Src42A is required for E-cadherin dynamics at cell junctions during *Drosophila* axis elongation

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Review timeline

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First revision received:	20 October 2022
Editorial decision:	28 November 2022
Second revision received:	2 December 2022
Accepted:	19 December 2022

Reviewer 1

Evidence, reproducibility and clarity

Highest priority:

1. The Src42A knockdown and germline clone experiments both cause defects in cellularization (Fig. 2B and 9A), which could result in differences in the state of the blastoderm epithelium (cell size, cell number, structural integrity, organization, etc.) between the experimental and control conditions. In addition, Src42A knockdown appears to affect the size and shape of the egg (Fig. 9A and 9C). The manuscript would be strengthened if the authors included data to demonstrate that the initial structure of the epithelium is mostly normal (quantifications of cell size, number, etc.) in the Src42A RNAi condition, as this would bolster the argument that germband extension, rather than due to indirect effects resulting from the cellularization defects. The authors may have relevant data to do this on-hand, for example using data associated with figures 1, 3, 6, and 9.
2. There is a discrepancy in the staging of embryos used between some of the analyses, which make it hard to interpret some of the data. For example, characterization of the knockdowns in Fig. 1A and B are based on stages 10 and 15, whereas the majority of the paper is focused on earlier stages 6 - 8 during germband extension (e.g., Fig. 1D). The analysis for Fig. 1B would be more meaningful if it was done on the same stages used for subsequent phenotypic analysis so they can be directly compared.
3. There is incongruence between figures in terms of which junctional pools (bAJs vs. tAJs) of beta-catenin and E-cadherin are quantified that makes it difficult to draw comparisons between analyses. For example pTyr levels are examined for both bAJs and tAJs in Figure 3, however, only tAJs are considered in Fig. 8. Similarly, in some cases planar cell polarity is considered (e.g., comparison of levels at AP vs DV bAJs in Fig. 6 and 9), and in other cases (e.g. Fig. 8) it is not.

Lower priority:

1. Introduction, 2nd paragraph - The modes of cell behaviours described to drive cell intercalation leaves out another clear example in the literature - Sun et al., 2017 - which describes a basolateral cell protrusion-based mechanism. While the authors cite this paper later, leaving it out when summarizing the state of the field misrepresents the current knowledge of the range of mechanisms responsible.

2. 'defective cytoplasm' - this term is confusing, and could perhaps be replaced with 'cellularization defect', or something similar.
3. Tests of statistical significance are not uniformly applied across the figures. For instance, Figures 3G + H indicate statistical significance, but Fig. 3D + E do not. Performing statistical tests throughout the paper, or clearly articulating a rationale when they are not used, would strengthen the manuscript. Specifically, the authors should consider this for Fig. 3D + E, and Fig. 7D + E, to support their arguments that rates of germband extension are different between conditions.
4. Page 12 - "We found that Src42A showed a distinct localization at the tAJs (Fig. 1B)": Figure 1B shows a quantification of levels at bAJs, not tAJs.
5. Figure 8 - in my opinion, using a FRAP or photoconversion approach would be a more convincing demonstration of differences in E-cadherin residency times/ turnover rate than time-lapse imaging of E-cadherin:GFP alone. Authors should decide whether this improvement is worth the investment.
6. Figure 8E - showing images of multiple tAJs, rather than z-slices of a single vertex, would better support the claim here, as the assertion is that Src42a levels are different between control and sdk RNAi conditions, and not that it varies in the z-dimension.

Significance

The manuscript by Backer et al. examines the function of Src42A in germband extension during Drosophila gastrulation. Prior studies in the field have shown that Src family kinases play an important role in the early embryo, including cellularization (Thomas and Wieschaus 2004), anterior midgut differentiation (Desprat et al. 2008), and germband extension (Sun et al. 2017; Tamada et al. 2021). In this study, the authors showed that Src42A was enriched at adherens junctions and was moderately enriched along junctions with myosin-I1. They then showed that maternal Src42A depletion exhibits phenotypes, starting with cellularization and including a defect in germband extension. The authors focus on defects in germband extension and found that Src42A was required for timely rearrangement of junctions and that the Src42A RNAi phenotype is enhanced by Abl RNAi. Finally the authors show that E- cadherin turnover is affect by Src42A depletion.

Overall, this study provided a higher resolution description of how Src42A regulates the behavior of junctions during germband extension. I thought the authors conclusions were well supported by the data and represent new insight in the field.

Reviewer 2

Evidence, reproducibility and clarity

Summary:

Chandran et al. investigate the role of Src42A in axis elongation during Drosophila gastrulation. Using maternal RNAi and CRISPR/Cas9-induced germline mosaics, they revealed that Src42A is required to contract junctions at anterior/posterior cell interfaces during cell intercalations. Using time-lapse imaging and image analysis, they further revealed the role of Src42A in E-Cad dynamics at cell junctions during this process.

By analyzing double knockdown embryos for Src42A and Abl, they further showed that Src42A might act in parallel to Abl kinase in regulating cell intercalations. The authors proposed that Src42A is involved in two processes, one affecting tension generated by myosin II and the other acting as a signaling factor at tricellular junctions in controlling E-Cad residence time.
Overall, the data are clear and nicely quantified. However, some data do not convincingly support the conclusion, and statistical analyses are missing for an experiment or two.
Methods for several quantifications also need improvement in writing. Also, several figures (Figures 6-8) do not match the citation in the text and need to be corrected.

Page and line numbers were not indicated in the manuscript. For my comments, I numbered pages starting from the title page (Title, page 1; Abstract, page 2, Introduction, pages 3-6; Results, pages 7-14; Discussion, pages 15-18; M&M, 19-23; Figure legends, 28-30) and restarted line numbers for each page. For Figures 6-8 that do not match the citation in the text, I still managed to look at the

potentially right panels. All the figure numbers I mention here are as cited in the text. My detailed comments are listed below.

Major comments:

1. b-Cat/E-Cad signals at the DN and A/P junctions in Src42Ai (Figs. 5-6). These data are critical for their major conclusion and should be demonstrated more convincingly.

In Fig. 5A, the authors said, "When the AP border was cut, the detached tAJs moved slower in Src42Ai embryos compared to control (Fig. 5A)". However, even control tAJs do not seem to move that much in the top panels, and I found the images not very convincing.

In Fig. 6A, b-Cat signals look fuzzier and dispersed and have more background signals in the control, compared to the Src42Ai background. Also, b-Cat signals in the control image do not seem to show enrichment at the D/V border, as shown in Tamada et al., 2012.

In Fig. 6B, C, it is not clear how the intensity was measured and how normalization was done. Was the same method used for these quantifications as "Protein levels at bicellular and tricellular AJs" on pages 21-22? Methods should be written more explicitly with enough details.

Does each sample (experimental repeat) for the D/V border in Fig. 6B match the one right below for the A/P border in Fig. 6C? It should be clearly mentioned in the figure legend. The ratio of the DV intensity to AP intensity will better show the compromised planar polarity of the b-Cat/E-Cad complex.
2. Based on the genetic interaction between Src42A and Abl using RNAi (Fig. 7), the authors argue that Src42A and Abl may act in parallel. However, the efficiency of Abl RNAi has not been tested. It can be done by RT-PCR or Abl antibody staining. Also, the effect of Abl RNAi alone on germband extension should be tested and compared with Src42A & Abl double RNAi embryos. I expect the experiments can be done within a few weeks without difficulty.

Minor comments:

Page 2, line 14 - The abbreviation for IAJs was not introduced before.

Page 7, line 6 - A reference should be cited for the Src42A26-1 allele.

Figure 1
- Fig. 1B: Src42A levels should be compared between control (Src42A/+) and Src42A/Src42A for each stage. It currently shows a comparison between Src42A/Src42A of stages 10 and 15.
- Fig. 1B: The figure legend says, "dotted line represents mean value and error bars," but there are no dotted lines shown in the figure. Also, what p-value is for ****? It should be mentioned in the figure legend. It also says Src42A levels were normalized against E-Cad intensity here (stages 10 and 15). They have shown that E-Cad levels are affected in Src42A RNAi during gastrulation (Fig. 6). Is E-Cad not affected in Src42A26-1 zygotic mutants at stages 1O and 15?

Page 7, lines 6-7 - The localization of Src42A in control should be described in more detail and more clearly here.

Supplemental Fig S1

- Fig. S1D: Based on the head structure and the segmental grooves, the embryo shown here is close to late stage 13/early stage 14, not stage 15.
- Fig S1E: It will be helpful if the predicted protein band and non-specific bands are indicated by arrows/arrowheads in the figure.

Page 7, lines 21-22

- "Src42A was slightly enriched at the AP interface" - To argue that, quantification should be provided.

Page 8, line 14

- "Embryos expressing TRiP04138 showed reduced hatching rates with variable penetrance and expressivity depending on the maternal Gal4 driver used (Fig. 2B)" - Fig. 2B doesn't seem to be a right citation for this sentence.

Fig. 2

- Fig. 2B: Higher magnification images of the defective cytoplasm can be shown as insets.
- Fig. 2C: It will be helpful to indicate two other non-specific bands in the figure with arrows/arrowheads with a description in the figure legend.
- Fig. 2E: A simple quantification of the penetrance of cuticle defects in Src42A mutants and RNAi will be helpful, as shown in Fig. S3.

Page 9, line 9

- This is the first lime that the fast and the slow phases of germband extension are mentioned. As these two phases are used to compare the Src42A and Src42A Abl double RNAi phenotypes, they should be introduced and explained better earlier, perhaps in Introduction.

Fig. 3

- Fig. 3A: It will be helpful to mark the starting and the ending points of germband elongation with different markers (arrows vs. arrowheads or filled vs. empty arrowheads).
- Fig. 3G and H should be cited in the text.
- Fig. 3C figure legend: R2 is wrongly mentioned in Fig. 3D, E. Also, R2 (coefficient of determination) needs to be defined either in the figure legend or Materials & Methods.
- Fig. 3D, E: statistical analysis is missing.
- Fig. 3F: It should be mentioned that the heat map is shown for pY20 signals in the figure legend, with an intensity scale bar in the figure.

Fig. 7A: Arrows can be added to mark the delayed germband extension.

Fig. 8A: It should be mentioned that the heat map is shown for E-Cad signals in the figure legend, with an intensity scale bar in the figure.

Fig. S3G: An arrowhead can be added to the gel image to indicate the band described in the legend.

Fig. 9

- Fig. 9A: Magnified views of the cytoplasmic clearing can be added as insets.
- Fig. 9B: Arrow/arrowheads can be added to show the absence of the signals in the nurse cells.
- Fig. 9C: Indicate the ending point of the germband extension by arrows.

