Non-cell autonomous regulation of petal initiation in *Arabidopsis thaliana*

Seiji Takeda, Yuki Hamamura, Tomoaki Sakamoto, Seisuke Kimura, Mitsuhiro Aida and Tetsuya Higashiyama
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Original submission

First decision letter

MS ID#: DEVELOP/2022/200684

MS TITLE: Non-cell autonomous regulation of petal initiation in Arabidopsis thaliana

AUTHORS: Seiji Takeda, Yuki Hamamura, Tomoaki Sakamoto, Seisuke Kimura, Mitsuhiro Aida, and Tetsuya Higashiyama

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

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision. If it would be helpful, you are welcome to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating your plans for addressing the referee’s comments, and we will look over this and provide further guidance.

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

Reviewer 1

Advance summary and potential significance to field

The manuscript presents two new pieces of data: 1) PTL and RBE are expressed in adjacent and non-overlapping cells and 2) Ablation of PTL-expressing cells prevents the formation of petals. Neither of these results is surprising. In addition, the authors identify UFO as a potential target of PTL using a PTL:PTL-GR transgene but there are few followup experiments to further investigate this possibility. Overall, I find the results rather incremental and do not think they add significantly to our understanding of how PTL works to promote petal initiation.

Comments for the author

Problems/Limitations with the study The adjacent and non-overlapping expression patterns of PTL (intersepal zone/sepal boundary) and RBE (petal primordia) were expected since previous work has shown that PTL-expressing cells are adjacent to DR5:GFP expression at presumed petal initiation sites (Lampugnani, E.R. et al., 2012). Furthermore that ablation of PTL-expressing cells results in a ptl phenotype (fused sepals fewer petals) is also not surprising as several pieces of evidence suggest communication between intersepal cells and adjacent petal primordial cells are required for petal initiation.
One of the identified upregulated genes after induction of PTL activity is UFO.
The authors performed epistasis using a weak ufo allele and show that the ptl-1 ufo-6 double mutant resembles ptl-1. This experiment should really use a strong ufo allele. UFO functioning in the same pathway as PTL is not surprising as previous work has shown that RBE and UFO function in the same genetic pathway (Krizek, B.A. et al., 2006) and that PTL and RBE function in the same petal initiation pathway (Lampugnani, E.R. et al., 2013). The authors do not present much follow-up work investigating whether UFO is a target of PLT regulation.
They did not see upregulation of UFO after dex+cyc induction of PTL-GR suggesting that UFO may be an indirect target of PTL. Furthermore, they did not examine UFO expression in ptl mutants. If PTL positively regulates UFO expression, one would expect to see reduced UFO expression in ptl mutants.
The manuscript could use editing as some sentences are not so clear, such as line 58.

Reviewer 2

Advance summary and potential significance to field

This manuscript describes non-cell-autonomous regulation of petal initiation by Arabidopsis PETAL LOSS (PTL) transcription factor. PTL gene encoding a GT2-clade trihelix transcription activator is expressed in boundaries between sepal primordia but not in the petal initiation sites. Thus, the petal loss phenotype in ptl appears non-cell-autonomous, either through interference with a mobile petal-initiation signal or movement of the PTL protein. First, the authors successfully visualized the PTL protein and showed that PTL protein is not expressed in petal initiation sites marked by the RABBIT EARS protein. This clearly denies a possibility of PTL protein movement to petal initiation sites. Secondly, they also showed that genetic ablation of PTL-expressing cells resulted in reduced petals. Thirdly, they created an inducible PTL line and showed that PTL induction at stage 5 is sufficient to initiate petal primordia. This is an interesting observation since PTL is expressed in the sepal boundaries from stage 3 floral buds. By RNA-seq, UNUSUAL FLORAL ORGANS (UFO) was identified as an upregulated gene 3 hours after PTL induction in ptl. At last, they showed that ptl is epistatic to ufo. Since UFO was previously shown to be involved in RBE activation via AP3, their data nicely connects the PTL activity to petal gene RBE through their solid and sophisticated approaches.

