Hippo signaling promotes Ets21c-dependent apical cell extrusion in the *Drosophila* wing disc

Xianlong Ai, Dan Wang, Junzhen Zhang and Jie Shen
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Original submission

First decision letter

MS ID#: DEVELOP/2020/190124

MS TITLE: Hippo signaling promotes Ets21c-dependent apical cell extrusion in the Drosophila wing disc

AUTHORS: Xianlong Ai, Dan Wang, Junzhen Zhang, and Jie Shen

I apologise for the long time before being able to come back to you. 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.

The overall evaluation is rather positive and we would like to publish a revised manuscript in Development, provided that the referees' comments can be satisfactorily addressed. I draw your attention to the need to provide robust quantification throughout and proper statistics as this is something I shall be very attentive to and essential for further consideration. Please attend to all of the reviewers' comments in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so.

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

Reviewer 1

Advance summary and potential significance to field

In this manuscript, Xialong Ai and colleagues describe a new process of Yki depleted clones elimination based on apical and basal cell extrusion, independent of caspase activation. By combining genetic perturbations with RNAseq, they identify independent mechanisms that can regulate either basal cell extrusion (specifically inhibited by Bantam expression), and apical extrusion (induced by Ets21c, a transcription factor upregulated by JNK signaling). Both extrusion processes are promoted by JNK signaling, which is activated downstream of Hippo pathway activation.
There are several interesting findings in this manuscript. First, it suggests that hippo activation (or yki depletion) can lead to clone elimination independently of apoptosis (while so far, it was thought to be triggered by Diap1 depletion). This is also to my knowledge one of the first case where there is a clear distinction between apical and basal cell extrusion including the identification of pathways that can block specifically one or the other (Bantam Vs Ets21c).

Yet, the novelty is a bit dampened by the numerous studies already showing the role of JNK in clone elimination in different genetic backgrounds (mostly Scribble clone elimination, work of Igaki lab). Accordingly, a previous study from Igaki lab characterized the effect of Slit and Robo on Scribble mutant clones elimination downstream of JNK and independently of caspases (Vaughem and Igaki 2016). Indeed, they already showed that JNK activation promotes clone extrusion (both apically and basally) mostly through Slit Robo upregulation. Similarly, it was already shown that JNK promotes apical extrusion of M6 deficient RasV12 clones (Dunn et al., 2018). Moreover, contrary to the studies mentioned above, this manuscript does not bring any understanding of the cellular effectors driving ACE or BCE downstream of Bantam and/or Ets21c. So my fear is that the novelty provided by this manuscript is at this stage rather incremental.

Comments for the author

1. Most of the data appears quite qualitative. There is a quantification of the proportion of ACE and BCE provided in Fig. S3 but there is no statistical analysis of these proportions. Also, there is no description in the methods of the criteria used to define ACE and BCE. Indeed, it seems to me that this can be sometimes quite ambiguous. For instance, this is not obvious to me that the two examples shown in Fig. 1 B and D (arrows) are indeed apically extruded cells (the one in D might still be in the epithelial layer). Once again, a better definition of the criteria used for the quantification would be very useful.

2. Similarly, when the authors mention the scarcity of yki RNAi clones and UAS-Ets21c clones (Fig. S1 and Fig. 5A), they only provide one picture of wing disc without quantification. They should provide at least the average number of clone per disc for these genetic backgrounds and associated controls for a proper number of wing discs (otherwise it will remain anecdotal).

3. It would be interesting to compare the results of this study with the role of Hippo/Yki in cell competition in the intro or the discussion (Tyler et al, Genetics 2007, Ziosi M et al, plos genetics 2001). In these examples, this is rather in conditions of Yki hyperactivation in clones. But this suggest that competition could also promote Yki depleted clone elimination. The process described in this study is probably different as it can be triggered cell autonomously, even when Yki is depleted in the full wing pouch.