Page 14, lines 9-10: More explicit description of the phenotype rather than just "stronger compared to Src42Ai" will be helpful.

Significance

This work revealed the role of Src42A in regulating germband extension. A previous study suggested the roles of Src42A and Src64 in this developmental process using a partial loss of both proteins (Tamada et al., 2021). Using different approaches, the authors demonstrated a role of Src42A in regulating E-Cad dynamic at cell junctions during Drosophila axis elongation. Most of the analyses were done with maternal knockdown using RNAi, but they successfully generated germline clones for the first time and confirmed the RNAi phenotypes. Overall, this work contains important and exciting novel findings.

This work will be of general interest to cell and developmental biologists, particularly researchers studying epithelial morphogenesis and junctional dynamics.

I have expertise in Drosophila genetics, epithelial morphogenesis, imaging, and quantitative image analysis.

Reviewer 3

Evidence, reproducibility and clarity

Chandran et al. report on the function of Src42A during cell intercalation in the early Drosophila gastrula. They create a Src42A-specific antibody (there are two Src genes in the fly genome) and examine the localization of Src42A and observe a planar-polarized distribution at cell interfaces. They then measure cell-contractile dynamics and show that T1 contraction is slower after Src42A disruption. The authors then argue that Src42A functions in a parallel pathway to the Abl protein, and that E-cadherin dynamics (turnover) is altered in Src42A disrupted embryos. Src function at these stages has been studied previously (though not to the degree that this study does), and in some respects the manuscript feels a little preliminary (please label figures with figure number!), but after editing this should be a polished study that merits publication in a developmentally-focused journal.

1. Does the argument that Src42A has two functions fully make sense? Myosin II function is known to affect E-cadherin stability (and vice versa), so it seems that Src42A could affect both Myall and Ecad by either decreasing Myosin 11 function/engagement at junctions or by destabilizing Ecad.

2. One obvious question that arises is the nature of cleavage defects that are mentioned that happen previously to intercalation. For example, is E-cad normal prior to intercalation initiating? How specific are the observed defects to GBE?

3. Pg. 10, "the shrinking junction along the AP axis strongly reduces its length with an average of 1.25 minute" - what is this measurement? How much is "strongly"?

4. Also pg. 10, "the AP junction was not markedly reduced after 1 minute" - what is the criteria for this statement? X%? 1 minute is very specific, it feels like how much of a reduction/non-reduction should also be specific.

5. It seemed odd to mention altered myosin levels but then skip over a measurement of myosin in favor of an indirect measurement such as interface recoil. Again (point 1), it seems that changes in Myosin II recruitment could cause changes in Ecad turnover.

Minor notes:

Page 4, missing comma after "For example"

Page 4, "inevitable" does not make sense in this context

Significance

This study gives a more detailed perspective on how Src proteins (Src42A in Drosophila) control epithelial stability and the contraction of specific surfaces of epithelial cells.

Author response to reviewers' comments

1. General Statements [optional]

We thank all reviewers for their constructive comments on our manuscript. We were very pleased to see that the reviewers found our study *'…represent new insight in the field'* (rev#1) and *'…contains important and exciting novel findings'* (rev#2), and *'…gives a more detailed perspective on how Src proteins (Src42A in Drosophila) control epithelial stability and the contraction of*

specific surfaces of epithelial cells' (rev#3). The reviewers raised a number of specific points that we partially addressed already in a preliminary revision of the manuscript. Some more points will require some additional experiments that we will incorporate in a fully revised version of the manuscript.

2. Description of the planned revisions

Insert here a point-by-point reply that explains what revisions, additional experimentations and analyses are <u>planned</u> to address the points raised by the referees.

Reviewer #1
(Evidence, reproducibility and clarity (Required)):
Highest priority:
1) The Src42A knockdown and germline clone experiments both cause defects in cellularization (Fig. 2B and 9A), which could result in differences in the state of the blastoderm epithelium (cell size, cell number, structural integrity, organization, etc.) between the experimental and control conditions. In addition, Src42A knockdown appears to affect the size and shape of the egg (Fig. 9A and 9C). The manuscript would be strengthened if the authors included data to demonstrate that the initial structure of the epithelium is mostly normal (quantifications of cell size, number, etc.) in the Src42A RNAi condition, as this would bolster the argument that germband extension, rather than due to indirect effects resulting from the cellularization defects. The authors may have relevant data to do this on-hand, for example using data associated with figures 1, 3, 6, and 9.

Response:
The cellularization phenotype of *src42A* knockdown embryos has a penetrance of about 50% and exhibits a variable expressivity. We attempted to characterize this phenotype in detail, but failed to identify any dramatic differences in cellularization of the *src42A* knockdown embryos compared to wild type. The localization of E-cadherin, in turn is not affected, but occasionally, nuclei are dropping out of the blastoderm before cellularization is accomplished. This can result in patches of irregular cellularization, but the blastoderm epithelium in stage 6 embryos did not display major defects in overall structure. We will present additional data on the cellularization phenotypes in the fully revised manuscript. As the referee suggested, we will analyze our data to determine potential effects on the cell size, cell number and overall organization of the blastoderm before germband extension. We plan to present these data as an additional Suppl. Mat. Figure in the full revision.

Lower priority:

5) Figure 8 - in my opinion, using a FRAP or photoconversion approach would be a more convincing demonstration of differences in E-cadherin residency times / turnover rate than time-lapse imaging of E-cadherin:GFP alone. Authors should decide whether this improvement is worth the investment.

Response:
We thank the reviewer for this comment. While we believe that the data presented in Fig. 8 demonstrates a significant difference in the E-cadherin residence time based on E-cadherin-GFP fluorescence intensity, we agree with the referee that FRAP analyses would provide additional evidence to support our conclusion. For the full revision, we will therefore attempt to perform FRAP-experiments on *src42A* knockdown embryos expressing E-cadherin-GFP and compare the recovery time to the wild type.

Reviewer #1 (Significance (Required)):

The manuscript by Backer et al. examines the function of Src42A in germband extension during Drosophila gastrulation. Prior studies in the field have shown that Src family kinases play an important role in the early embryo, including cellularization (Thomas and Wieschaus 2004), anterior midgut differentiation (Desprat et al. 2008), and germband extension (Sun et al. 2017; Tamada et al. 2021). In this study, the authors showed that Src42A was enriched at adherens junctions and was moderately enriched along junctions with myosin-II. They then showed that maternal Src42A depletion exhibits phenotypes, starting with cellularization and including a defect in germband

extension. The authors focus on defects in germband extension and found that Src42A was required for timely rearrangement of junctions and that the Src42A RNAi phenotype is enhanced by Abl RNAi. Finally the authors show that E-cadherin turnover is affect by Src42A depletion.

Overall, this study provided a higher resolution description of how Src42A regulates the behavior of junctions during germband extension. I thought the authors conclusions were well supported by the data and represent new insight in the field.

Reviewer #2 (Evidence, reproducibility and clarity (Required)):

Summary:
Chandran et al. investigate the role of Src42A in axis elongation during Drosophila gastrulation. Using maternal RNAi and CRISPR/Cas9-induced germline mosaics, they revealed that Src42A is required to contract junctions at anterior/posterior cell interfaces during cell intercalations. Using time-lapse imaging and image analysis, they further revealed the role of Src42A in E-Cad dynamics at cell junctions during this process.

By analyzing double knockdown embryos for Src42A and Abl, they further showed that Src42A might act in parallel to Abl kinase in regulating cell intercalations. The authors proposed that Src42A is involved in two processes, one affecting tension generated by myosin II and the other acting as a signaling factor at tricellular junctions in controlling E-Cad residence time.
Overall, the data are clear and nicely quantified. However, some data do not convincingly support the conclusion, and statistical analyses are missing for an experiment or two. Methods for several quantifications also need improvement in writing. Also, several figures (Figures 6-8) do not match the citation in the text and need to be corrected.

Page and line numbers were not indicated in the manuscript. For my comments, I numbered pages starting from the title page (Title, page 1; Abstract, page 2, Introduction, pages 3-6; Results, pages 7-14; Discussion, pages 15-18; M&M, 19-23; Figure legends, 28-30) and restarted line numbers for each page. For Figures 6-8 that do not match the citation in the text, I still managed to look at the potentially right panels. All the figure numbers I mention here are as cited in the text. My detailed comments are listed below.

Response (*already addressed in transferred manuscript*):
We apologize for the lack of organization of the manuscript and the figure numbering. In the revised version we have added page numbers, line numbers and we corrected the figure numbers.

Major comments:

1. b-Cat/E-Cad signals at the D/V and A/P junctions in Src42Ai (Figs. 5-6). These data are critical for their major conclusion and should be demonstrated more convincingly.

In Fig. 5A, the authors said, "When the AP border was cut, the detached tAJs moved slower in Src42Ai embryos compared to control (Fig. 5A)". However, even control tAJs do not seem to move that much in the top panels, and I found the images not very convincing.

Response:
We thank the referee for commenting on the lack of clarity in the presentation of the data. The overall movement within the first 10 seconds after the laser cut (determined by movement of adjacent D/V tAJs from each other) was about 2 µm in the wildtype, while in the mutant it was 1 µm. Despite this 50% difference, it may be difficult to appreciate this difference from looking at Fig. 5A in our original submission. The yellow lines in Fig 5A only showed the region of the cut, but did not indicate the movement of the tAJ from each other, which may have led to a distraction from the actual movement. We will change the annotation and the marks within the figure to visualize the movement much more clearly in the full revision. In the fully revised manuscript, we will also add movies from the experiments including marks of the tricellular junctions to follow the displacement as part of the Supplemental Material.

2. Based on the genetic interaction between Src42A and Abl using RNAi (Fig. 7), the authors argue that Src42A and Abl may act in parallel. However, the efficiency of Abl RNAi has not been tested. It can be done by RT-PCR or Abl antibody staining. Also, the effect of Abl RNAi alone on germband extension should be tested and compared with Src42A & Abl double RNAi embryos. I expect the experiments can be done within a few weeks without difficulty.

Response:
We agree with the referee that it is important to determine the level of depletion in Abl RNAi embryos in order to interpret the genetic relationship between Abl and Src42A. In the full revision of the manuscript, we will follow the advice of the referee and analyze the knockdown, preferably by antibody labeling with an anti-Abl antibody. We will also generate single knockdowns of *abl* in embryos and determine their effect on germband extension compared to wildtype and *src42/abl* double knockdown.

