Notch signaling enhances bone regeneration in the zebrafish mandible

Jessica M. Kraus, Dion Giovannone, Renata Rydzik, Jeremy L. Balsbaugh, Isaac L. Moss, Jennifer L. Schwedler, Julien Y. Bertrand, David Traver, Kurt D. Hankenson, Gage Crump and Daniel W. Youngstrom
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Original submission

First decision letter

MS ID#: DEVELOP/2021/199995

MS TITLE: Notch Signaling Enhances Bone Regeneration in the Zebrafish Mandible

AUTHORS: Jessica M. Kraus, Dion Giovannone, Renata Rydzik, Jeremy L. Balsbaugh, Isaac L. Moss, Jennifer L. Schwedler, Julien Y. Bertrand, David Traver, Kurt D. Hankenson, Gage Crump, and Daniel W. Youngstrom

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

As you will see, the referees express considerable interest in your work, but have some significant criticisms and suggestions for improving your manuscript. If you are able to revise the manuscript along the lines suggested, I will be happy receive a revised version of the manuscript. Please also note that Development will normally permit only one round of major revision.

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

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

Reviewer 1

Advance summary and potential significance to field

Humans lack the ability to repair larger bone defects and there is an unmet clinical need to boost bone regeneration following major skeletal trauma. This study shows that modulation of Notch signaling during mandibular bone healing in zebrafish cause lasting changes to regenerative outcome. Inhibition of Notch signaling prevents regeneration, while over-activation improves bone repair. Overall, this study is a novel and important contribution to the bone regeneration field. However, there are several points to be addressed, as explained below.

Comments for the author

Major points:
1) Why does callus formation fail in DBZ treated fish? Is recruitment or expansion of osteochondro-progenitor cells impaired? Is there an increase in cell death etc.? I think the authors should provide some mechanistic insights.
2) The authors compared callus gene expression between DBZ and DMSO treated fish at 10 dpr. This analysis is difficult to interpret, because DBZ treatment inhibits callus formation. Fig. 3 B shows that instead of a callus, there are mainly epidermal and few connective tissue cells in DBZ treated fish. It would be more informative to show immunostainings and demonstrate presence/absence of cell types.
3) Sox9a positivity in hsp:NICD+/- fish. Quantification of Sox9a+ cells is missing. There seem to be more Sox9a+ cells in NICD+ fish at 4 dpr.
4) α-PCNA IHC in hsp:NICD+/- fish. Quantification is missing.
5) The authors demonstrate increased bone quantity in hsp:NICD+ fish. Notch signaling has been shown to control osteoclast formation and resorbtive activity. Osteoclasts should be examined to exclude that the phenotype is not caused by decreased osteoclast numbers/activity.
6) The shift from chondroid to intramembranous bone formation in hsp:NICD+ fish is very interesting. However, the underlying mechanism remains unknown. I think the conclusion “our work specifically implicates Notch signaling in encoding bone fate and metabolism in regenerative Sox9a+ cells” (line 255) is inappropriate. The authors favor a model in which progenitor cells that would otherwise have made chondrocytes (or hybrid bone cells) instead directly differentiate into osteoblasts. An alternative explanation is that different subsets of progenitors within the periosteum can generate chondrocytes and osteoblasts during mandible regeneration. In hsp:NICD+ fish, those progenitors that would have made chondrocytes might simply fail to expand and differentiate, whereas recruitment or expansion of those that differentiate into osteoblast might be enhanced. I think the authors should be more careful in drawing conclusions and discuss alternative explanations for their findings.

Minor points:
1) Please indicate injury margins in all images.
2) Line 164. “suggesting increased osteoblast activity”. Please provide data or discuss alternative explanations (e.g. decreased osteoclast activity, activation of osteoblast progenitors).

Reviewer 2

Advance summary and potential significance to field

In this manuscript the authors demonstrate that manipulation of Notch signaling during regeneration of the mandible following injury leads to long lasting effects on bone quantity, suggesting that there are time windows during the early regenerative process that are highly sensitive to Notch signaling which direct the regeneration process.

The results are interesting and novel and the case for potential applications to address a clinical need are well made.