4. Other points (typos, references and statements)

- Page 2, second paragraph : “yki mutant clones rare and invaded into the lumen (Fig.1B, arrow).” I guess it should be "mutant clones were rare".

- Page 10 second paragraph: PIEZIO (should be PIEZO). Also the reference is probably not correct (Coste et al. 2010). The role of Piezo in crowding-induced extrusion and the contribution of S1P was shown in Eisenhoffer et al 2012

- Page 10 end of second paragraph. To my knowledge, the only cases of spontaneous apical extrusion in flies are in the adult midgut (e.g. Martin JL et al., elife, 2018, O Brien lab). Maybe the authors should mention that when referring to “normal conditions” (which is not really the case for the conditions studied in this manuscript).

- Page 11: “In normal cells, slit, robo2, and ena-overexpression only results in BCE when cell death isblocked (Vaughen and Igaki, 2016). Therefore Slit-Robo2-Ena do not function as downstream targets of the Hpo pathway to regulate ACE.”

I believe this might be an overstatement. It is true that Slit Robo overexpression in WT conditions rather lead to BCE. But it does not exclude that Slit Robo could promote apical extrusion in the Yki RNAi condition. Indeed, Slit Robo overexpression massively promote apical extrusion of Scribble mutant clones (Vaughen and Igaki 2016). So at this stage, nothing exclude a contribution of Slit Robo in the ACE of active Hpo clones. To prove that, the authors would need to test if Slit Robo are differentially expressed upon Hpo activation and show that Slit Robo downregulation does not prevent ACE in Yki RNAI/active Hpo clones.

- Page 12, second paragraph: “Meanwhile, in normal cells, Mmp1 upregulation when Robo2 and Ena are overexpressed only induces BCE (Vaughen and Igaki, 2016).”
I am not sure that this statement is correct. To my knowledge Vaughen and colleagues didn’t use any conditions upregulating MMP1 (they used MMP1 staining as a readout of JNK activation). They did show though that inhibition of caspases (UAS-p35) reveals a lot of BCE in clones overexpressing Robo2 and Ena.
So I guess the author should replace Mmp1 by p35 in this sentence.

- Fig 5B: the green label cannot be read.

- Fig 5: How did the authors make sure that the GFP levels of ets21c are comparable between WT and yki depleted discs ? (fixed in parallel ?). It might be good to give more details in the Methods

- Fig S7: The absence of cleaved C3 staining is not a strong proof of absence of apoptosis (this antibody has very variable sensitivity). Although this result is in good agreement with the capacity of JNK to trigger ACE despite inhibition of caspases, I would remain cautious on its interpretation (to make a strong statement, the authors should trigger clones overexpressing both Ets21c and p35 and quantifiy ACE).

Reviewer 2

Advance summary and potential significance to field

The manuscript by Shen and colleagues addresses the mechanisms by which cells extrude from Drosophila wing imaginal discs. The authors show that activation of Hippo signaling (in knockdowns of yki) results in Diap1 and JNK-dependent cell extrusion both from the apical and basal sides of the epithelium. Basal cell extrusion further requires the known Yki-target gene bantam. Apical cell extrusion is mediated by the transcription factor Ets21c downstream of JNK.

The manuscript provides interesting and novel insights into the mechanisms by which Hippo signaling affects epithelial integrity. The data is of high quality and well presented.

Comments for the author

Specific comments:
- Fig. 1F, S2. Yki mutant clones are poorly recovered from the wing disc pouch, but seem to be recovered (to some extent) in the hinge (see Fig. S1B). Yet, apical extrusion of Yki depleted cells is mainly observed in the hinge (but not pouch). What is then the role/significance of apical cell extrusion for the low recovery rate of Yki depleted cells? The authors should discuss this.

Fig. 2, S3. The authors claim that bantam expression, but not expression of Diap1, dMyc or CycE, partially suppresses basal cell extrusion in a yki knockdowns. Comparing the images in Fig. 2B, 2D, 2F, 2H, this difference between bantam and the other three genes is not so obvious. The authors should explain in the Materials and Methods how they score wing discs positive for basal cell extrusion.