Minor comments:

Fig. 2
- Fig. 2B: Higher magnification images of the defective cytoplasm can be shown as insets.

Response:
We will add some higher magnification images of the cellularization phenotype in the full revision of the manuscript. In addition, as mentioned in the response to reviewer #1, we will provide a more detailed analysis of the cellularization in *src42A*^{i} embryos in the fully revised manuscript.

- Fig. 2E: A simple quantification of the penetrance of cuticle defects in Src42A mutants and RNAi will be helpful, as shown in Fig. S3.

Response:
In the full revision, we will add the quantification of the occurrence of the different classes of cuticle phenotypes.

Fig. 9
- Fig. 9A: Magnified views of the cytoplasmic clearing can be added as insets.

Response: As described in our response to the comments made by referee #1, we will add a more detailed analysis of the cellularization phenotype in the full revision.

Page 14, lines 9-10: More explicit description of the phenotype rather than just "stronger compared to Src42Ai" will be helpful.

Response:
In the full revision, we will add a more detailed description of the phenotype and re-analyze and present data on the hatching rate, stage of lethality and cuticle phenotypes.

Reviewer #2 (Significance (Required)):
This work revealed the role of Src42A in regulating germband extension. A previous study suggested the roles of Src42A and Src64 in this developmental process using a partial loss of both proteins (Tamada et al., 2021). Using different approaches, the authors demonstrated a role of Src42A in regulating E-Cad dynamic at cell junctions during Drosophila axis elongation. Most of the analyses were done with maternal knockdown using RNAi, but they successfully generated germline clones for the first time and confirmed the RNAi phenotypes. Overall, this work contains important and exciting novel findings.
This work will be of general interest to cell and developmental biologists, particularly researchers studying epithelial morphogenesis and junctional dynamics.
I have expertise in Drosophila genetics, epithelial morphogenesis, imaging, and quantitative image analysis.

Reviewer #3 (Evidence, reproducibility and clarity (Required)):

Chandran et al. report on the function of Src42A during cell intercalation in the early Drosophila gastrula. They create a Src42A-specific antibody (there are two Src genes in the fly genome) and examine the localization of Src42A and observe a planar-polarized distribution at cell interfaces. They then measure cell-contractile dynamics and show that T1 contraction is slower after Src42A disruption. The authors then argue that Src42A functions in a parallel pathway to the Abl protein, and that E-cadherin dynamics (turnover) is altered in Src42A disrupted embryos. Src function at these stages has been studied previously (though not to the degree that this study does), and in some respects the manuscript feels a little preliminary (please label figures with figure number!), but after editing this should be a polished study that merits publication in a developmentally-focused journal.

1) Does the argument that Src42A has two functions fully make sense? Myosin II function is known to affect E-cadherin stability (and vice versa), so it seems that Src42A could affect both MyoII and Ecad by either decreasing Myosin II function/engagement at junctions or by destabilizing Ecad.

Response:
We thank the referee for raising an important point that we may not have discussed appropriately in our initial submission. We agree that the reciprocal relationship between actomyosin and E-cadherin might not be reflected equivocally in our manuscript. As the referee points out, Src42A could affect both MyoII planar localization and E-cadherin dynamics through the same pathway. Previous studies showed that Src is involved in translating the planar polarized distribution of the Toll-2 receptor by recruiting Pi3-Kinase activity to the Toll-2 receptor complex resulting in planar polarized distribution of MyoII at the A/P interfaces. These data, however do not address the possibility that a well-known Src target, the E-cadherin/ß-Catenin complex, which is extensively remodeled in germband extension contributes to the delay in germband extension. The observed defects in both studies can be attributed to both a defect in abnormal planar polarization of MyoII and the abnormal dynamics of the E-cadherin/ß-catenin complex. In either of these cases, we suggest that Src42A phosphorylates distinct substrates, the Toll-2 intracellular domain in the MyoII planar polarity pathway and the E-cad/ß-Cat complex controlling E-cad dynamics. Given the relationship between MyoII and E-cadherin, however, it is not possible to decide whether these two effects are independent functions of Src42A or are consequences of each other. Since we cannot resolve a possible epistatic relationship between these potential two activities of Src42A, we decided to extend the discussion on this topic by taking both possible scenarios into account and discussing them appropriately. We will add this discussion in the full revision of the manuscript.

2) One obvious question that arises is the nature of cleavage defects that are mentioned that happen previously to intercalation. For example, is E-cad normal prior to intercalation initiating? How specific are the observed defects to GBE?

Response:
see response to referee #1

3) Pg. 10, "the shrinking junction along the AP axis strongly reduces its length with an average of 1.25 minute" - what is this measurement? How much is "strongly"?

Response:
We thank the referee for pointing out our inappropriate qualitative statement of the experimental data, which was indeed misleading. The measurement of the shrinking junction was based upon the time it takes for the AP interface junction between two adjacent vertices on the DV axis to shrink into a single 4-cell vertex. The time for this contraction was on average 1 minute 25 seconds. The data in Fig.4 A',C show that after 2 minutes in the control embryo 100% of the observed AP junctions have collapsed and the extension of the new DV junction along AP axis has begun. At the same timepoint of 2 minutes in the src42A knockdown, we show in Fig. 4B',D that the shrinking of the AP junction interface has still not been completed in 60% of the cases.
In the full revision, we will remove the qualitative statement and replace it with a correct description of the measurements taken and will refer to the data described in Fig. 4 A-D.

4) Also pg. 10, "the AP junction was not markedly reduced after 1 minute" - what is the criteria for this statement? X%? 1 minute is very specific, it feels like how much of a reduction/non-reduction should also be specific.

Response:
See response to point 3.

Reviewer #3 (Significance (Required)):

This study gives a more detailed perspective on how Src proteins (Src42A in Drosophila) control epithelial stability and the contraction of specific surfaces of epithelial cells.

3. Description of the revisions that have already been incorporated in the transferred manuscript

Please insert a point-by-point reply describing the revisions that were already carried out and included in the transferred manuscript. If no revisions have been carried out yet, please leave this section empty.

Reviewer #2 and #3 noted that the manuscript was somewhat unorganized with regard to lacking the numbering of pages, lines and figures. We also noted that in the submission process the figures were not presented in the correct order. In the preliminary revision of the manuscript, we fixed these problems to facilitate the evaluation of our transferred manuscript by editorial boards.

In addition, we also addressed issues that the referees mentioned by editing the text according to their comments. We also addressed problems regarding the presentation of the figures and statistical analyses of the data. The following changes were made:

a) We added page numbers and line numbers.
b) We added figure numbers to the figure panels.
c) We corrected ordering of figures in the transferred manuscript.
d) We addressed the following comments by statistical analyses, editing the text and the figures:

Regarding comments from Reviewer #1:

Highest Priority:
2) There is a discrepancy in the staging of embryos used between some of the analyses, which make it hard to interpret some of the data. For example, characterization of the knockdowns in Fig. 1A and B are based on stages 10 and 15, whereas the majority of the paper is focused on earlier stages 6 - 8 during germband extension (e.g., Fig. 1D). The analysis for Fig. 1B would be more meaningful if it was done on the same stages used for subsequent phenotypic analysis so they can be directly compared.

Response:
We thank the referee for pointing out an apparent misunderstanding caused by the description of Fig. 1A,B. The data presented in Fig.1A and 1B do not show RNAi knockdown experiments, but show a comparison between embryos that are heterozygous or homozygous for the loss-of-function allele *src42A*$^{26\text{-}1}$. These data were intended to demonstrate that zygotic mutants still maintain levels of maternal Src42A protein up until late stages of development. Data for embryos at an earlier stage (stage 5) were shown in the Supplementary Fig. S1E, where no difference in protein levels of Src42A can be observed between heterozygous and homozygous zygotic *src42A*$^{26\text{-}1}$ embryos.
At the beginning of the results sections 1 and 2 of the preliminary revised manuscript, we added a sentence to address the referee's concern that earlier stages exhibit no difference in protein levels and will refer to Fig. S1E. We also more explicitly spelled that out that the experiment (referring to Fig.1A,B and S1) was intended to look at zygotic mutants and to demonstrate that our novel Src42A antibody was able to detect the reduction of maternal Src42A protein in mid- to late-stage homozygous zygotic embryos.

3) There is incongruence between figures in terms of which junctional pools (bAJs vs. tAJs) of beta-catenin and E-cadherin are quantified that makes it difficult to draw comparisons between analyses. For example, pTyr levels are examined for both bAJs and tAJs in Figure 3, however, only tAJs are considered in Fig. 8. Similarly, in some cases planar cell polarity is considered (e.g., comparison of levels at AP vs DV bAJs in Fig. 6 and 9), and in other cases (e.g. Fig. 8) it is not.

Response:
We thank the referee for commenting on the different readouts for different pools of cell junctions in our experiments. In our study we considered effects on src42A on both, bAJs and tAJs by RNAi knockdown of src42A. We decided to present the data for bAJ and tAJ in separate figures for clarity and structure. For example, the data for the effect of src42A knockdown on the planar polarized distribution on bAJs of E-cadherin were presented in Fig.6, while the effect on E-cadherin residence time in tAJs were presented in Fig.8. The analysis pTyr levels considered both pools in order to determine whether src42A knockdown leads to an overall reduction of pTyr levels or to a reduction in a specific junctional pool. From our data we conclude that pTyr levels show a similar reduction in both, the bAJ and the tAJ junctions.

In order to address the reviewer's comment, we have linked the figures more stringently with the results text of the preliminary revision. We only referred to the reduction in PTyr levels in Fig. 3 to point out that both junctional pools are affected by reduced PTyr in *src42*^{i} embryos. Furthermore, we referred to the individual figure panels when addressing junctional pools and explain the rationale to focus on particular pools (bAJs or tAJ) in the experiments in detail. For Fig. 6 we point out in the preliminary revised manuscript that we focus the analyses on the known planar polarized distribution of beta-catenin and E-Cadherin.

Lower priority:
1) Introduction, 2nd paragraph - The modes of cell behaviors described to drive cell intercalation leaves out another clear example in the literature - Sun et al., 2017 - which describes a basolateral cell protrusion-based mechanism. While the authors cite this paper later, leaving it out when summarizing the state of the field misrepresents the current knowledge of the range of mechanisms responsible.

Response:
We thank the referee for this remark. In the preliminary revision, we have added to the introduction that the cell behaviors associated with germband elongation include apical and basolateral rearrangements of the cells indicating that basolateral protrusions also contribute to the set of mechanisms that drive germ band elongation.