Comments for the author

In general the paper is well written and easy to read. It has obvious significance for the field and the experiments in which manipulation of notch signaling during regeneration leads to altered bone phenotypes are compelling.
The major comment I have relates to cell types and timing. The authors make a number of statements about how different modes of ossification are giving rise to the effects of reduced or enhanced bone observed (in relation to the degree to which they believe the regeneration is occuring via a chondral intermediat), but dont really go into which of the Notch responsive cell populations 'labelled by their transgene they think are responsible. '
The authors discuss the degree to which regeneration is proceeding via a chondral intermediate. However they have done relatively few experiments to show which cells are responsible for the differences, instead they have observed differences at the tissue level in terms of expression levels via qPCR (10dpr)/ proteomics (performed at 4dpr) and combined these with histology and immunohistochemistry performed at different stages. While these data are interesting and informative they don't really address which cells Notch is affecting and when. Previous work from some of the same authors (Paul et al 2016, Development) showed beautiful imaging of multiple markers of cartilage and bone (spp1, sp7, col2a1, runx2 etc). Not looking at any of these markers, rather relying on Safranin O, sox9 and CT alone feels like a missed opportunity to more conclusively address this issue and identify which cells are responsible.
The proteomic data feels a bit mistimed, as the authors themselves state early Notch manipulation "results in sustained osteoanabolic activity'. Why then look at a time point so early, when as the authors then point out çlassical chondroblast or osteoblast markers were not captured in this experiment due to the early 4dpr harvest' if the goal was to look at the 'molecular basis of the osteoanabolism' why look before osteoblast markers are present. Ideally, one would hope to see some of the proteomic hits taken through to later stages to test whether they are responsible for the sustained osteoanabolism, or (even better) labelling using the markers from the previous Paul et al paper at key stages.

Minor comments. As results come before methods in Development it would be helpful to define acronyms at first use (in results) e.g. page 6 IHC, ICD etc.
Some extra annotation of Fig 1B would be helpful, when e.g. discussion differetnces between chondrocytes/ stromal cells etc, it would be aid visualisation to put arrows to key cellular populations, e.g. the 'small number of her6+ cells lining the bone injury margins'. Similarly where histological sections don't match up as well with the sections used for IHC extra annotation would be helpful
The sentence from line 130-133 is somewhat confusing, as jag1b while most highly expressed was not significantly upregulated according to the data.
Minor rewording could help clarity. While ideally it would be desirable to see the spatial localisation of some of the proteins rather than qPCR as the authors say it is a heterogenous environment in the repair callus, the qPCR is useful.
It would be good to have some explanation as to the choice of timing for the blockade and activation of the Notch pathway. Given that highest signal from the notch reporter line was seen at 10dpr which cells do the authors think are responsible for the differences observed and do the authors think that different effects would be observed if Notch was manipulated during other time windows. There is some discussion of this in relation to 1-3dpr heat shock vs 1-5 (lines 151-152) but this could be expanded to discuss what is happening at a cellular level.

First revision

Author response to reviewers' comments

We extend our sincere thanks to the Reviewers for volunteering their time and expertise. We believeaddressing your critiques has improved the mechanistic clarity of our manuscript.

Reviewer 1

Reviewer 1 Advance Summary and Potential Significance to Field...

Humans lack the ability to repair larger bone defects and there is an unmet clinical need to boost bone regeneration following major skeletal trauma. This study shows that modulation of Notch signaling during mandibular bone healing in zebrafish cause lasting changes to regenerative outcome. Inhibition of Notch signaling prevents regeneration, while over-activation improves bone repair. Overall, this studyis a novel and important contribution to the bone regeneration field. However, there are several points tobe addressed, as explained below.

Reviewer 1 Comments for the Author...

Major points:

1) Why does callus formation fail in DBZ treated fish? Is recruitment or expansion of osteochondro- progenitor cells impaired? Is there an increase in cell death etc.? I think the authors should provide somemechanistic insights.