-Fig. 3D: The authors should state in the legend the number of technical/biological replicates and whether error bars refer to sd or sem. Also, statistical testing to reveal significances should be provided.

-Fig. 5F,S7: The authors claim that expression of Ets21c induces apical cell extrusion. I can see only very few nuclei (marked by the arrows) that may indicate cells undergoing apical extrusion. This seems to be in contrast to the activation of JNK signaling which leads to more widespread apical cell extrusion. The authors may want to dampen their statement on page 9 that “Ets21c is a key mediator of Hpo-Yki-JNK-dependent ACE”.

-The manuscript would benefit from an additional main figure providing a model or summary of the findings.

Reviewer 3

Advance summary and potential significance to field

Cell extrusion is a biological phenomenon essential for epithelial cell turnover by providing a mechanism to eliminate cells in a spatiotemporally controlled manner. Hippo signalling and its target transcriptional effector Yorkie/YAP have merged as key regulators of tissue biology (proliferation, migration, cell death, healing, etc.) in response to mechanical stimuli.
In this manuscript, Ai et al combine transgenesis, fluorescence imaging, reporter constructs, and RNAseq to find that Hippo signalling promotes basal and apical extrusion in Drosophila wing disc epithelia by activating JNK signalling. In addition, they identify Ets21c as a transcription factor in charge of a putative transcriptional programme required for apical extrusion and ban as a microRNA controlling basal extrusion.
In general, the article is clearly written, and the ideas presented in a comprehensive way, making the logic of the sequence of experiments easy to follow. Figures are clear, understandable, and prove the points made by the authors. The identification of a transcription factor required for apical extrusion suggests a path to future identification of extrusion-related genes. The manuscript outlines important molecular players controlling fundamental processes, apical and basal cell extrusion. It will be interesting if these are also conserved in different species.

Comments for the author

Major comments
- Lack of image quantification and statistical analysis
- The discussion could be tightened up and led by a schematic.

Minor comments
- Introduction, paragraph 1, line 3: coactivator should be substituted by coregulator. Yorkie’s ortholog YAP has repressor functions in given contexts: 10.1016/j.celrep.2015.03.015
- Introduction, paragraph 1, line 13: Yap should be substituted by YAP, as in line 13. Additionally, explaining its relation to Yorkie would be informative for the non-expert reader.
- Introduction, paragraph 3, line 1: the lonely reference Gudipaty and Rosenblatt, 2017 can be merged with the following block of references in line 3.
- Would the authors be open to replacing the red-green combination for a magenta-green one? This change would facilitate figure appreciation and analysis by us, the colour-blind scientific cohort.
- Fig.1A, third picture from the left, Bacal should read Basal.
- RT-PCR should read RT-qPCR to include the term quantitative, written by the authors.
- Discussion, paragraph 3: line 5. PIEZIO should read PIEZO1. Coste’s reference does not work to S1P-S1P2 pathway in crowding ACE. Maybe meant Eisenhoffer et al., 2012. Also, Slattum and

Jody, 2014 should be corrected to Slattum and Rosenblatt, 2014. Or maybe even better, substituted by the original works showing the relationship between oncogenes and extrusion directionality. In general, this and the following paragraph could be rewritten for clarity: the S1P-S1P2 pathway regulates both apoptosis-induced and apoptosis-independent ACE. A single sentence would be clearer than the current phrasing.

First revision

Author response to reviewers' comments

Referee1
1.Most of the data appears quite qualitative. There is a quantification of the proportion of ACE and BCE provided in Fig. S3 but there is no statistical analysis of these proportions. Also, there is no description in the methods of the criteria used to define ACE and BCE. Indeed, it seems to me that this can be sometimes quite ambiguous. For instance, this is not obvious to me that the two examples shown in Fig. 1 B and D (arrows) are indeed apically extruded cells (the one in D might still be in the epithelial layer). Once again, a better definition of the criteria used for the quantification would be very useful.