Comments for the author

The introduction section is very well-written and was easy to follow. The results are logically laid out, and the overall conclusion is nicely supported by their data. I do, however, have some serious reservations that I hope the authors can address. Below are several points the authors may want to consider.

Major comments:
1) Although they propose that the dynamic expression pattern of UFO may enable non-cell-autonomous signaling, it is unclear whether PTL activates UFO non-cell autonomously or not. How is UFO expressed in ptl mutant, and where is UFO induced in pPTL:PTL-GR? In situ hybridization or reporter analysis of UFO in the pPTL:PTL-GR ptl in 3 hours after mock and DEX treatment should be performed.
2) Auxin pathway is previously suggested to play a critical role downstream PTL. Loss of PTL function disrupts DR5 expression in petal initiation zones, and further, a PTL promoter-driven auxin biosynthetic gene could complement the petal defects in ptl (Lampugnani et al., 2013). Thus, one intriguing hypothesis is that PTL regulates auxin signaling through UFO or other downstream targets. Is UFO a key downstream gene of PTL? Does the ectopic induction of UFO under a PTL promoter rescue the petal loss phenotype? If it does, how is the DR5 reporter expressed in that line? The authors should at least discuss their findings in terms of PTL-dependent auxin regulation.
Minor comments:
1) In line 42, “cells where PTL and RBE were translated did not overlap” should read “cells where PTL and RBE were localized did not overlap.” They examined not the translation sites but the protein localization.
2) In line 86, “the double mutants have sepalloid second whorl organs” should be “the double mutants have the reduced number of sepalloid second whorl organs.” Without the description of identity-independent organ number regulation, this sentence does not make sense.
3) In lines 93, 95, and others (including Table S3), “petal primordia” should read “petal initiation sites.” PTL and RBE are expressed from stage 3 when the petal primordia are invisible.

First revision

Author response to reviewers' comments

Reviewer 1 Comments for the author

Problems/Limitations with the study
The adjacent and non-overlapping expression patterns of PTL (intersepal zone/sepal boundary) and RBE (petal primordia) were expected since previous work has shown that PTL-expressing cells are adjacent to DR5:GFP expression at presumed petal initiation sites (Lampugnani, E.R. et al., 2012). Furthermore, that ablation of PTL-expressing cells results in a ptl phenotype (fused sepals, fewer petals) is also not surprising as several pieces of evidence suggest communication between intersepal cells and adjacent petal primordial cells are required for petal initiation.

[Response] As reviewer points out, it has been shown that PTL- and DR5-expressing cells are adjacent and not overlapped: our data showed the relation between speal boundary and petal initiation sites more directly. First, we used translational fusions for both PTL and RBE expression, so that the data showed the cells where these transcription factors act. We believe that it is worth to show this expression relation directly by using translational fusions, suggesting that PTL carries a non-cell autonomous function on petal initiation (Lampugnani et al 2012 and 2013 used PTL promoter-reporter line, not translational fusions). Second, DR5-expressing cells (namely cells where auxin response occurs) contains petal primordia cells but not limited to them, since auxin response is not limited to the petal primordia in the floral buds. RBE is expressed specifically in the petal initiation sites at stage 4-5, so our data show the spatio-temporal relation between inter-sepals and petal initiation sites.

One of the identified upregulated genes after induction of PTL activity is UFO. The authors performed epistasis using a weak ufo allele and show that the ptl-1 ufo-6 double mutant resembles ptl-1. This experiment should really use a strong ufo allele. UFO functioning in the same pathway as PTL is not surprising as previous work has shown that RBE and UFO function in the same genetic pathway (Krizek, B.A. et al., 2006) and that PTL and RBE function in the same petal initiation pathway (Lampugnani, E.R. et al., 2013). The authors do not present much follow-up work investigating whether UFO is a target of PLT regulation. They did not see upregulation of UFO after dex+cyc induction of PTL-GR suggesting that UFO may be an indirect target of PTL. Furthermore,

they did not examine UFO expression in ptl mutants. If PTL positively regulates UFO expression, one would expect to see reduced UFO expression in ptl mutants.

