need to revise their reasoning.*
Response: As requested, we have modified the sentence to now read: "In *sup*, *CUC2* expression was also observed in the FM region (Figure S4B and D), in a domain where *SUP* is not normally expressed, suggesting that *CUC2* is not a direct target of SUP." We also added high-resolution images of *CUC2* to Supplemental Figure S4.

3. *P9 when using PCIB: the authors should use DR5 and DII-VENUS to analyze the effect of the treatment and understand how the rescue occurs*.
Response: We have analyzed *DR5, DII-VENUS* and *CUC2* (the latter in response to a request from reviewer 1; see above) reporter lines in *sup-5* and wild-type flowers after PCIB treatment. We noticed that *DR5* expression is markedly reduced in *sup-5* at 4-6 hours after the PCIB treatment, which is consistent with the observed rescue of *sup-5* by PCIB. In contrast, we did not detect clear differences for DII-VENUS perhaps because any effect on its activity would be indirectly caused by the PCIB treatment. We have provided the images of *DR5* (and *CUC2*) at the Supplemental Figure S6.

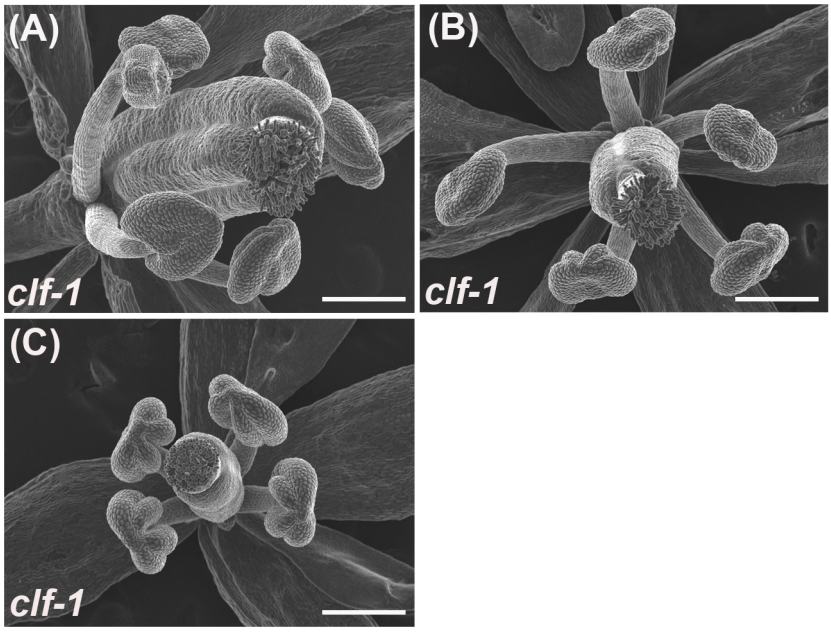
4. *Fig 2H-J and corresponding text P9: the control is said to be the untreated wild-type. A wild-type control treated with IAM is needed.*
Response: We agree, did the experiment and have added this control to Figure 2J.

5. *P10 when identifying the auxin-related targets: the authors discard PIN3/4 because their results are not fully consistent but transport regulation makes a lot of sense for obtaining a spatio-temporal specificity in an auxin-mediated regulation. The authors actually discuss it at the end of the discussion without even mentioning again that they identified the two putative targets. A better justification for not analyzing these two genes is required and they need to be mentioned in the discussion.*
Response: We fully agree with the reviewer that polar auxin transport may also be important for SUP's function in floral meristem regulation. We tested the effects that treatments with the polar auxin transport inhibitor N-1-naphthylphthalamic acid (NPA) have on *sup* mutant flowers and found that the application of NPA at stage 4-6 can partially rescue both the carpel morphology and stamen number defects (Supplemental Figure S13). This suggests that after the depression of *YUC1/4,* auxin transport is required to bring about morphological defects. As already described above (see our reply to Question 5 from Referee #1), we determined the expression pattern of two auxin efflux carrier-coding genes, *PIN1* and *PIN3*, in *sup-5* and wild-type flowers but did not detect any clear differences at the levels of reporter expression.