As suggested, we added statistical analysis in Fig. S3 and the criteria to define ACE and BCE in the method (See L390-396, L420-430). Now the cell extrusion should be obvious.

2.Similarly, when the authors mention the scarcity of yki RNAi clones and UAS-Ets21c clones (Fig. S1 and Fig. 5A), they only provide one picture of wing disc without quantification. They should provide at least the average number of clone per disc for these genetic backgrounds and associated controls for a proper number of wing discs (otherwise it will remain anecdotal).

We calculated the clone number and analyzed the data in Fig. S1 (See Fig. S1 and L420-430).

3.It would be interesting to compare the results of this study with the role of Hippo/Yki in cell competition in the intro or the discussion (Tyler et al, Genetics 2007, Ziosi M et al, plos genetics 2001). In these examples, this is rather in conditions of Yki hyperactivation in clones. But this suggest that competition could also promote Yki depleted clone elimination. The process described in this study is probably different as it can be triggered cell autonomously, even when Yki is depleted in the full wing pouch.

We compared their research with our results in the discussion. (See L242-249)

4.Other points (typos, references and statements)

- Page 2, second paragraph : “yki mutant clones rare and invaded into the lumen (Fig.1B, arrow).” I guess it should be "mutant clones were rare".

Corrected.

-Page 10 second paragraph: PIEZIO (should be PIEZO). Also the reference is probably not correct (Coste et al. 2010). The role of Piezo in crowding-induced extrusion and the contribution of S1P was shown in Eisenhoffer et al 2012

We rephrased the sentence. (See L257-260)

-Page 10 end of second paragraph. To my knowledge, the only cases of spontaneous apical extrusion in flies are in the adult midgut (e.g. Martin JL et al., elife, 2018, O Brien lab). Maybe the authors should mention that when referring to “normal conditions” (which is not really the case for the conditions studied in this manuscript).

We added the cases of spontaneous apical extrusion in Drosophila. (See L266-268)

-Page 11: “In normal cells, slit, robo2, and ena-overexpression only results in BCE when cell death is blocked (Vaughen and Igaki, 2016). Therefore, Slit-Robo2-Ena do not function as downstream targets of the Hpo pathway to regulate ACE.”
I believe this might be an overstatement. It is true that Slit Robo overexpression in WT conditions rather lead to BCE. But it does not exclude that Slit Robo could promote apical extrusion in the Yki RNAi condition. Indeed, Slit Robo overexpression massively promote apical extrusion of Scribble mutant clones (Vaughen and Igaki 2016). So at this stage, nothing exclude a contribution of Slit Robo in the ACE of active Hpo clones. To prove that, the authors would need to test if Slit Robo are differentially expressed upon Hpo activation and show that Slit Robo downregulation does not prevent ACE in Yki RNAI/active Hpo clones.

In our RNA-Seq results, slit, robo2, and ena were not changed in the Yki depleted Drosophila wing disc, which means Slit-Robo2-Ena does not associate with Hpo pathway to regulate ACE. We added the sentence. (See L272-274)

-Page 12, second paragraph: “Meanwhile, in normal cells, Mmp1 upregulation when Robo2 and Ena are overexpressed only induces BCE (Vaughen and Igaki, 2016).”
I am not sure that this statement is correct. To my knowledge Vaughen and colleagues didn’t use any conditions upregulating MMP1 (they used MMP1 staining as a readout of JNK activation). They did show though that inhibition of caspases (UAS-p35) reveals a lot of BCE in clones overexpressing Robo2 and Ena. So I guess the author should replace Mmp1 by p35 in this sentence.

Corrected. (See L316)

-Fig 5B: the green label cannot be read.

The label was modified.