This was an important unanswered question in our original submission, and we thank the Reviewer for the opportunity to address this. We added an additional time point—6 dpr, so as to capture earliest detectable changes by histology—in which we treated DMSO±DBZ animals with BrdU prior to euthanasia. This allowed us to concurrently stain for *her6*:mCherry, BrdU, and Caspase-3 with DAPI by4-color fluorescent IHC in single sections. We detected a 30.7% reduction in BrdU (p=0.021) but no change in Caspase-3. This data was added to Figure 3. The ratios of BrdU (or Caspase-3) to DAPI or to mCherry were not significantly different, indicating that Notch influences the number of proliferating cells, but not necessarily the ratio of proliferating to total cells within the callus. Furthermore, our new data show that *col2a1a*-positive cartilage callus cells fail to express *col1a1a* following DBZ treatment, suggesting that Notch promotes both initial callus formation/growth and later conversion of the cartilagecallus to *col1a1a*-positive bone.

2) The authors compared callus gene expression between DBZ and DMSO treated fish at 10 dpr. Thisanalysis is difficult to interpret, because DBZ treatment inhibits callus formation. Fig. 3 B shows that instead of a callus, there are mainly epidermal and few connective tissue cells in DBZ treated fish. It would be more informative to show immunostainings and demonstrate presence/absence of cell types.

To address the question of cell types and callus differentiation state, we have supplemented the qPCR data with in situ hybridization for *col1a1a* and *col2a1a*, as these transcripts are abundantly co-expressedin callus progenitor cells (Paul, et al. 2016). This data was added to Figure 3. We now know that, while DBZ results in drastically reduced callus cellularity, the remaining callus cells have an increased *col2a1a*/*col1a1a* ratio, suggesting a difference in transition of *col2a1a*+ cells to bone. We hope that thisaddresses the question as to presence/absence of regenerative cell types.

3) Sox9a positivity in hsp:NICD+/- fish. Quantification of Sox9a+ cells is missing. There seem to be more Sox9a+ cells in NICD+ fish at 4 dpr.

We have now quantified Sox9a, NICD and PCNA IHC at 6, 8, and 10 dpr. This allowed us to capture critical new data. Between 6-10 dpr, Sox9a increases in the *hsp:NICD*(-) group but decreases in the *hsp:NICD*+ group. This shows that increased Notch signaling causes an increased rate of osteogenesis: i.e. earlier loss of Sox9a positivity. As suggested by the reviewer, there also appears to be earlier activation of Sox9a+ cells in the *hsp:NICD*+ group. As quantification at day 4 was not

practical becausethere are relatively few mesenchymal cells at this early time point, we now only show 6-10 dpr time- points in Figure 4 and Supp. Figure 5.

4) α-PCNA IHC in hsp:NICD+/- fish. Quantification is missing.

Continuing from the previous comment, we also quantified PCNA from 6-10 dpr (Supp. Fig. 5). We observed an earlier peak in proliferation in the *hsp:NICD*+ group, peaking at 6 dpr instead of 10 dpr.This was not appreciated during initial qualitative assessment, and we thank the Reviewer for the suggestion.

5) The authors demonstrate increased bone quantity in hsp:NICD+ fish. Notch signaling has been shownto control osteoclast formation and resorptive activity. Osteoclasts should be examined to exclude that the phenotype is not caused by decreased osteoclast numbers/activity.

To address this important point, we performed TRAP staining on 32 dpr samples in the *hsp:NICD*± experiment (Supp. Fig. 4D). We did not detect changes in osteoclast number, bone surface coverage, orany other outcomes from static histomorphometry in mid-callus fields. While we lack functional data regarding resorptive activity of these osteoclasts, taken together with the rest of this body of work, we believe the TRAP data supports a primarily osteoblast-lineage phenotype in this healing model.

6) The shift from chondroid to intramembranous bone formation in hsp:NICD+ fish is very interesting. However, the underlying mechanism remains unknown. I think the conclusion “our work specifically implicates Notch signaling in encoding bone fate and metabolism in regenerative Sox9a+ cells” (line 255) is inappropriate. The authors favor a model in which progenitor cells that would otherwise have made chondrocytes (or hybrid bone cells) instead directly differentiate into osteoblasts. An alternative explanation is that different subsets of progenitors within the periosteum can generate chondrocytes andosteoblasts during mandible regeneration. In hsp:NICD+ fish, those progenitors that would have made chondrocytes might simply fail to expand and differentiate, whereas recruitment or expansion of those that differentiate into osteoblast might be enhanced. I think the authors should be more careful in drawing conclusions and discuss alternative explanations for their findings.