[Response] Strong ufo allele, such as ufo-2, shows the phenotype similar to the class B mutants such as ap3 or pi. The double mutant of ptl ap3 shows additive phenotype (Griffith et al 1999), so that we thought that the double mutant of ptl and strong ufo allele would show the same phenotype. This suggests that strong ufo allele masks its function in petal initiation. To see this point clearly, we used weak allele of ufo mutant, ufo-6. The relation between RBE-UFO and PTL-RBE were suggested previously as reviewer said, but we showed the direct relation by generating ptl ufo double mutant, which has not been shown before. We checked the UFO expression in mock-treated PTLg:GR, corresponding to ptl mutant, and found that UFO was expressed before stage 3 at cup-shaped domain but not petal primordia at stage 4 (we added this data to Fig. 4A-B). This supports that UFO is not expressed at petal initiation sites (and maybe inter-sepal cells) in ptl mutant.

The manuscript could use editing as some sentences are not so clear, such as line 58.

[Response] The sentence is corrected.

Reviewer 2 Comments for the author
The introduction section is very well-written and was easy to follow. The results are logically laid out, and the overall conclusion is nicely supported by their data. I do, however, have some serious reservations that I hope the authors can address. Below are several points the authors may want to consider.

Major comments:
1)Although they propose that the dynamic expression pattern of UFO may enable non-cell-autonomous signaling, it is unclear whether PTL activates UFO non-cell autonomously or not. How is UFO expressed in ptl mutant, and where is UFO induced in pPTL:PTL-GR? In situ hybridization or reporter analysis of UFO in the pPTL:PTL-GR ptl in 3 hours after mock and DEX treatment should be performed.

[Response] Thank you for this important suggestion. We checked the UFO expression pattern in DEX-treated PTLg:GR plants after 3 hours. In the mock plants, UFO was not expressed in inter-sepal or petal initiation sites, but it was in the DEX-treated plants. We added this data to Fig. 4. This results clearly show that UFO is locally upregulated within 3 hours by PTL.

2)Auxin pathway is previously suggested to play a critical role downstream PTL. Loss of PTL function disrupts DR5 expression in petal initiation zones, and further, a PTL promoter-driven auxin biosynthetic gene could complement the petal defects in ptl (Lampugnani et al., 2013). Thus, one intriguing hypothesis is that PTL regulates auxin signaling through UFO or other downstream targets. Is UFO a key downstream gene of PTL? Does the ectopic induction of UFO under a PTL promoter rescue the petal loss phenotype? If it does, how is the DR5 reporter expressed in that line? The authors should at least discuss their findings in terms of PTL-dependent auxin regulation.

[Response] We agree that auxin is a critical factor on petal initiation, and PTL regulates this pathway. From our RNA-seq results, however, there were no auxin-related genes other than IAA29, which is downregulated by PTL activation. We checked the IAA29 expression by RT-qPCR, but it was not significant difference. Therefore, we consider that auxin pathway is later than early response to PTL action. We added the IAA29 RT-qPCR data to Supplemental data (Fig. S3) and discussion part. The rescue experiment of UFO by PTL promoter is quite interesting and important, we agree, but this needs several months to examine, so we think this is our future work. Since some alleles of ufo (ufo-11 and ufo-12) lack petals (Durfee et al 2003), and together with our data, we suggest that UFO is a major player for petal initiation under the PTL regulation.

Minor comments:
1)In line 42, “cells where PTL and RBE were translated did not overlap” should read “cells where PTL and RBE were localized did not overlap.” They examined not the translation sites but the protein localization.

[Response] Thank you for this comment, we corrected the word to ‘localized’.

2)In line 86, “the double mutants have sepalloid second whorl organs” should be “the double mutants have the reduced number of sepalloid second whorl organs.” Without the description of identity-independent organ number regulation, this sentence does not make sense.