-Fig 5: How did the authors make sure that the GFP levels of ets21c are comparable between WT and yki depleted discs ? (fixed in parallel ?). It might be good to give more details in the Methods

We added the details in the method. (See L387-389)

-Fig S7: The absence of cleaved C3 staining is not a strong proof of absence of apoptosis (this antibody has very variable sensitivity). Although this result is in good agreement with the capacity of JNK to trigger ACE despite inhibition of caspases, I would remain cautious on its interpretation (to make a strong statement, the authors should trigger clones overexpressing both Ets21c and p35 and quantifiy ACE).

Our lab has experience on cleaved C3 staining (Fan et al., 2020; Liu et al., 2016; Lu et al., 2017; Shen et al., 2010; Shen et al., 2008; Shen et al., 2014; Sun et al., 2020; Tsai et al., 2015). We rephrased the sentence to avoid over statement. (See L213-216)

Referee 2
Specific comments:
- Fig. 1F, S2. Yki mutant clones are poorly recovered from the wing disc pouch, but seem to be recovered (to some extent) in the hinge (see Fig. S1B). Yet, apical extrusion of Yki depleted cells is mainly observed in the hinge (but not pouch). What is then the role/significance of apical cell extrusion for the low recovery rate of Yki depleted cells? The authors should discuss this.

We are sure that the extrusion area was within the wing pouch. We have checked y-z sections for definition of Drosophila wing pouch (Butler et al., 2003; Johnstone et al., 2013; Widmann and Dahmann, 2009), most of the apical extrusion of Yki depleted cells occurred within the pouch and near the hinge, it greatly damaged the integrity of epithelium. So it seems like the location of apical cell extrusion was in the hinge, but it is not true. To better observe the location of apical extrusion of Yki depleted cells, we outlined wing pouch region in Fig. S2 B.

Fig. 2, S3. The authors claim that bantam expression, but not expression of Diap1, dMyc or CycE, partially suppresses basal cell extrusion in a yki knockdowns. Comparing the images in Fig. 2B, 2D, 2F, 2H, this difference between bantam and the other three genes is not so obvious. The authors should explain in the Materials and Methods how they score wing discs positive for basal cell extrusion.

We added the criteria to define ACE and BCE in the method (See L390-396).

-Fig. 3D: The authors should state in the legend the number of technical/biological replicates and whether error bars refer to sd or sem. Also, statistical testing to reveal significances should be provided.

We added the number of replicates and the error bar represents SD. Necessary statistical tests were provided. (See L690-693)

-Fig. 5F,S7: The authors claim that expression of Ets21c induces apical cell extrusion. I can see only very few nuclei (marked by the arrows) that may indicate cells undergoing apical extrusion. This seems to be in contrast to the activation of JNK signaling which leads to more widespread apical cell extrusion. The authors may want to dampen their statement on page 9 that “Ets21c is a key mediator of Hpo-Yki-JNK-dependent ACE”.

Ets21c indued ACE was repeatedly observed in our experiments. Fig. 5F showed three cells undergoing ACE, now were all indicated by arrows. We rephrased the conclusion to avoid over statement (See L216).

-The manuscript would benefit from an additional main figure providing a model or summary of the findings.

We provided a model as Fig. 6

Referee 3

Major comments
•Lack of image quantification and statistical analysis
The necessary quantification and statistical analysis were provided in Fig.S3.

•The discussion could be tightened up and led by a schematic.
We try to tighten up the discussion. A schematic model in Fig. 6 was added.

Minor comments
•Introduction, paragraph 1, line 3: coactivator should be substituted by coregulator. Yorkie’s ortholog YAP has repressor functions in given contexts: 10.1016/j.celrep.2015.03.015
We corrected the word and added the reference. (See L36-37)

•Introduction, paragraph 1, line 13: Yap should be substituted by YAP, as in line 13. Additionally, explaining its relation to Yorkie would be informative for the non-expert reader.
We corrected the word and explained its relation to Yorkie(See L44)

•Introduction, paragraph 3, line 1: the lonely reference Gudipaty and Rosenblatt, 2017 can be merged with the following block of references in line 3.
We merged the references. (See L63)

•Would the authors be open to replacing the red-green combination for a magenta-green one? This change would facilitate figure appreciation and analysis by us, the colour-blind scientific cohort.
We replaced the colour combination as suggested.