We have softened the language to include statements such as, “While we favor a model in which Notchsignaling causes accelerated ossification of cartilage, an alternative explanation is that Notch signaling selects for a subpopulation of particularly osteogenic progenitor cells.” At 8 dpr, both hsp:NICD(-) and hsp:NICD+ samples appear to have similar Sox9a positivity and cartilage calluses. However, by 10 dpr, hsp:NICD+ fish have developed much reduced Sox9a/cartilage and increased bone. We know from our previous paper that the callus starts out cartilage-like but co-expresses *col2a1a*/*col1a1a* and other cartilage/bone markers. Then, by 10-14 dpr, this hybrid callus shuts off cartilage genes and matures into bone. We now have several lines of evidence that NICD accelerates this conversion from hybrid to bone.Conversely, DBZ treatment results in *col2a1a*+ cells with less *col1a1a*, suggesting a delay in this conversion. Differential recruitment of distinct progenitors could be alternative model but is less supported by the current data. Future studies could incorporate single-cell technologies and in vitro assays to dive deeper into these models.

Minor points:

1) Please indicate injury margins in all images.

Injury margins have now been marked in all histology images using yellow arrows.

2) Line 164. “suggesting increased osteoblast activity”. Please provide data or discuss alternative explanations (e.g. decreased osteoclast activity, activation of osteoblast progenitors).

This language was changed to “suggesting global changes to skeletal metabolism,” as we did not perform histomorphometry on these vertebrae (they were destroyed during paraffin histology of themandible). We plan to more specifically study changes in vertebral bone in future investigations.

Reviewer 2

Reviewer 2 Advance Summary and Potential Significance to Field...

In this manuscript the authors demonstrate that manipulation of Notch signaling during regeneration ofthe mandible following injury leads to long lasting effects on bone quantity, suggesting that there are time windows during the early regenerative process that are highly sensitive to Notch signaling which direct the regeneration process.

The results are interesting and novel and the case for potential applications to address a clinical need arewell made.

Reviewer 2 Comments for the Author...

In general the paper is well written and easy to read. It has obvious significance for the field and theexperiments in which manipulation of notch signaling during regeneration leads to altered bone phenotypes are compelling.

The major comment I have relates to cell types and timing. The authors make a number of statements about how different modes of ossification are giving rise to the effects of reduced or enhanced bone observed (in relation to the degree to which they believe the regeneration is occurring via a chondral intermediate), but don't really go into which of the Notch responsive cell populations 'labelled by theirtransgene they think are responsible.'

We have completed new time point experiments and completed IHC for molecular markers, and this haspermitted us to refine our proposed model. New data suggests that Notch accelerates conversion of the hybrid callus to bone, as well as being required for early proliferation and expansion of the callus.

The authors discuss the degree to which regeneration is proceeding via a chondral intermediate. However they have done relatively few experiments to show which cells are responsible for the differences, instead they have observed differences at the tissue level in terms of expression levels via qPCR (10dpr)/ proteomics (performed at 4dpr) and combined these with histology and immunohistochemistry performed at different stages. While these data are interesting and informative they don't really address which cells Notch is affecting and when. Previous work from some of the sameauthors (Paul et al 2016, Development) showed beautiful imaging of multiple markers of cartilage and bone (spp1, sp7, col2a1, runx2 etc). Not looking at any of these markers, rather relying on Safranin O, sox9 and CT alone feels like a missed opportunity to more conclusively address this issue and identify which cells are responsible.