2) 'defective cytoplasm' - this term is confusing, and could perhaps be replaced with 'cellularization defect', or something similar.

Response:
We agree that the term we applied for the cellularization defect may be misleading. The observation, we intended to describe with the term was a defect in the cytoplasmic clearing which occurs in the last syncytial division and the beginning of the cell formation process. We changed the description of this observation according now refer to the defect in the preliminary revised manuscript as '*cytoplasmic clearing defect*'.

3) Tests of statistical significance are not uniformly applied across the figures. For instance, Figures 3G + H indicate statistical significance, but Fig. 3D + E do not. Performing statistical tests throughout the paper, or clearly articulating a rationale when they are not used, would strengthen the manuscript. Specifically, the authors should consider this for Fig. 3D + E, and Fig. 7D + E, to support their arguments that rates of germband extension are different between conditions.

Response:
We agree with the reviewer and have provided statistical analysis for the data displayed in Fig. 3D,E and Fig. 7D,E in the preliminary revision of the manuscript.

4) Page 12 - "We found that Src42A showed a distinct localization at the tAJs (Fig. 1B)": Figure 1B shows a quantification of levels at bAJs, not tAJs.

Response:
In the preliminary version of the revised manuscript, we added a quantification of the localization of Src42A at the tAJs as a part of Suppl Fig. S4. In Fig. S4A-C we show that Src42A is enriched in comparison to the bAJs.

Regarding comments from reviewer #2:

Major Comments:

In Fig. 6A, b-Cat signals look fuzzier and dispersed and have more background signals in the control, compared to the Src42Ai background. Also, b-Cat signals in the control image do not seem to show enrichment at the D/V border, as shown in Tamada et al., 2012.

Response:
We agree with the referee that the image in Fig. 6A for the control is fuzzier and looks dispersed. This is due to the fixation method that we used. In this experiment we did not apply heat fixation, but used formaldehyde fixation in which b-catenin protein, in addition to the junctional pool, is also maintained in the cytoplasm creating the fuzzy cytoplasmic staining. We chose to do this in order to be able to co-immunolabel the embryos with b-catenin and E-cadherin antibodies; the latter staining is not working with the heat fixation applied in the Tamada et al. 2012 paper. Despite the slightly lower quality of the staining, the quantification of the data clearly indicated an effect of src42A knockdown on the planar polarized distribution of E-cad/b-cat complex does show an enrichment. In the preliminary revision added a note to the figure legend to indicate the fact that the fixation procedure was not optimized for b-catenin junctional staining. In the preliminary revision we also added a quantification of live imaging data recording E-cadherin-GFP in wild-type and *src42A*^{i} embryos. We present these additional data in Fig. S5 in the preliminary revision of the manuscript. These data are consistent with the results in Fig. 6 from the immunolabeling and support our conclusion that E-cadherin AP/DV ratio is increased in Src42A knockdown embryos.

In Fig. 6B, C, it is not clear how the intensity was measured and how normalization was done. Was the same method used for these quantifications as "Protein levels at bicellular and tricellular AJs" on pages 21-22? Methods should be written more explicitly with enough details.

Response:
We thank the referee for pointing out the lack of detail in explaining how the quantification was done. In the preliminary revision of the manuscript, we extended a paragraph entitled '*Protein levels at bicellular and tricellular junctions'* in the methods section that will serve this purpose and describe the methods that were applied for each quantification and the method as to how the data were normalized.

Does each sample (experimental repeat) for the D/V border in Fig. 6B match the one right below for the A/P border in Fig. 6C? It should be clearly mentioned in the figure legend. The ratio of the DV intensity to AP intensity will better show the compromised planar polarity of the b-Cat/E-Cad complex.

Response:
We thank the reviewer for pointing out a lack of clarity in our presentation. The experimental repeats for each measurement do indeed match, *i.e.* the measurement of the DV border matches the same adjacent 4-cell pair in the same embryo and in total 5 distinct embryos were analyzed for each experiment. In the preliminary revision of the manuscript, we explain this detail of the experimental design in the figure legend. In the preliminary revision, we also determined the ratios of DV/AP cell interfaces for b-Cat and E-Cad and added this quantification as panel 6C and 6E for a clearer presentation of the data.

Minor notes:
Page 4, missing comma after "For example"
Response: The text was edited accordingly.

Page 4, "inevitable" does not make sense in this context
Response: We eliminated ‘inevitable’ and replaced it with ‘critical’ to better indicate the importance of Canoe protein for germband elongation.

Page 7, lines 6-7 - The localization of Src42A in control should be described in more detail and more clearly here.
Response: In the preliminary revised manuscript, we extended our description of the distribution of Src42A in more detail pointing out its dynamics and differential distribution at distinct plasma membrane domains.

Supplemental Fig S1
- Fig. S1D: Based on the head structure and the segmental grooves, the embryo shown here is close to late stage 13/early stage 14, not stage 15.
- Fig S1E: It will be helpful if the predicted protein band and non-specific bands are indicated by arrows/arrowheads in the figure.

Response:
We thank the referee for their careful observation of the embryonic stage. We agree that the embryo was actually a younger stage. In the preliminary revision, we replaced the images with an example of an older stage. We will also add clear annotations as arrows to clearly mark the specific protein bands in Fig. S1E.

Page 7, lines 21-22
- "Src42A was slightly enriched at the AP interface" - To argue that, quantification should be provided.

Response:
We thank the referee for pointing out a qualitative statement that we made with regard to the distribution of Src42A at the AP cell interfaces. In the preliminary revision of the manuscript, we present an additional quantification of the imaging data of Src42A immunolabeling. In Figure S4A-C, we now present a quantification of the enrichment of Src42A at the tricellular junctions. In addition, the new Fig. S4D,E shows a quantification of the planar polarized distribution of Src42A at the AP cell interfaces.

Figure 1
- Fig. 1B: Src42A levels should be compared between control (Src42A/+) and Src42A/Src42A for each stage. It currently shows a comparison between Src42A/Src42A of stages 10 and 15.

Response:
We thank the referee for the comment. As indicated in our response to referee #1, the point of this analysis was to (1) provide evidence for the specificity of our new anti-Src42A antibody and (2) to demonstrate the presence of substantial material contribution of Src42A protein in zygotic mutant. We do not see the advantage to provide a detailed developmental Western-blot analysis, but we provide data in Suppl. Mat Fig S1E showing that the level of Src42A is unimpaired in stage 6 zygotic src42A[26-1] homozygous mutant embryos.

- Fig. 1B: The figure legend says, "dotted line represents mean value and error bars," but there are no dotted lines shown in the figure. Also, what p-value is for ****? It should be mentioned in the figure legend. It also says Src42A levels were normalized against E-Cad intensity here (stages 10 and 15). They have shown that E-Cad levels are affected in Src42A RNAi during gastrulation (Fig. 6). Is E-Cad not affected in Src42A26-1 zygotic mutants at stages 10 and 15?

Response:
We thank the referee for pointing out inaccuracies in the presentation and the description of Fig.1B. In the preliminary revision, we emphasized the marks on the graph and provide p-values throughout. Regarding the E-Cadherin levels: E-cadherin levels were altered in *src42A* RNAi knockdown embryos, but not in zygotic mutants, even at later developmental stages.

Page 8, line 14
- "Embryos expressing TRiP04138 showed reduced hatching rates with variable penetrance and expressivity depending on the maternal Gal4 driver used (Fig. 2B)" - Fig. 2B doesn't seem to be a right citation for this sentence.

Response:
We agree with the referee and in the preliminary revised manuscript we corrected the reference to the conclusion drawn from Figure 2A', which does show the relationship of hatching rate to the various maternal Gal4 drivers.

- Fig. 2C: It will be helpful to indicate two other non-specific bands in the figure with arrows/arrowheads with a description in the figure legend.

Response:
In the preliminary revision, we added an arrow to mark the band specific for Src42A and asterisks to mark unspecific bands in Fig 2C.

Page 9, line 9
- This is the first time that the fast and the slow phases of germband extension are mentioned. As these two phases are used to compare the Src42A and Src42A Abl double RNAi phenotypes, they should be introduced and explained better earlier, perhaps in Introduction.

Response:
We thank the referee for pointing out that the two phases of germband extension were not introduced. We added a sentence to introduce and define the distinct phases of extension movements in the preliminary revision.

Fig. 3
- Fig. 3A: It will be helpful to mark the starting and the ending points of germband elongation with different markers (arrows vs. arrowheads or filled vs. empty arrowheads).

Response:
In the preliminary revision, we added distinct markers to indicate the start and endpoints of germband elongation to make this figure easier to read.

- Fig. 3C figure legend: R2 is wrongly mentioned in Fig. 3D, E. Also, R2 (coefficient of determination) needs to be defined either in the figure legend or Materials & Methods.

Response:
We thank the referee for pointing this misleading reference to us. In the preliminary revision we corrected the reference to R2 in Fig,3D,E and will describe the definition of R2 in the figure legend.

- Fig. 3D, E: statistical analysis is missing.

Response:
In the preliminary revision, we included a statistical analysis of the data (see ref #1). We changed the figure to indicate the data sets that were analyzed and added the p-values to the figure legend.

- Fig. 3G and H should be cited in the text.
Response:
In the preliminary revision, we added references to Fig 3G,H in the result section to the annotation of Fig.3F).

- Fig. 3F: It should be mentioned that the heat map is shown for pY20 signals in the figure legend, with an intensity scale bar in the figure.

Response:
In the preliminary revision, we added an intensity scale bar to the figure panel and mentioned the relationship to the PY20 signal.

Fig. 7A: Arrows can be added to mark the delayed germband extension.

Response:
In the preliminary revision, we added arrows to mark the anterior and posterior extent of the germband.

Fig. 8A: It should be mentioned that the heat map is shown for E-Cad signals in the figure legend, with an intensity scale bar in the figure.

Response:
In the preliminary revision, we added an intensity scale to the heat map and mention the relationship to the E-cadherin signal in the figure legend.

Fig. S3G: An arrowhead can be added to the gel image to indicate the band described in the legend.

Response:
In the preliminary revision, we added an arrow to help annotating the Src42A-specific bands on the Western blot.

- Fig. 9B: Arrow/arrowheads can be added to show the absence of the signals in the nurse cells.

Response:
In the preliminary revision, we added markers to help recognizing the reduced signal in the nurse cells and the oocyte.

- Fig. 9C: Indicate the ending point of the germband extension by arrows.

Response: In the preliminary revision, we added arrows to mark the anterior and posterior extent of the germband.

Regarding comments from reviewer #3:

Minor notes:

Page 4, missing comma after "For example"
Response: The text was edited accordingly.