•Fig.1A, third picture from the left, Bacal should read Basal.
Corrected.

•RT-PCR should read RT-qPCR to include the term quantitative, written by the authors. Corrected.

•Discussion, paragraph 3: line 5. PIEZIO should read PIEZO1. Coste's reference does not work to S1P-S1P2 pathway in crowding ACE. Maybe meant Eisenhoffer et al., 2012. Also, Slattum and Jody, 2014 should be corrected to Slattum and Rosenblatt, 2014. Or maybe even better, substituted by the original works showing the relationship between oncogenes and extrusion directionality. In general, this and the following paragraph could be rewritten for clarity: the S1P-S1P2 pathway regulates both apoptosis-induced and apoptosis-independent ACE. A single sentence would be clearer than the current phrasing.

We corrected the references and substituted by the original works showing the relationship between oncogenes and extrusion directionality. Aslo, we rephrased the sentence. (See L257-263)

Second decision letter

MS ID#: DEVELOP/2020/190124

MS TITLE: Hippo signaling promotes Ets21c-dependent apical cell extrusion in the Drosophila wing disc

AUTHORS: Xianlong Ai, Dan Wang, Junzhen Zhang, and Jie Shen

I have now received all the referees reports on the above manuscript, and have reached a decision. The overall evaluation is very positive and we would like to publish your manuscript in Development. However, before we can formaly do so can you please attend to the few remaining minor comments.

Reviewer 1

Advance summary and potential significance to field

The authors have adressed most of my concerns and the manuscript has been improved by providing quantifications and further methods details.

Comments for the author

I only have few minor comments that will only require some text/figure modifications.

-Fig S1F,G : how many replicate were used for this analysis ?

-Fig S2 B and D: Could the authors provide the number of discs used for the C765 GFP control in the legend like the other genotypes ?

-Fig 6 (scheme) : The scheme is not totally correct as the authors have shown that JNK signaling promotes both apical and basal cell extrusion (necessary and sufficient for both). To fully represent the data, the scheme should also include the link between JNK and basal cell extrusion. Also the epistatic link between Ets21c and JNK is only based on the induction of Ets21c by JNK. Nothing exludes at this stage that JNK also promotes apical extrusion independently of Ets21c (to prove that, one would need to deplete Ets21C in hepCA wing pouch). I would suggest to correct the figure accordingly by adding a dashed line and an arrow connecting JNK and ACE directly.

-In the discussion (line 343-345), the authors relate the pro-tumoural role of Ets21c with its impact on apical cell extrusion. It might be worth mentioning that apical extrusion is rather associated

with the elimination of tumoural cells in mammals (as for the elimination of Ras activated cells through EDAC :
Epithelial Defense Against Cancer, see the articles from Yasu Fujita group), while aberrant basal extrusion is traditionally associated with higher invasive capacity. It is therefore difficult to infer a putative protumoral functions of Et21c in mammals through its effect on apical extrusion (where this would rather lead to tumour cell elimination in the lumen).

Reviewer 2

Advance summary and potential significance to field

As previously.

Comments for the author

The authors have satisfactorily addressed my comments.

I have one minor issue:

Line 390: The authors now describe the 'cell extrusion criteria'. However, the wording is in part confusing. "Normal DP cell nucleus […] never break through the apical membrane toward lumen". How can a nucleus break through the apical membrane [of a cell]? A phrase like "In controls, DP cell nuclei were observed in the middle/center of the cell layer, but never close to the basal membrane or within the overlying wing disc lumen" might be more appropriate. Similar for the next sentence (lines 394-396) and the legend to Fig. 6 (line 720).

Reviewer 3

Advance summary and potential significance to field

I am now fine with the changes made.