We agree that the initial gene expression, proteomics, μCT, histology and IHC data provided bulk characterization but left some unanswered questions with respect to early progenitor cell biology. This isa critique shared by Reviewer 1, and we have followed your suggestion to incorporate in situ hybridization, as well as quantitative BrdU and Caspase-3 analysis, to assess the activation and multipotentiality of these cells during the early stages of healing. We elected to stain for *col1a1a* and *col2a1a* transcript as per Paul, et al. 2016 because they are specific to the callus mesenchyme and allow us to monitor the conversion of the hybrid callus to bone, which we find delayed in DBZ. Throughout the manuscript, we now also have immunostaining at cellular resolution for *her6*:mCherry, Sox9a, PCNA, Caspase-3, and endogenous NICD. Since the majority of the wild-type callus stains for *her6*:mCherry, and various combination of *col1a1a* and/or *col2a1a*, we infer a primary contribution to the remaining bulk mRNA/protein data from Notch+ osteochondral repair cells. Incorporating the suggested experiments has provided improved cellular mechanism to our manuscript, and we thank the reviewer for their suggestion. Specifically, our data support a biphasic role of Notch signaling in callus cell recruitment and the conversion of cartilage to bone. We recognize that some knowledge gaps remainregarding the source and potential subpopulations of callus cells. The text has been substantially revised to emphasize what we have discovered and hypothesize, while acknowledging remaining questions that will be fruitful areas of future investigation.

The proteomic data feels a bit mistimed, as the authors themselves state early Notch manipulation ‘’results in sustained osteoanabolic activity’. Why then look at a time point so early, when as the authorsthen point out classical chondroblast or osteoblast markers were not captured in this experiment due to the early 4dpr harvest’ if the goal was to look at the ‘molecular basis of the osteoanabolism’ why look before osteoblast markers are present. Ideally, one would hope to see some of the proteomic hits taken through to later stages to test whether they are responsible for the sustained osteoanabolism, or (even better) labelling using the markers from the previous Paul et al paper at key stages.

We prioritized a time point for proteomic analysis 3 hours after the last heat shock so that we could capture changes on the time scale of hours post-Notch transgene activation, rather than changes incidental to downstream recomposition of callus tissue days later. We have added a line explaining thisto the results/discussion. The *col2a1a/col1a1a* ISH following DBZ treatment at 6 dpr shows that this is an active time for callus differentiation. Unfortunately, sampling multiple timepoints is beyond the scope of this study. We have emphasized how early Notch signaling patterns the callus (up to 10 dpr) but agree that, considering the proteomic data, the healing trajectory at intermediate timepoints would beinteresting to examine in the future.

Minor comments.

As results come before methods in Development it would be helpful to define acronyms at first use (in results) e.g. page 6 IHC, ICD etc. Some extra annotation of Fig 1B would be helpful, when e.g. discussion differences between chondrocytes/ stromal cells etc, it would be aid visualisation to put arrows to key cellular populations, e.g. the 'small number of her6+ cells lining the bone injury margins'.Similarly where histological sections don't match up as well with the sections used for IHC extra annotation would be helpful

Abbreviations for NICD, DBZ, DMSO, IHC, and ISH have now been moved to place of first mention (body instead of methods). µCT outcomes like BV are redundantly defined in the body and methods, subject to editorial revision. Some additional annotations have been added to Figure 1B and defined inthe caption for: Meckel’s chondrocytes (MC), stromal/mesenchymal cells (SM), and repair chondroblasts (RC). Also, injury margins have now been marked in all histology images using yellowarrows to better orient the reader.

The sentence from line 130-133 is somewhat confusing, as jag1b while most highly expressed was notsignificantly upregulated according to the data. Minor rewording could help clarity. While ideally it would be desirable to see the spatial localisation of some of the proteins rather than qPCR as the authors say it is a heterogenous environment in the repair callus, the qPCR is useful.

For clarity, this line has been changed, to include “However, *jag2b* and *dlc* were upregulated in the context of injury, while *jag1b* was not.” We completely agree that spatial localization would be valuablehere, considering the heterogeneity of the tissue biopsies for qPCR. We plan to further dissect the extracellular component of Notch signaling in the context of bone regeneration in future studies, to potentially include in situ hybridization for the various ligands and receptors in callus mesenchyme.

It would be good to have some explanation as to the choice of timing for the blockade and activation ofthe Notch pathway. Given that highest signal from the notch reporter line was seen at 10dpr which cellsdo the authors think are responsible for the differences observed and do the authors think that different effects would be observed if Notch was manipulated during other time windows. There is some discussion of this in relation to 1-3dpr heat shock vs 1-5 (lines 151-152) but this could be expanded to discuss what is happening at a cellular level.