Page 4, "inevitable" does not make sense in this context
Response:
In the preliminary revision, we eliminated ‘inevitable’ and replaced it with ‘critical’ to better indicate the importance of Canoe protein for germband elongation.

4. **Description of analyses that authors prefer not to carry out**

Referee #1 point2 and Referee#2 minor comment figure 1. Both referees suggest that figure 1 AB should include earlier developmental stages according to the stages looked at in the RNAi knockdown experiment.

Response:
The referees’ comments are likely based on a misunderstanding. The data that the reviewer are referring to present analyses of the zygotic phenotype of embryos homozygous for the *src42A*$^{26\text{-}1}$ loss of function allele. They are not related to the maternal RNAi knockdown experiments, but were meant to demonstrate the existence and extent of a maternal pool of Src42A protein, that persists even to late stages in development. The maternal knockdown mutants are analyzed in detail at the appropriate stages in Fig. 2.

As described in our response above, we don’t feel that a detailed developmental stage Western analysis of wildtype and *src42A*$^{26\text{-}1}$ embryos would provide significant additional insights. As mentioned in our response above, data for an earlier developmental stage (before germband elongation, as requested by the referees, were provided in Suppl. Fig. S1E.

Referee #1 Point 6) Figure 8E - showing images of multiple tAJs, rather than z-slices of a single vertex, would better support the claim here, as the assertion is that Src42a levels are different between control and sdk RNAi conditions, and not that it varies in the z-dimension.

Response:
The image series of Fig. 8E shows one representative example of multiple tAJs that have been imaged for this experiment (n=6 for wild type and n=10 for sdk RNAi). We think that the presentation of Z-slices for this experiment is important as the protein distribution needs to be considered for a larger area along the apical-lateral cell interface. In addition the quantification of the data for multiple tAJs was presented in Fig. 8F,G as a graph. We would therefore rather not change this figure in the revised manuscript.

Referee #3 suggests that anti MyoII staining should accompany the analysis of tension measurements in the germband.

As this analysis has already been performed by Tamada et al. 2021, we decided not to reproduce these data, but rather extend the analysis towards tension measurements, which support the findings by Tamada et al. 2021 on a functional level. We do not see the added value of adding MyoII labeling.

Original submission

First decision letter

MS ID#: DEVELOP/2022/201119

MS TITLE: Src42A is required for E-cadherin dynamics at cell junctions during Drosophila axis elongation

AUTHORS: Lenin Chandran, Wilko Backer, Seyed Amir Hamze Beati, Deqing Kong, Stefan Luschnig, and H.-Arno Muller

Following Thomas' email inviting you to revise the paper according to your plan outlined, this is just the formal decision letter. Please do note the various formatting guidelines below.

We look forward to receiving your revision.
All the best,
Katherine

Katherine Brown, PhD
Executive Editor
Development

First revision

Author response to reviewers' comments

Response to the reviewers

We thank all reviewers for their constructive comments on our manuscript. We were very pleased to see that the reviewers found our study '…represent new insight in the field' (rev#1) and '…contains important and exciting novel findings' (rev#2), and '…gives a more detailed perspective on how Src proteins (Src42A in Drosophila) control epithelial stability and the contraction of specific surfaces of epithelial cells' (rev#3).

Reviewer #2 and #3 noted that the original manuscript was somewhat unorganized with regard to lacking the numbering of pages, lines and figures. We also noted that in the submission process the figures were not presented in the correct order. In the revised manuscript, we fixed these problems by making the following changes:

a) We added page numbers and line numbers.
b) We added figure numbers to the figure panels.
c) We corrected ordering of figures in the revised manuscript submission.

Response to reviewer #1

reviewer #1:
1) The Src42A knockdown and germline clone experiments both cause defects in cellularization (Fig. 2B and 9A), which could result in differences in the state of the blastoderm epithelium (cell size, cell number, structural integrity, organization, etc.) between the experimental and control conditions. In addition, Src42A knockdown appears to affect the size and shape of the egg (Fig. 9A and 9C). The manuscript would be strengthened if the authors included data to demonstrate that the initial structure of the epithelium is mostly normal (quantifications of cell size, number, etc.) in the Src42A RNAi condition, as this would bolster the argument that germband extension, rather than due to indirect effects resulting from the cellularization defects. The authors may have relevant data to do this on-hand, for example using data associated with figures 1, 3, 6, and 9.

Response:
The cellularization phenotype of Src42A knockdown embryos has a penetrance of about 40% and exhibits a variable expressivity. We attempted to characterize this phenotype in more detail, but failed to identify any penetrant dramatic differences in cellularization of the Src42A knockdown embryos compared to wild type. The localization of E-cadherin was not affected, but occasionally, nuclei were dropping out of the blastoderm before cellularization was completed. This can result in patches of irregular cellularization, but the blastoderm epithelium in stage 6 embryos did not display major defects in overall structure or polarity.
We also analyzed germband cell numbers and apical cell areas at the onset of germband elongation. We find that the sizes of individual apical cell areas are more variable and overall larger in Src42Ai embryos and the cell number is reduced. We suspect that these effects may be consequences of the nuclear drop out during cellularization, which may cause the appearance of fewer cells with larger apical domains. The altered cell number and associated increase in apical cell area are unlikely to affect germband elongation. It was shown previously that embryos lacking post-blastoderm divisions extend their germband normally (Irvine and Wieschaus, 1994; Edgar and O'Farrell, 1989). Therefore, we conclude that any effects on cellularization are unlikely to be the cause of the defects described in germband extension of Src42Ai embryos. Embryos derived from Src42A germline clones do show changes in egg shape and more penetrant clearing defects in cellularization as compared to Src42A knockdown embryos. We cannot exclude that these defects result in the observed enhancement of the germband elongation phenotype when comparing Src42Ai with Src42AGLC embryos. In the original manuscript, we also describe effects on egg chamber development in Src42A germline clone embryos. We interpret these more penetrant and severe phenotypes as an indication that Src42A may have other requirements for development that occur prior to germband elongation and we mention this conclusion in the revised manuscript.
We describe these data in two additional paragraphs in the results section (lines 179ff, 236ff, and 335ff) and present the new data in supplemental figures S4, S5, S9 and discuss them appropriately, in order to take these potential limitations in the interpretation of our data into account.

reviewer #1:
2) There is a discrepancy in the staging of embryos used between some of the analyses, which make it hard to interpret some of the data. For example, characterization of the knockdowns in Fig. 1A and B are based on stages 10 and 15, whereas the majority of the paper is focused on earlier stages 6 - 8 during germband extension (e.g., Fig. 1D). The analysis for Fig. 1B would be more meaningful if it was done on the same stages used for subsequent phenotypic analysis so they can be directly compared.

Response:
We thank the referee for pointing out an apparent misunderstanding caused by our description of Fig. 1A,B in the original manuscript. The data presented in Fig.1A and 1B do not show RNAi knockdown experiments, but show a comparison between embryos that are heterozygous or homozygous for the loss-of-function allele src42A26-1. These data were intended to demonstrate that zygotic mutants still maintain maternal Src42A protein up until late stages of development. Data for embryos at an earlier stage (stage 5) were shown in the supplementary Fig. S1E, where no difference in protein levels of Src42A was observed between heterozygous and homozygous zygotic src42A26-1 embryos. The data on protein depletion in Src42Ai embryos at stage 5 (cellularization) are presented in Fig. 2C.
At the beginning of the results sections 1 (line130f) and 2 (line158f) of the revised manuscript, we added a sentence each to address the referee's concern that earlier stages exhibit no difference in protein levels and referred to Fig. S1E. We also more explicitly spelled that out that the experiment (referring to Fig.1A,B and S1) was intended to look at zygotic mutants and to demonstrate that our novel Src42A antibody was able to detect the reduction of maternal Src42A protein in mid- to late-stage homozygous zygotic embryos. We added this conclusion in the results section (line 136f).

reviewer #1:
3) There is incongruence between figures in terms of which junctional pools (bAJs vs. tAJs) of beta-catenin and E-cadherin are quantified that makes it difficult to draw comparisons between analyses. For example pTyr levels are examined for both bAJs and tAJs in Figure 3, however, only tAJs are considered in Fig. 8. Similarly, in some cases planar cell polarity is considered (e.g., comparison of levels at AP vs DV bAJs in Fig. 6 and 9), and in other cases (e.g. Fig. 8) it is not.

Response:
We thank the referee for commenting on the different readouts for distinct pools of cell junctions in our experiments and their representation in the figures. In our study we considered the requirements of Src42A on both, bAJs and tAJs by RNAi knockdown. We decided to present the data for bAJ and tAJ in separate figures for clarity and structure. For example, the data for the effect of src42A knockdown on the planar polarized distribution on bAJs of E-cadherin were presented in Fig.6, while the effect on E-cadherin residence time in tAJs were presented in Fig.8. The analysis pTyr levels considered both pools in order to determine whether Src42A knockdown leads to an overall reduction of pTyr levels or to a reduction in a specific junctional pool. From our data we conclude that pTyr levels show a similar reduction in both, the bAJ and the tAJ junctions.
In order to address the reviewer's concern, we have linked the figures more stringently with the results text of the revised manuscript. We only referred to the reduction in PTyr levels in Fig. 3 to point out that both junctional pools are affected by reduced PTyr in Src42i embryos. Furthermore, we referred to the individual figure panels when addressing junctional pools and explain the rationale to focus on particular pools (bAJs or tAJ) in the experiments. For Fig. 6 we point out in the revised manuscript that we focus the analyses on the known planar polarized distribution of beta-catenin and E-cadherin.

reviewer #1:
Lower priority:
1) Introduction, 2nd paragraph - The modes of cell behaviours described to drive cell intercalation leaves out another clear example in the literature - Sun et al., 2017 - which describes a basolateral cell protrusion-based mechanism. While the authors cite this paper later, leaving it out when summarizing the state of the field misrepresents the current knowledge of the range of mechanisms responsible.

Response:
We thank the referee for this remark. In the revision, we have added to the introduction that the cell behaviors associated with germband elongation include apical and basolateral rearrangements of the cells indicating that basolateral protrusions also contribute to the set of mechanisms that drive germ band elongation (line 62f).

2) 'defective cytoplasm' - this term is confusing, and could perhaps be replaced with 'cellularization defect', or something similar.

Response:
We agree that the term we applied for the cellularization defect may be misleading. The observation, we intended to describe with the term was a defect in the cytoplasmic clearing which occurs in the last syncytial division and the beginning of the cell formation process. We changed the description of this observation according now refer to the defect in the revised manuscript as 'cytoplasmic clearing defect' (line172 ff).