Comments for the author

The schematic helps the reader, although the discussion could still be tightened up further to walk the reader through the findings, especially in the first paragraph that many readers often read. Additionally, while it is good to provide the statistics now, they are typically accompanied with the data discussed in each figure. I would recommend putting in that order than all in one figure. Most people don't do it that for a reason--it is hard to read each figure. These changes will make the paper more readable, as most people skimming papers tend to read: The title, the abstract, and glance at figures and maybe read the first paragraph of the discussion. However, I will leave this up to you.

Second revision

Author response to reviewers' comments

Referee1
Reviewer 1 Advance summary and potential significance to field
The authors have adressed most of my concerns and the manuscript has been improved by providing quantifications and further methods details.

Reviewer 1 Comments for the author

-Fig S1F,G : how many replicate were used for this analysis ?

We added the number of replicates in the method. (See L434-435)

-Fig S2 B and D: Could the authors provide the number of discs used for the C765 GFP control in the legend like the other genotypes ?

We added the number in the legend of Fig. S3.

-Fig 6 (scheme) : The scheme is not totally correct as the authors have shown that JNK signaling promotes both apical and basal cell extrusion (necessary and sufficient for both). To fully represent the data, the scheme should also include the link between JNK and basal cell extrusion. Also the epistatic link between Ets21c and JNK is only based on the induction of Ets21c by JNK. Nothing exludes at this stage that JNK also promotes apical extrusion independently of Ets21c (to prove that, one would need to deplete Ets21C in hepCA wing pouch). I would suggest to correct the figure accordingly by adding a dashed line and an arrow connecting JNK and ACE directly.

Additional links between JNK and BCE/ACE were added according to your suggestion (new Figure 6).

-In the discussion (line 343-345), the authors relate the pro-tumoural role of Ets21c with its impact on apical cell extrusion. It might be worth mentioning that apical extrusion is rather associated with the elimination of tumoural cells in mammals (as for the elimination of Ras activated cells through EDAC : Epithelial Defense Against Cancer, see the articles from Yasu Fujita group), while aberrant basal extrusion is traditionally associated with higher invasive capacity. It is therefore difficult to infer a putative protumoral functions of Et21c in mammals through its effect on apical extrusion (where this would rather lead to tumour cell elimination in the lumen).

Accepted, we added such discussion according to your suggestion (See L352-358)

Reviewer 2 Advance summary and potential significance to field As previously.
Reviewer 2 Comments for the author
The authors have satisfactorily addressed my comments.

I have one minor issue:

Line 390: The authors now describe the ‘cell extrusion criteria’. However, the wording is in part confusing. “Normal DP cell nucleus […] never break through the apical membrane toward lumen”. How can a nucleus break through the apical membrane [of a cell]? A phrase like “In controls, DP cell nuclei were observed in the middle/center of the cell layer, but never close to the basal membrane or within the overlying wing disc lumen” might be more appropriate. Similar for the next sentence (lines 394-396) and the legend to Fig. 6 (line 720).

Accepted, we rephrased the description (L405-409, L736-737)

Reviewer 3 Advance summary and potential significance to field
I am now fine with the changes made.

Reviewer 3 Comments for the author
The schematic helps the reader, although the discussion could still be tightened up further to walk the reader through the findings, especially in the first paragraph that many readers often read. Additionally, while it is good to provide the statistics now, they are typically accompanied with the data discussed in each figure. I would recommend putting in that order than all in one figure. Most people don't do it that for a reason--it is hard to read each figure. These changes will make the paper more readable, as most people skimming papers tend to read: The title, the abstract, and glance at figures and maybe read the first paragraph of the discussion. However, I will leave this up to you.

We rephrased the first paragraph in discussion part (See L220-228). We put the statistics together for easy comparison and for the convenient figure composition.

Third decision letter

MS ID#: DEVELOP/2020/190124

MS TITLE: Hippo signaling promotes Ets21c-dependent apical cell extrusion in the Drosophila wing disc

AUTHORS: Xianlong Ai, Dan Wang, Junzhen Zhang, and Jie Shen
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.