[Response] Corrected.

3)In lines 93, 95, and others (including Table S3), “petal primordia” should read “petal initiation sites.” PTL and RBE are expressed from stage 3 when the petal primordia are invisible.

[Response] We appreciate this important comment, and corrected through the manuscript.

Second decision letter

MS ID#: DEVELOP/2022/200684

MS TITLE: Non-cell autonomous regulation of petal initiation in Arabidopsis thaliana

AUTHORS: Seiji Takeda, Yuki Hamamura, Tomoaki Sakamoto, Seisuke Kimura, Mitsuhiro Aida, and Tetsuya Higashiyama

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

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision. If it would be helpful, you are welcome to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating your plans for addressing the referee’s comments, and we will look over this and provide further guidance.

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

Reviewer 1

Advance summary and potential significance to field

The manuscript presents the following advances: 1) PTL and RBE proteins are present in adjacent and non-overlapping cells, the inter-sepal zone and petal initiation cells, respectively; 2) Ablation of PTL-expressing cells prevents the formation of petals, indicating that the intersepal zone cells are require for petal initiation; and 3) UFO appears to act downstream of PLT in petal initiation. Even with the finding that PTL is required for UFO expression in petal initiation sites in stage 4 flowers, I find the results in the manuscript incremental. UFO was already known to play a role in petal initiation and whether it is a direct or indirect target of PLT regulation remains unclear.

Comments for the author

The authors have added one additional piece of evidence supporting their hypothesis that PTL acts upstream of UFO to promote petal initiation. They now present in situ hybridization showing that UFO expression in petal initiation sites of stage 4 flowers is missing in mock treated pPTL:PTL-GR ptl-1 but restored in dex-treated pPTL:PTL-GR ptl-1. The authors previously reported that UFO expression was not upregulated in pPTL:PTL-GR ptl treated with dex and chx.
This may be because whole inflorescences were used in the RT-qPCR. Why not also perform in situ hybridization on the chx and chx+dex tissue as they did for mock and dex? The addition of RT-qPCR of IAA29(Fig S3) does not add anything to the manuscript.
It is unclear to me how PTL expressed in the inter-sepal zone activates UFO expression in the petal initiation sites in stage 4 flowers. Perhaps the regulation is not direct; in this case we are still missing the factor that moves between the sepal boundary and the adjacent petal initiation sites. The authors explanation in the discussion (lines 330-339) on this point is not clear to me.
I have concerns with the ptl ufo genetic data. The choice of the ufo-6 allele is still unclear; there are four ufo alleles (ufo-11, ufo-12, ufo-13, and ufo-14) which show a specific defect in petal initiation. These four ufo partial loss of function alleles produce less than one petal per flower (earlier-arising flowers can have more than one while later-producing flowers produce few petals). These alleles seem like a better choice to investigate the combined loss of PTL and UFO on petal initiation compared with the ufo-6 which makes 3.5 petals/flower.
The ufo-11 allele was used in the rbe ufo double mutant referenced on line 299. Furthermore, I think there should be a careful analysis of ufo-11 ptl double mutants by performing floral organ counts (flowers 1-20) and counting the instances of sepal fusion.
No wild type plants are shown for comparison in FIgure 2.
What are the error bars in Fig S3?
Table S3: In stage 4, I think UFO is only expressed in petal initiation site; also in Lee 1997, it doesn't look like UFO is expressed in sepals in stage 3 flowers The manuscript needs extensive editing. Many of the sentences are awkward. Lots of "the" or "a" are missing. "Alternative" should be replaced with "alternate" throughout the manuscript. For example, in the Abstract, change the second sentence to "In many flowering plants, petals initiate in the second whorl in alternate positions with the first whorl sepals, suggesting possible signaling between the sepal boundaries and petal initiation sites". In line 40 of the Abstract, you say "Here we show the molecular link between PTL and RBE during petal initiation". Thus, the reader expects the next sentence to describe this molecular link but instead you talk about the localization of PTL and RBE proteins. The protein localization sentence should be moved ahead of the "molecular link" sentence since you had just described the location of the transcripts and it would make sentence to then present the protein localization.
This protein localization sentence is also very awkward. In line 42, you don't really mean genetic ablation of inter-sepal cells by the PTL promoter; perhaps you could say "Genetic ablation of inter-sepal cells by expression of DT-A under the control of the PTL promoter...". This kind of unclear writing is present throughout the manuscript (additional examples below; this is not a complete list).