3) Tests of statistical significance are not uniformly applied across the figures. For instance, Figures 3G + H indicate statistical significance, but Fig. 3D + E do not. Performing statistical tests throughout the paper, or clearly articulating a rationale when they are not used, would strengthen the manuscript. Specifically, the authors should consider this for Fig. 3D + E, and Fig. 7D + E, to support their arguments that rates of germband extension are different between conditions.

Response:
We thank the reviewer for spotting this inconsistency. We have provided statistical analysis for the data displayed in Fig. 3D,E and Fig. 7D,E in the revision of the manuscript.

4) Page 12 - "We found that Src42A showed a distinct localization at the tAJs (Fig. 1B)": Figure 1B shows a quantification of levels at bAJs, not tAJs.

Response:
In the revised manuscript, we added a quantification of the localization of Src42A at the tAJs as a part of supplementary Fig. S3. In Fig. S3A-C we show that Src42A is enriched at the tAJs in comparison to the bAJs.

5) Figure 8 - in my opinion, using a FRAP or photoconversion approach would be a more convincing demonstration of differences in E-cadherin residency times / turnover rate than time-lapse imaging of E-cadherin:GFP alone. Authors should decide whether this improvement is worth the investment.

Response:
We thank the reviewer for this comment. While we believe that the data presented in Fig. 8 demonstrates a significant difference in the E-cadherin residence time based on E-cadherin-GFP fluorescence intensity. We agree with the referee that FRAP analyses can provide additional evidence to support our conclusion. We conducted FRAP-experiments on control and Src42A knockdown embryos expressing E-cadherin-GFP and compared the recovery times. The relative recovery rates were rather similar compared to controls, however in the case of bAJs in the Src42Ai we observed an overshoot compared to the control saturation curve. At the time when fluorescence recovery in controls enters an equilibrium, in Src42A embryos, fluorescence intensity was still increasing. This result is consistent with an effect on E-cadherin turnover in a way that leads to increasing levels at the junctions. Whether this increase depends on defects in removing E-cadherin from the junctions or an increased incorporation of E-cadherin remains an open question. We added the FRAP results in Fig.8E,F and in supplemental Fig. S7.

6) Figure 8E - showing images of multiple tAJs, rather than z-slices of a single vertex, would better support the claim here, as the assertion is that Src42a levels are different between control and sdk RNAi conditions, and not that it varies in the z-dimension.

Response:
The image series of now Fig. 8G shows one representative example of multiple tAJs that have been imaged for this experiment (n=6 for wild type and n=10 for sdk RNAi). We think that the presentation of Z-slices for this experiment is important as the protein distribution needs to be considered for a larger area along the apical-lateral cell interface. In addition the quantification of the data for multiple tAJs was presented in Fig. 8H,I as a graph. We would therefore prefer not to change this figure.

Reviewer #1 (Significance (Required)):
The manuscript by Backer et al. examines the function of Src42A in germband extension during Drosophila gastrulation. Prior studies in the field have shown that Src family kinases play an important role in the early embryo, including cellularization (Thomas and Wieschaus 2004), anterior

midgut differentiation (Desprat et al. 2008), and germband extension (Sun et al. 2017; Tamada et al. 2021). In this study, the authors showed that Src42A was enriched at adherens junctions and was moderately enriched along junctions with myosin-II. They then showed that maternal Src42A depletion exhibits phenotypes, starting with cellularization and including a defect in germband extension. The authors focus on defects in germband extension and found that Src42A was required for timely rearrangement of junctions and that the Src42A RNAi phenotype is enhanced by Abl RNAi. Finally the authors show that E-cadherin turnover is affect by Src42A depletion.

Overall, this study provided a higher resolution description of how Src42A regulates the behavior of junctions during germband extension. I thought the authors conclusions were well supported by the data and represent new insight in the field.

Response to reviewer 2

Reviewer #2 Summary:
Chandran et al. investigate the role of Src42A in axis elongation during Drosophila gastrulation. Using maternal RNAi and CRISPR/Cas9-induced germline mosaics, they revealed that Src42A is required to contract junctions at anterior/posterior cell interfaces during cell intercalations. Using time-lapse imaging and image analysis, they further revealed the role of Src42A in E-Cad dynamics at cell junctions during this process.
By analyzing double knockdown embryos for Src42A and Abl, they further showed that Src42A might act in parallel to Abl kinase in regulating cell intercalations. The authors proposed that Src42A is involved in two processes, one affecting tension generated by myosin II and the other acting as a signaling factor at tricellular junctions in controlling E-Cad residence time.
Overall, the data are clear and nicely quantified. However, some data do not convincingly support the conclusion, and statistical analyses are missing for an experiment or two. Methods for several quantifications also need improvement in writing. Also, several figures (Figures 6-8) do not match the citation in the text and need to be corrected.
Page and line numbers were not indicated in the manuscript. For my comments, I numbered pages starting from the title page (Title, page 1; Abstract, page 2, Introduction, pages 3-6; Results, pages 7-14; Discussion, pages 15-18; M&M, 19-23; Figure legends, 28-30) and restarted line numbers for each page. For Figures 6-8 that do not match the citation in the text, I still managed to look at the potentially right panels. All the figure numbers I mention here are as cited in the text. My detailed comments are listed below.

Response:
We apologize for the lack of organization of the manuscript and the figure numbering. In the revised version we have added page numbers, line numbers and we corrected the figure numbers.

Reviewer #2:
1. b-Cat/E-Cad signals at the D/V and A/P junctions in Src42Ai (Figs. 5-6). These data are critical for their major conclusion and should be demonstrated more convincingly.
In Fig. 5A, the authors said, "When the AP border was cut, the detached tAJs moved slower in Src42Ai embryos compared to control (Fig. 5A)". However, even control tAJs do not seem to move that much in the top panels, and I found the images not very convincing.

Response:
We thank the referee for commenting on the lack of clarity in the presentation of the data. The overall movement within the first 10 seconds after the laser cut (determined by movement of adjacent D/V tAJs from each other) was about 2 µm in the wildtype, while in the mutant it was 1 µm. Despite this 50% difference, it may be difficult to appreciate this difference by looking at Fig. 5A in our original submission. The yellow lines in Fig 5A only showed the region of the cut, but did not indicate the movement of the tAJ from each other, which may have led to a distraction from the actual movement. We will change the annotation and the marks within the figure to visualize the movement much more clearly in the full revision.

Reviewer #2: In Fig. 6A, b-Cat signals look fuzzier and dispersed and have more background signals in the control, compared to the Src42Ai background. Also, b-Cat signals in the control image do not seem to show enrichment at the D/V border, as shown in Tamada et al., 2012.

Response:
We agree with the referee that the image in Fig. 6A for the control appears fuzzier and looks dispersed. This is due to the fixation method that we used. In this experiment we did not apply heat fixation, but used formaldehyde fixation in which beta-catenin protein, in addition to the junctional pool, is also maintained in the cytoplasm creating a diffuse cytoplasmic staining. We chose to do this in order to be able to co-immunolabel the embryos with beta-catenin and E-cadherin antibodies; the latter staining is not working in our hands with the heat fixation applied in the Tamada et al. 2012 paper. Despite the slightly lower quality of the staining, the quantification of the data clearly indicated an effect of the Src42A knockdown on the planar polarized distribution of the E-cad/b-cat complex. In the revision, we added a note to the figure legend (line 851ff) indicating that the fixation procedure was not optimized for beta-catenin junctional staining. In the revision we also added a quantification of live-imaging data recording E-cadherin-GFP in wild-type and src42Ai embryos. We present these additional data in Fig. S6 in the revision of the manuscript. These data are consistent with the results in Fig. 6 from the immunolabeling and support our conclusion that E-cadherin AP/DV ratio is increased in Src42A knockdown embryos.

Reviewer #2:
In Fig. 6B, C, it is not clear how the intensity was measured and how normalization was done. Was the same method used for these quantifications as "Protein levels at bicellular and tricellular AJs" on pages 21-22? Methods should be written more explicitly with enough details.

Response:
We thank the referee for pointing out the lack of detail in explaining how the quantification was done. In the revision of the manuscript, we extended a paragraph entitled ‘Protein levels at bicellular and tricellular junctions’ in the methods section (line 540ff) that will serve this purpose and describe the methods that were applied for each quantification and the methods as to how the data were normalized.

Reviewer #2:
Does each sample (experimental repeat) for the D/V border in Fig. 6B match the one right below for the A/P border in Fig. 6C? It should be clearly mentioned in the figure legend. The ratio of the DV intensity to AP intensity will better show the compromised planar polarity of the b-Cat/E-Cad complex.

Response:
We thank the reviewer for pointing out a lack of clarity in our presentation. The experimental repeats for each measurement do indeed match, i.e. the measurement of the DV border matches the same adjacent 4-cell pair in the same embryo and in total 5 distinct embryos were analyzed for each experiment. In the revision of the manuscript, we explain this detail of the experimental design in the figure legend (line 855ff). In the revision, we also determined the ratios of DV/AP cell interfaces for beta-cat and E-cad and added this quantification as panel 6C and 6E for a clearer presentation of the data.

Reviewer #2:
2. Based on the genetic interaction between Src42A and Abl using RNAi (Fig. 7), the authors argue that Src42A and Abl may act in parallel. However, the efficiency of Abl RNAi has not been tested. It can be done by RT-PCR or Abl antibody staining. Also, the effect of Abl RNAi alone on germband extension should be tested and compared with Src42A & Abl double RNAi embryos. I expect the experiments can be done within a few weeks without difficulty.

Response:
We agree with the referee that it is important to determine the level of depletion in Abl RNAi embryos in order to interpret the genetic relationship between Abl and Src42A. We have performed immunolabeling of Src42Ai and Abli embryos and found that Abl levels were significantly reduced, but still detectable at the onset of germband extension. Interestingly, Abl levels were also slightly reduced after Src42A knockdown. Together with published data that indicated Src42A acting upstream of Abl in the control of epithelial plasticity, we revised our initial conclusion and propose that Src42A and Abl act in concert in germband elongation and discuss that they might be acting in a common pathway. We have also conducted single knockdown experiments for both Src42A and Abl

and double knockdown experiments. We find that Abl single knockdown does not affect the fast phase of germband extension but it does affect the slow phase.

Reviewer #2:
Minor comments:
Page 2, line 14 - The abbreviation for tAJs was not introduced before.

Response: Thanks for spotting this. We have now introduced the abbreviation in the abstract (line 35).