6. *P12: the authors compare YUC translational and translational to demonstrate a need for the coding region of the YUC genes. This experiment does not support their conclusion at all. The GUS activity from a translational fusion is regulated by post-transcriptional mechanisms. The authors*

need another approach to approach the problem (mutation of some of the sequences?) and this is an important part of the analysis.
Response: We agree with the reviewer and tested the transcription level of *GUS* in the GUS reporter lines by Q-PCR (Supplemental Figure S10F). We confirmed that the transcription level correlates with the GUS staining, indicating the coding region that is covered by H3K27me3 has the negative effect for transcription. We also carefully analyzed the reporter lines in the *sup* mutant background. SUP was reported to bind to DNA regions with the AGT core motif, by its single Cys_2–His_2 zinc finger domain, and two basic regions located on either side (Dathan N. et al., (2002) Nucleic Acids Res., **30**(22):p4945–4951). SUP binds the wider regions of *YUC1* and *YUC4* loci, and thus it was not possible to do mutagenesis-type approach for the binding sites as the high frequent presence of the AGT motif. Recent genome-wide analysis identified both CLF and TFL2 are essential for H3K27me3 mark spread (Wang et al., (2016) PLoS Genet, **12**(1): p. e1005771). We have included the argument in the discussion about the possibility whether SUP is also involved in the spreading of H3K27me3 mark at *YUC1/4*. We hope that these amendments are satisfactory.

7. *CLF and PcG proteins have been involved in regulation of the stem cell niche activity in the flower (direct regulation of AG). Mentioning this would add an interesting dimension to the discussion*.
Response: We fully agree with the reviewer and have added the discussion about the function of the interaction of SUP with CLF/TFL2.
The analysis of the genetic interaction between *sup-5* and *clf-1* is complicated by the multi-functionality of CLF. CLF has been shown to be involved in the repression of *AG* and *STM* (Schubert et al. (2006) *EMBO J*. **25**, 4638-49), the AG-dependent repression of *WUS* in floral meristems (Liu *et al.* (2011) *Plant Cell* **23**, 3654-70), as well as in other processes during plant development, including PIN-dependent establishment of auxin maxima (Gu *et al.* (2014) *Mol. Plant.* **7**, 977-88). Accordingly, we occasionally observed a reduced number of stamens, and an increased carpel number in *clf-1*, which could be associated with *WUS* activity (see images below).



More minor comments:
1- *Ref 22 needs to be completed*
Response: We have corrected this mistake.

2. *P7 when discussing non-cell autonomy: the authors seems to suggest that if the SUP protein was moving that would argue against non-cell autonomy. This needs to be rephrased.*
Response: We have modified the paragraph as requested.

3. *P10 "biotic and abiotic stress": how does this relate to what is shown on Fig 3A?*

Response: To address this point, we have re-analyzed the data from the transcriptomics experiments and modified Figure 3A and the Material and Methods section accordingly.

2nd Editorial Decision 12 March 2018

Thank you for submitting a revised version of your manuscript. The manuscript has now been seen by the three original referees, who find that their main concerns have been addressed and are now in favour of publication of the manuscript. There remain only a few minor mainly editorial issues that have to be addressed before I can extend formal acceptance of the manuscript.

1. Please implement the final textual clarifications requested by referee #3.

2nd Revision - authors' response 25 March 2018

Referee's comments:

Referee #3 (Remarks to the Author):

The revised manuscript has been streamlined and the authors have addressed adequately most of my concerns.
Q1: However the authors have overlooked one of my comments on the connection between stem cell regulation and stamen number regulation. There might be something I did not get but the manuscript often describes both cell autonomous and non-cell autonomous effects of SUP without clearly separating them. This blurs the message and could be easily addressed. The data in the manuscript (such as the one with DR5) clearly shows that extra stamens arise in the whorl 3/4 boundary, indicating that the regulation is cell autonomous while the one on the stem cells is clearly non cell-autonomous (the revised manuscript is much more convincing on this aspect). This is never really stated anywhere and is rather important when authors looks at complementation of the sup phenotype with auxin-producing transgenics or drugs. I think they should clarify this in the manuscript as it would help the reader to fully understand their findings.