line 50: typo; UNUSUAL line 56: do you mean to say that flower morphology can be important for attracting pollinators; this sentence needs re-writing line 62: "Different organ identities in each whorl of the flower are specified by distinct protein complexes of floral homeotic proteins."
line 66: I am not sure this is generally true.
lines 67-69: The abaxial sepal arises first followed by the adaxial sepal and the lateral sepals.
lines 70-72: awkward line 88: "Several pieces of evidence"
lines 98-99: "We used the glucocorticoid induction system to enable temporal activation..."
line 112: "or a gift from another laboratory"
line 116: "PTL constructs"; line 199 should be "construct" not construction line 151: "Microscopy"
line 153: "glass slides"
line 160: The PTLg:GR construct was transformed into the ptl-1 mutant". This nomenclature is kind of confusing, why not PLT:PLT-GR plt-1?
line 161: glufosinate ammonium is not an antibiotic lines 171-174: confusing, do you mean that you selected genes that fulfilled all three criteria?
line 195: "lacks petals or has deformed petals" "suggesting that PTL regulates"
line 203: "correspond to" rather than "contain"

line 209: "plants to fluorescent line expressed in boundary domains"
line 243: delete "with"
line 245: delete "own"

Reviewer 2

Advance summary and potential significance to field

This manuscript describes non-cell-autonomous regulation of petal initiation by Arabidopsis PETAL LOSS (PTL) transcription factor.

Comments for the author

I requested to clarify whether PTL activates UFO non-cell autonomously or not. By performing in situ hybridization of UFO in the pPTL:PTL-GR ptl, they claimed “UFO is rapidly induced at four clusters including sepal boundaries and petal initiation sites upon PTL induction (Fig. 4)”. However, whether arrowheads point to sepal boundaries or petal initiation sites is unclear. Please mark them separately in more explicit and magnified images. It is important to distinguish them clearly and make a clearer statement on spatial regulation of UFO by PTL. On the other points, they nicely revised the manuscript.

Second revision

Author response to reviewers' comments

Point-by-point responses to reviewers

Reviewer 1 Comments for the author
The authors have added one additional piece of evidence supporting their hypothesis that PTL acts upstream of UFO to promote petal initiation. They now resent in situ hybridization showing that UFO expression in petal initiation sites of stage 4 flowers is missing in mock treated pPTL:PTL-GR ptl-1 but rstored in dex-treated pPTL:PTL-GR ptl-1. The authors previously reported that UFO expression was not upregulated in pPTL:PTL-GR ptl treated with dex and chx. This may be because whole inflorescences were used in the RT-qPCR. Why not also perform in situ hybridization on the chx and chx+dex tissue as they did for mock and dex? The addition of RT-qPCR of IAA29(Fig S3) does not add anything to the manuscript.

[Response] Thank you for this comment. It may be true that RT-qPCR with whole inflorescence may make it hard to detect the UFO up-regulation in CHX-DEX plants. However, with the same experimental condition with whole inflorescence with DEX plants, the UFO expression was detected, so if UFO were upregulated in CHX-DEX plants, it should have been detected. Therefore, we concluded that UFO is not upregulated in CHX-DEX plants in 3 hours.
According to the first revision, the reviewer 2 suggested to include the discussion of auxin. We added this in the revised version (Discussion part), and mentioned to IAA29 which appeared in the down-regulated gene list of PTL induction. Unfortunately, this expression was not significant by RT-qPCR analysis (we added this to Fig. S3), so this data supported our hypothesis that auxin is not involved in early response to PTL action.