Page 7, line 6 - A reference should be cited for the Src42A26-1 allele.

Response: We added the reference Takahashi et al., 2005, where this allele was first described (line 129).

Reviewer #2:
Figure 1
- Fig. 1B: Src42A levels should be compared between control (Src42A/+) and Src42A/Src42A for each stage. It currently shows a comparison between Src42A/Src42A of stages 10 and 15.

Response:
We thank the reviewer for the comment. As indicated in our response to reviewer #1, the point of this analysis was to provide evidence for the specificity of our new anti-Src42A antibody and to demonstrate the presence of substantial maternal contribution of Src42A protein in the zygotic mutant. We do not see the advantage to provide a detailed developmental Western-blot analysis, but we provide data in the supplemental Fig S1E showing that the level of Src42A is unimpaired in stage 6 zygotic Src42A[26-1] homozygous mutant embryos.
In the revised manuscript, we made the rationale and the data more accessible to the reader. At the beginning of the results sections 1 (line130f) and 2 (line158f) of the revised manuscript, we added a sentence each to address the referee's concern that earlier stages exhibit no difference in protein levels and referred to Fig. S1E. We also more explicitly spelled that out that the experiment (referring to Fig.1A,B and S1) was intended to look at zygotic mutants and to demonstrate that our novel Src42A antibody was able to detect the reduction of maternal Src42A protein in mid- to late-stage homozygous zygotic embryos. We added this conclusion in the results section (line 136f).

Reviewer #2:
- Fig. 1B: The figure legend says, "dotted line represents mean value and error bars," but there are no dotted lines shown in the figure. Also, what p-value is for ****? It should be mentioned in the figure legend. It also says Src42A levels were normalized against E-Cad intensity here (stages 10 and 15). They have shown that E-Cad levels are affected in Src42A RNAi during gastrulation (Fig. 6). Is E-Cad not affected in Src42A26-1 zygotic mutants at stages 10 and 15?

Response:
We thank the referee for pointing out inaccuracies in the presentation and the description of Fig.1B. In the preliminary revision, we emphasized the marks on the graph and provide p-values throughout. Regarding the E-cadherin levels: E-cadherin levels were altered in src42A RNAi knockdown embryos, but not in zygotic mutants, even at later developmental stages.

Reviewer #2:
Page 7, lines 6-7 - The localization of Src42A in control should be described in more detail and more clearly here.

Response: In the revised manuscript, we extended our description of the distribution of Src42A in more detail pointing out its dynamics and differential distribution at distinct plasma membrane domains (line 139ff).

Reviewer #2:
Supplemental Fig S1

- Fig. S1D: Based on the head structure and the segmental grooves, the embryo shown here is close to late stage 13/early stage 14, not stage 15.
- Fig S1E: It will be helpful if the predicted protein band and non-specific bands are indicated by arrows/arrowheads in the figure.

Response:
We thank the referee for their careful observation of the embryonic stage. We agree that the embryo was actually a younger stage. In the revision, we replaced the images with an example of an older stage. We will also add clear annotations as arrows to clearly mark the specific protein bands in Fig. S1E.

Reviewer #2:
Page 7, lines 21-22
- "Src42A was slightly enriched at the AP interface" - To argue that, quantification should be provided.

Response:
We thank the referee for pointing out a qualitative statement that we made with regard to the distribution of Src42A at the AP cell interfaces. In the revision of the manuscript, we present an additional quantification of the imaging data of Src42A immunolabeling. In supplementary figure S3A-C, we now present a quantification of the enrichment of Src42A at the tricellular junctions. In addition, the new Fig. S3D,E shows a quantification of the planar polarized distribution of Src42A at the AP cell interfaces.

Reviewer #2:
Page 8, line 14
- "Embryos expressing TRiP04138 showed reduced hatching rates with variable penetrance and expressivity depending on the maternal Gal4 driver used (Fig. 2B)" - Fig. 2B doesn't seem to be a right citation for this sentence.

Response:
We agree with the referee and in the revised manuscript we corrected the reference to the conclusion drawn from Fig. 2A', which does show the relationship of hatching rate to the various maternal Gal4 drivers.

Reviewer #2:
Fig. 2
- Fig. 2B: Higher magnification images of the defective cytoplasm can be shown as insets

Response:
Rather than producing higher magnification of bright field whole mounts of living embryos, we added an analysis of cellularization stage embryos using markers for membrane domains and show these data as supplementary Fig. S4.

Reviewer #2:
- Fig. 2C: It will be helpful to indicate two other non-specific bands in the figure with arrows/arrowheads with a description in the figure legend.

Response:
In the revision, we added an arrow to mark the band specific for Src42A and asterisks to mark unspecific bands in Fig 2C.

Reviewer #2:
- Fig. 2E: A simple quantification of the penetrance of cuticle defects in Src42A mutants and RNAi will be helpful, as shown in Fig. S3.

Response:
We added the quantification of the occurrence of the different classes of cuticle phenotypes as an additional panel in Fig 2E.

Reviewer #2:
Page 9, line 9
- This is the first time that the fast and the slow phases of germband extension are mentioned. As these two phases are used to compare the Src42A and Src42A Abl double RNAi phenotypes, they should be introduced and explained better earlier, perhaps in Introduction.

Response:
We thank the referee for pointing out that the two phases of germband extension were not introduced. In the revised manuscript, we added a sentence to introduce and define the distinct phases of extension movements (line 200).

Reviewer #2:
Fig. 3
- Fig. 3A: It will be helpful to mark the starting and the ending points of germband elongation with different markers (arrows vs. arrowheads or filled vs. empty arrowheads).

Response:
In the revision, we added distinct markers to indicate the start and endpoints of germband elongation and annotated those in the figure legends to improve accessibility of the data.

Reviewer #2:
- Fig. 3G and H should be cited in the text.

Response:
In the revision, we added references to Fig 3G,H in the result section to the annotation of Fig.3F (line 210).

Reviewer #2:
- Fig. 3C figure legend: R2 is wrongly mentioned in Fig. 3D, E. Also, R2 (coefficient of determination) needs to be defined either in the figure legend or Materials & Methods.

Response:
We thank the referee for pointing this misleading reference to us. In the revised manuscript, we corrected the reference to R2 in Fig,3D,E and described the definition of R2 in the figure legend.

Reviewer #2:
- Fig. 3D, E: statistical analysis is missing.

Response:
In the revision, we included a statistical analysis of the data (see ref #1). We changed the figure to indicate the data sets that were analyzed and added the p-values to the figure legend.

Reviewer #2:
- Fig. 3F: It should be mentioned that the heat map is shown for pY20 signals in the figure legend, with an intensity scale bar in the figure.

Response:
In the revision, we added an intensity scale bar to the figure panel and mentioned the relationship to the PY20 signal.

Reviewer #2:
Fig. 7A: Arrows can be added to mark the delayed germband extension.

Response:
In the revision, we added arrows to mark the anterior and posterior extent of the germband.

Reviewer #2:
Fig. 8A: It should be mentioned that the heat map is shown for E-Cad signals in the figure legend, with an intensity scale bar in the figure.

Response:
In the revision, we added an intensity scale to the heat map and mention the relationship to the E-cadherin signal in the figure legend.

Reviewer #2:
Fig. S3G: An arrowhead can be added to the gel image to indicate the band described in the legend.

Response:
In the revision, we added an arrow to help annotating the Src42A-specific bands on the Western blot. This figure panel is now supplementary Fig. S8G in the revised submission.

Reviewer #2:
Fig. 9
- Fig. 9A: Magnified views of the cytoplasmic clearing can be added as insets.

Response: Rather than producing higher magnification of bright field whole mounts of living embryos, we added an analysis of cellularization stage embryos using markers for membrane domains and added these data as supplementary Fig. S4.

Reviewer #2:
- Fig. 9B: Arrow/arrowheads can be added to show the absence of the signals in the nurse cells.

Response:
In the revision, we added markers to help recognizing the reduced signal in the nurse cells and the oocyte.

Reviewer #2:
- Fig. 9C: Indicate the ending point of the germband extension by arrows.

Response: In the preliminary revision, we added arrows to mark the anterior and posterior extent of the germband.

Reviewer #2:
- Page 14, lines 9-10: More explicit description of the phenotype rather than just "stronger compared to Src42Ai" will be helpful.

Response:
In the revised manuscript, we added data showing that cellularization defects in Src42A knockdown embryos result in a more pronounced reduction of cell nuclei in the blastoderm (Fig. S9A,B). In addition, we present data on the hatching of Src42Ai embryos and from Src42A germline clone derived embryos to demonstrate that compared to Src42Ai, embryos derived from Src42A26-1 germline clones do not hatch (Fig. S9C).

Reviewer #2 (Significance (Required)):

This work revealed the role of Src42A in regulating germband extension. A previous study suggested the roles of Src42A and Src64 in this developmental process using a partial loss of both proteins (Tamada et al., 2021). Using different approaches, the authors demonstrated a role of Src42A in regulating E-Cad dynamic at cell junctions during Drosophila axis elongation. Most of the analyses were done with maternal knockdown using RNAi, but they successfully generated germline clones for the first time and confirmed the RNAi phenotypes. Overall, this work contains important and exciting novel findings.

This work will be of general interest to cell and developmental biologists, particularly researchers studying epithelial morphogenesis and junctional dynamics.

I have expertise in Drosophila genetics, epithelial morphogenesis, imaging, and quantitative image analysis.
Response to reviewer 3

Reviewer #3 (Evidence, reproducibility and clarity (Required)):
Chandran et al. report on the function of Src42A during cell intercalation in the early Drosophila gastrula. They create a Src42A-specific antibody (there are two Src genes in the fly genome) and examine the localization of Src42A and observe a planar-polarized distribution at cell interfaces. They then measure cell-contractile dynamics and show that T1 contraction is slower after Src42A disruption. The authors then argue that Src42A functions in a parallel pathway to the Abl protein, and that E-cadherin dynamics (turnover) is altered in Src42A disrupted embryos. Src function at these stages has been studied previously (though not to the degree that this study does), and in some respects the manuscript feels a little preliminary (please label figures with figure number!), but after editing this should be a polished study that merits publication in a developmentally-focused journal.

Reviewer #3
1) Does the argument that Src42A has two functions fully make sense? Myosin II function is known to affect E-cadherin stability (and vice versa), so it seems that Src42A could affect both MyoII and Ecad by either decreasing Myosin II function/engagement at junctions or by destabilizing Ecad.