Response: Thank you for your comments. Our work presents that SUP cell-autonomously represses auxin biosynthesis, leading to a non-cell autonomous effect in the floral meristem (FM) at the spatial and temporal aspects. Our data show that both cell-autonomous and non-cell-autonomous effects contribute to the increase in stamen number in *sup*:
(1) local derepression of auxin biosynthesis/class B gene expression (Prunet et al, 2017 PNAS) causes the formation of a few extra stamens at the boundary between whorls 3 and 4
(2) as these extra stamens emerge, they form a new boundary with the FM, but this boundary lacks SUP function, which causes the ectopic expression of YUCCAs and class B genes, and the formation of more extra stamens. It's an iterative phenotype.
(3) the increase in the number and prolonged maintenance of floral stem cells replenish the FM, and allow for the consecutive production of several whorls of extra stamens.
We have clarified the importance of both cell-autonomous and non-cell-autonomous effects in the results and discussion.

This clarification effort should include:

Q2- the introduction: the section on Page 4 that starts with "the number and position of floral organs ..." is really confusing and the link between boundary regions and WUS/CLV3 difficult to understand. The connection to the previous section on floral indeterminacy is also not clear and this participate to blurring the message.

Response: We thank the reviewer for the suggestions and comments. In the introduction, we have added one sentence "FM activity is associated with the number of floral organs" in the second section on floral indeterminacy, to make the second and third sections better connected. In the third

section, we reviewed the knowledge about the relationship between boundary gene and auxin, and auxin's function in meristem regulation. We have modified the third section to make the logic flow smoother.

Q3- The first section of results that starts with "To better understand the role of SUP in FM regulation, we first tested whether the formation of supernumerary stamens in sup mutants is associated with WUS function" but no conclusion is reached on this question. The authors should make sure that the logic they follow is clear in this section and probably in the following ones on that aspect.

Response: We have made the logic flow smoother. To make the conclusion clearer, we also have changed it to "*wus-1* is fully epistatic to *sup,* suggesting that the *sup* phenotype of supernumerary stamens is dependent on *WUS* function".

Q4- In the results clearly stating what part of the phenotype are related to cell autonomous and non-cell autonomous effects.

Response: Thank you. Please see the A1.

A more minor comment:

Q5- Fig 3A is difficult to read and the p-values should be added on the figure.

Response: We have enlarged the font size, changed the color of the words, and added the p-values in the Figure 3A. We thank the reviewer for all the suggestions and comments.

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PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Toshiro Ito
Journal Submitted to: EMBO J
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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 $\chi 2$ 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?	For statistical analysis (i.e. the student's t-test), enough sample size was ensured (at least 20 plants per experiment) with2-3 replicates each.
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?	Only experimental group and control group were included in the analysis. Any plant without these traits was excluded by genotyping.
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.	Random selection was not conducted. Plants in either experimental group or control group were included in the study. These two groups were distinct from each other; experimental group was compared with control group for phenotyping/expression.
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.	The plants were grown side by side to reduce any possible bias.
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?	The Student's t-test is appropriate to compare the means of two samples from different populations with different numbers of replicates. One way ANOVA test is used for auxin measurement with three repeats.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	NA

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Is there an estimate of variation within each group of data?	The Student's t-test makes no assumptions about the distribution of the population. Despite its small sample size, the t-test is considered robust when sampled independently from the two populations being compared (but large deviations from the assumptions may be possible).
Is the variance similar between the groups that are being statistically compared?	There is no variation within the group; variance between the groups is statistically compared using the tests described earlier.

C- Reagents

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).	anti-H3K4me3 (Millipore, #07473), anti-H3K27me3 (Millipore, #07449), anti-GFP (Life Technologies, #A11122), Anti-GFP (Santa Cruz Biotechnology, #SC-8334) and anti-HA (Santa Cruz Biotechnology, #SC-7392) antibodies were used.
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
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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	The microarray data are available at the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE92729.
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

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