It is unclear to me how PTL expressed in the inter-sepal zone activates UFO expression in the petal initiation sites in stage 4 flowers. Perhaps the regulation is not direct; in this case we are still missing the factor that moves between the sepal boundary and the adjacent petal initiation sites. The authors explanation in the discussion (lines 330-339) on this point is not clear to me.

[Response] Thank you for this critical comment. Whether four clusters where UFO is expressed in stage 4 include inter-sepal cells, or just petal presumptive cells, is still unclear. As reviewer

suggests, it is possible that PTL regulates UFO indirectly, since our CHX-DEX experiment did not detect UFO expression. We modified this part in the Discussion.

I have concerns with the ptl ufo genetic data. The choice of the ufo-6 allele is still unclear; there are four ufo alleles (ufo-11, ufo-12, ufo-13, and ufo-14) which show a specific defect in petal initiation. These four ufo partial loss of function alleles produce less than one petal per flower (earlier-arising flowers can have more than one while later-producing flowers produce few petals). These alleles seem like a better choice to investigate the combined loss of PTL and UFO on petal initiation compared with the ufo-6 which makes 3.5 petals/flower. The ufo-11 allele was used in the rbe ufo double mutant referenced on line 299. Furthermore, I think there should be a careful analysis of ufo-11 ptl double mutants by performing floral organ counts (flowers 1-20) and counting the instances of sepal fusion.

[Response] As the reviewer suggests, some ufo alleles lack petals. We predicted that double mutant with two petal-lacking mutants (ufo-allele and ptl) would result in petal-lacking flowers, and in this case we could not conclude the genetic relation between PTL and UFO. To see this clearly, we dared to selected the weak ufo-6 allele which remained petals. In ufo-6, PTL activity remains so that it produces petals, and this effect is gone in ptl-1 ufo-6 double mutant. Conversely, in ptl-1, UFO activity is lost since there is no petals: so that ptl-1 ufo-6 double mutant resembles ptl-1 single mutant. This is consistent with our hypothesis that PTL regulates UFO, shown by RNA-sequencing, RT-qPCR and in situ hybridization results.

No wild type plants are shown for comparison in Figure 2.

[Response] Figure 2 show the phenotype of PTL overexpression, and the phenotype of wild-type is trivial, so that we only show the transformants phenotype.

What are the error bars in Fig S3?

[Response] Thank you for this point. The error bars indicate the SD. We added this to the legend. Also we noticed that this information lacked in Fig. 3, so we added there too.

Table S3: In stage 4, I think UFO is only expressed in petal initiation site; also in Lee 1997, it doesn't look like UFO is expressed in sepals in stage 3 flowers

[Response] Thank you for this point. We changed the UFO expression as: cone-shaped domain at stage 3, and four clusters at stage 4.

The manuscript needs extensive editing. Many of the sentences are awkward. Lots of "the" or "a" are missing. "Alternative" should be replaced with "alternate" throughout the manuscript. For example, in the Abstract, change the second sentence to "In many flowering plants, petals initiate in the second whorl in alternate positions with the first whorl sepals, suggesting possible signaling between the sepal boundaries and petal initiation sites".

[Response] Thank you for your detail revision of the manuscript. We checked through the manuscript by English Editing Service and corrected whole manuscript. ‘Alternative’ words were replaced by ‘Alternate’. The abstract sentence was corrected as suggested.

In line 40 of the Abstract, you say "Here we show the molecular link between PTL and RBE during petal initiation". Thus, the reader expects the next sentence to describe this molecular link but instead you talk about the localization of PTL and RBE proteins. The protein localization sentence should be moved ahead of the "molecular link" sentence since you had just described the location of the transcripts and it would make sentence to then present the protein localization. This protein localization sentence is also very awkward.

[Response] We removed the sentence, and modified the Abstract.

In line 42, you don't really mean genetic ablation of inter-sepal cells by the PTL promoter; perhaps you could say "Genetic ablation of inter-sepal cells by expression of DT-A under the control of the

PTL promoter...". This kind of unclear writing is present throughout the manuscript (additional examples below; this is not a complete
list).