Response:
We fully agree with the reviewer and realize that the reciprocal relationship between actomyosin and E-cadherin might not be reflected equivocally in our manuscript. As the reviewer points out, one possibility is that Src42A could affect both MyoII planar localization and E-cadherin dynamics through the same pathway. Previous studies showed that Src is involved in translating the planar polarized distribution of the Toll-2 receptor by recruiting Pi3-Kinase activity to the Toll-2 receptor complex resulting in planar polarized distribution of MyoII at the A/P interfaces. These data, however do not exclude another possible mechanisms by which Src42A directly affects the dynamics of a well-known Src target, the E-cadherin/beta-catenin complex and thereby contributes to the delay in germband extension. The observed defects in both studies can be attributed to both a defect in abnormal planar polarization of MyoII and the abnormal dynamics of the E-cadherin/ß-catenin complex. In either of these cases, we suggest that Src42A phosphorylates distinct substrates, the Toll-2 intracellular domain in the MyoII planar polarity pathway and the E-cad/beta-cat complex or components of the machinery controlling E-cad dynamics at cell junctions. Given the relationship between MyoII and E-cadherin, however, it is not possible to decide whether these two effects are independent functions of Src42A or are consequences of each other. Since we cannot resolve a possible epistatic relationship between these two potential activities of Src42A, we decided to extend the discussion on this topic by taking both possible scenarios into account and discussing them appropriately in the revised manuscript. We therefore rewrote parts of the discussion and in the concluding paragraph propose a model that in addition to the function of Src42B as signaling component that translates Toll receptor activity into planar polarity of MyoII it may also act more directly in signal transduction at tAJs controlling the dynamics of the E-cadherin/beta catenin complex (lines 434ff).

Reviewer #3
2) One obvious question that arises is the nature of cleavage defects that are mentioned that happen previously to intercalation. For example, is E-cad normal prior to intercalation initiating? How specific are the observed defects to GBE?

Response:
We thank the referee for bringing up this important point. As indicated in our response to the reviewer #1, we have provided a more extensive analysis of the cellularization phenotypes in the revised manuscript. Our data revealed that the defects in cellularization are mild, leading to occasional dropping of nuclei from the cortex. However, the overall polarity and structure of the blastoderm epithelium remained largely normal, in particular the localization of E-cadherin appeared unimpaired. We also analyzed cell numbers and apical cell areas at the onset of germband elongation. We find that the apical cell areas of germband cells is much more variable and overall larger in Src42Ai embryos and that the cell number is reduced. We suspect that these effects might be consequences of nuclei dropping out defect during cellularization, that may lead to fewer cells with larger apical domains. The altered cell number and associated increase in apical cell area are unlikely to affect germband elongation, as it was shown before that embryos lacking post-blastoderm divisions extend their germband normally (Irvine and Wieschaus, 1994; Edgar and O'Farrell, 1989). Therefore, we conclude that any effects on cellularization in Src42A knockdown

embryos are unlikely to cause the defects described in germband extension. Embryos derived from Src42A germline clones do show changes in egg shape and more penetrant clearing defects in cellularization as compared to Src42A knockdown embryos. We cannot exclude that these defects result in the observed enhancement of the germband elongation phenotype.
We describe these data in two additional paragraphs in the results section (lines 179ff, 236ff, and 335ff) and present the new data in supplemental figures S4, S5, S9 and discuss them appropriately, in order to take these potential limitations in the interpretation of our data into account.

Reviewer #3
3) Pg. 10, "the shrinking junction along the AP axis strongly reduces its length with an average of 1.25 minute" - what is this measurement? How much is "strongly"?

Reviewer #3
4) Also pg. 10, "the AP junction was not markedly reduced after 1 minute" - what is the criteria for this statement? X%? 1 minute is very specific, it feels like how much of a reduction/non-reduction should also be specific.

Response:
We thank the referee for pointing out our inappropriate qualitative statement of the experimental data, which was indeed misleading. The measurement of the shrinking junction was based upon the time it takes for the AP interface junction between two adjacent vertices on the DV axis to shrink into a single 4-cell vertex. The time for this contraction was on average 1 minute 25 seconds. The data in Fig.4 A',C show that after 2 minutes in the control embryo 100% of the observed AP junctions have collapsed and the extension of the new DV junction along AP axis has begun. At the same timepoint of 2 minutes in the src42A knockdown, we show in Fig. 4B',D that the shrinking of the AP junction interface has still not been completed in 60% of the cases. In the revision, we have removed the qualitative statements and replaced them with a correct description of the measurements taken and referred to the data described in Fig. 4 A-D.

Reviewer #3
5) It seemed odd to mention altered myosin levels but then skip over a measurement of myosin in favor of an indirect measurement such as interface recoil. Again (point 1), it seems that changes in Myosin II recruitment could cause changes in Ecad turnover.

Response:
As this analysis has already been performed by Tamada et al. 2021, we decided not to reproduce these data, but rather extend the analysis towards tension measurements, which support the findings by Tamada et al. 2021 on a functional level. We do not see the added value of adding MyoII labeling.

Reviewer #3 Minor notes:

Page 4, missing comma after "For example"
Response: The text was edited accordingly.

Page 4, "inevitable" does not make sense in this context

Response: In the revision, we eliminated 'inevitable' and replaced it with 'critical' to better indicate the importance of Canoe protein for germband elongation.

Reviewer #3 (Significance (Required)):

This study gives a more detailed perspective on how Src proteins (Src42A in Drosophila) control epithelial stability and the contraction of specific surfaces of epithelial cells.

Second decision letter

MS ID#: DEVELOP/2022/201119

MS TITLE: Src42A is required for E-cadherin dynamics at cell junctions during Drosophila axis elongation

AUTHORS: Lenin Chandran, Wilko Backer, Raphael Schleutker, Deqing Kong, Seyed Amir Hamze Beati, Stefan Luschnig, and H.-Arno Muller

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 very positive and we would like to publish your manuscript in Development. Before we can proceed with this, please address the requested clarifications on statistics being used, as documented by all 3 reviewers.

Reviewer 1

Advance summary and potential significance to field

The manuscript examines the function of Src42A in germband extension during Drosophila gastrulation. The authors showed that Src42A was enriched at adherens junctions and was moderately enriched along junctions with myosin-II. They then showed that maternal Src42A depletion exhibits phenotypes, starting with cellularization and including a defect in germband extension. The authors focus on defects in germband extension and found that Src42A was required for timely rearrangement of junctions and that the Src42A RNAi phenotype is enhanced by Abl RNAi. Finally the authors show that E-cadherin turnover is affect by Src42A depletion.

Comments for the author

The authors have thoroughly addressed my comments from the round of review from Review Commons (I was Reviewer #1). One point they should fix before publication is to define the error bars in all figure plots (e.g. is it standard error or standard deviation). Also, when the student's t test is used, the authors should point out whether they tested for normality in the data.

Reviewer 2

Advance summary and potential significance to field

Chandran et al. investigate the role of Src42A in axis elongation during Drosophila gastrulation. Using maternal RNAi and CRISPR/Cas9-induced germline mosaics, they revealed that Src42A is required to contract junctions at anterior/posterior cell interfaces during cell intercalations. Using time-lapse imaging and image analysis, they further revealed the role of Src42A in E-Cad dynamics at cell junctions during this process.

The authors have carefully addressed all my previous questions. The addition of new experimental results, data quantification, and discussion have greatly improved the manuscript. I support the publication of this work in Development.

Comments for the author

I have a few minor comments that the authors may wish to address.

1. Sample numbers in Fig. 3G,H & Fig. S3E Is n=5 the number of embryos? How many junctions have been measured for each embryo? Please provide the total number of junctions measured as well.

From the p values <0.0001 (Fig. 3G,H), I'd assume that the authors used the number of individual data points (junctions) for their statistical analysis rather than the number of embryos.

2. Embryonic stages for Fig. S1 & S2 Fig.S1B and S2B – Based on the morphology, including the tracheal pits, these embryos look like stage 11, not stage 10.
Fig.S1D – based on the thin foregut, compartmentalized midgut, and long convoluted hindgut, this embryo must be stage 16+.
If the authors have used these embryos for their western experiment in Fig. 1S it would be appropriate to label them as stages 10/11 and stages 15+.

3. Fig.7A.
Please use arrows/arrowheads for the start/end site of germband extension as in other figures.

4. Fig. 8D X- and Y-axis labels are inaccurate, and I couldn't really figure out what I should look at in this graph.

Reviewer 3

Advance summary and potential significance to field

Chandran et al. report on the function of Src42A during cell intercalation in the early Drosophila gastrula.
They create a Src42A-specific antibody (there are two Src genes in the fly genome) and examine the localization of Src42A and observe a planar-polarized distribution at cell interfaces. They then measure cell-contractile dynamics and show that T1 contraction is slower after Src42A disruption. The authors then examine the potential contributions of Src42A functions to both Ecad and Myosin behaviors.

Comments for the author

Apologies if I missed it, but are n numbers consistently reported across figures and figure legends? I found it hard to find this info in several instances. Otherwise, the authors have seriously considered the review critiques and responded appropriately (in my opinion). Aside from any minor edits, I think the manuscript is appropriate for publication. Congrats to the authors on a very nice study!

Second revision

Author response to reviewers' comments

We thank the referees for a very positive response to our first revision. In the second revision, we addressed all the remaining concerns of the referees. In brief, here are the changes that we made in the second revision:

Addressing comments by Referee#1: We added a paragraph to the methods section to indicate our application of either D'Agostino & Pearson tests or Kolmogorov-Smirnov tests to determine normality in the data sets, when student's t-tests were used. We added the definition of the error bars for all plots in the respective figure legends.

Addressing comments by Referee#2: We clarified the sample size identity for the quantification of the data in figure panels 3G,H and S3E. We adopted the staging as proposed by referee#2 for Fig. S1, S2. We added arrows in Fig. 7A to point out the posterior extent of the germband as suggested by referee#2. We separated the plots in Fig. 8D in order to avoid confusion with the labeling of the Y axis. We eliminated a mislabeling of the X axis for the difference in means of the estimation plot.

Addressing comments by Referee#2/#3: We also carefully checked that all sample sizes are shown and, where missing, we added them to the figure legends.

Third decision letter

MS ID#: DEVELOP/2022/201119

MS TITLE: Src42A is required for E-cadherin dynamics at cell junctions during Drosophila axis elongation

AUTHORS: Lenin Chandran, Wilko Backer, Raphael Schleutker, Deqing Kong, Seyed Amir Hamze Beati, Stefan Luschnig, and H.-Arno Muller
ARTICLE TYPE: Research Article

Thank you for sending your manuscript to Development through Review Commons.

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