[Response] We changed the sentence as suggested.

line 50: typo; UNUSUAL

[Response] Corrected.

line 56: do you mean to say that flower morphology can be important for attracting pollinators; this sentence needs re-writing

[Response] Corrected.

line 62: "Different organ identities in each whorl of the flower are specified by distinct protein complexes of floral homeotic proteins."

[Response] Corrected.

line 66: I am not sure this is generally true.

[Response] It is true that ‘ABCDE model’ is not generally accepted. We changed here to ‘ABCE model’ which have appeared in many manuscripts.

lines 67-69: The abaxial sepal arises first followed by the adaxial sepal and the lateral sepals.

[Response] We removed ‘first’ from this sentence.

lines 70-72: awkward

[Response] Sentence changed.

line 88: "Several pieces of evidence"

[Response] Corrected.

lines 98-99: "We used the glucocorticoid induction system to enable temporal activation..."

[Response] Corrected.

line 112: "or a gift from another laboratory"

[Response] Corrected.

line 116: "PTL constructs"; line 199 should be "construct" not construction

[Response] Corrected.

line 151: "Microscopy"

[Response] Corrected.

line 153: "glass slides"

[Response] Corrected.

line 160: The PTLg:GR construct was transformed into the ptl-1 mutant". This nomenclature is kind of confusing, why not PLT:PLT-GR plt-1?

[Response] To make the construct name shorter, we defined their name on above section.

line 161: glufosinate ammonium is not an antibiotic

[Response] ‘antibiotics’ deleted.

lines 171-174: confusing, do you mean that you selected genes that fulfilled all three criteria?

[Response] Yes. We modified the sentence.

line 195: "lacks petals or has deformed petals" "suggesting that PTL regulates"

[Response] Corrected.

line 203: "correspond to" rather than "contain"

[Response] We would like to remain ‘contain’, since here we want to examine whether PTL-expressing cells are restricted to inter-sepals cells or contain some of the petal primordia cells. We modified the sentence.

line 209: "plants to fluorescent line expressed in boundary domains"

[Response] Corrected.

line 243: delete "with"

[Response] Deleted.

line 245: delete "own"

[Response] Deleted.

Reviewer 2 Advance summary and potential significance to field
This manuscript describes non-cell-autonomous regulation of petal initiation by Arabidopsis PETAL LOSS (PTL) transcription factor.

Reviewer 2 Comments for the author
I requested to clarify whether PTL activates UFO non-cell autonomously or not. By performing in situ hybridization of UFO in the pPTL:PTL-GR ptl, they claimed “UFO is rapidly induced at four clusters including sepal boundaries and petal initiation sites upon PTL induction (Fig. 4)”. However, whether arrowheads point to sepal boundaries or petal initiation sites is unclear. Please mark them separately in more explicit and magnified images. It is important to distinguish them clearly and make a clearer statement on spatial regulation of UFO by PTL. On the other points, they nicely revised the manuscript.

[Response] Thank you for this point. We considered the UFO expression pattern part after the revision, and now we realize that It is difficult to separate the inter-sepal cells and petal presumptive cells at stage 4. Therefore, we show ‘four clusters at stage 4’ with arrows and ‘petal primordia at stage 5’ with arrowheads in Figure 4.

Third decision letter

MS ID#: DEVELOP/2022/200684

MS TITLE: Non-cell autonomous regulation of petal initiation in Arabidopsis thaliana

AUTHORS: Seiji Takeda, Yuki Hamamura, Tomoaki Sakamoto, Seisuke Kimura, Mitsuhiro Aida, and Tetsuya Higashiyama
ARTICLE TYPE: Research Article

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

Reviewer 2

Advance summary and potential significance to field

This manuscript describes the non-cell-autonomous regulation of petal initiation by Arabidopsis PETAL LOSS (PTL) transcription factor.

Comments for the author

I have no more comments.