The greatest quantity of her6:mCherry+ cells are present at 10 dpr, but we wanted to block the activationof these cells entirely by early blockade of Notch signaling over the first several dpr. As you mention, we observed similar effects with slightly modified early blockade. We are very interested in what Notch inhibition/overactivation would do if initiated later on in the healing process, and plan to explore this in the future. We believe there is stronger translational potential in focusing on perioperative interventions.As a result of this comment and others of the review, we have increased the specificity of our mechanism/conclusions throughout the manuscript, and thank

you for your insights. Specifically, our data now support two roles of Notch: 1) required for early callus expansion/proliferation, 2) necessaryand sufficient to promote the conversion of the (hybrid) cartilage callus to bone. It is likely that *her6* does not reflect this early role in expansion, and it is possible/likely that Notch impacts distinct target genes during its roles in early versus late mandibular bone regeneration.

Summary of Changes

In addition to extensive changes throughout the text, we have incorporated the following new data:

A new DMSO±DBZ experiment was performed with a 6 dpr harvest preceded by BrdU treatment. Thisfacilitated quantitative assessment of proliferation and death of progenitors resulting from early DBZ treatment. Our finding of reduced BrdU+ cells provided novel mechanism to the non-union phenotype.

In situ hybridization for *col1a1a* and *col2a1a* was performed on both new and existing 10 dpr slides forthe DBZ experiment. The Paul, et al. 2016 protocol was replicated in the Youngstrom Lab, resulting in important mechanistic insights into the effect of Notch signaling dose on the differentiation states of thehybrid cartilage/bone progenitor cells characteristic of this healing model. Specifically, Notch signalingdose regulates the conversion of the *col2a1a*/*col1a1a* callus into bone.

IHC figures were quantified for Sox9a, PCNA, NICD, BrdU, and Caspase-3. Our initial qualitative findings related to Sox9a were corroborated by this new data, but in the process, we also identified amore subtle shift in the temporal activity of PCNA in our gain-of-function experiment. These data support *hsp:NICD*+ accelerating osteogenesis, again adding to mechanism.

TRAP staining was performed on existing 32 dpr slides from the NICD experiment to rule out the possibility of bone accrual through inhibition of osteoclasts. While we have not ruled out changes in osteoclast activity earlier in the healing process, this negative data argues against bone accrual by NICDoverexpression being largely due to impaired bone resorption.

We again would like to thank the Reviewers and the Editorial team for their feedback and for the opportunity to share our exciting work in Development.

Second decision letter

MS ID#: DEVELOP/2021/199995

MS TITLE: Notch Signaling Enhances Bone Regeneration in the Zebrafish Mandible

AUTHORS: Jessica M. Kraus, Dion Giovannone, Renata Rydzik, Jeremy L. Balsbaugh, Isaac L. Moss, Jennifer L. Schwedler, Julien Y. Bertrand, David Traver, Kurt D. Hankenson, Gage Crump, and Daniel W. Youngstrom

I have now received all the referees reports and there are just a couple of minor points to address before we proceed to publication. 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.

Reviewer 1

Advance summary and potential significance to field

see previous

Comments for the author
The authors' response and the incorporated new data have addressed all my concerns. I am satisfied with the revisions made on the manuscript.
I believe that this manuscript is a novel and important contribution to the field with obvious potential applications to address clinical needs.

Minor comments:
1) Some of the figure labels are very small and/or difficult to read.
2) Line 186-198. The increase in col2a1/col1a1 ratio in DBZ treated fish is an important finding. It would be good to have some explanation as to the choice of this markers and the ratio. The readers may not know that col2a1 and col1a1 are chondrocyte and osteoblast markers, respectively.

Reviewer 2

Advance summary and potential significance to field

The authors have satisfactorily addressed the issues raised during the review, and I support acceptance of the article

Comments for the author

The authors have satisfactorily addressed the issues raised during the review, and I support acceptance of the article

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Second revision

Author response to reviewers' comments

Reviewer 1

Reviewer 1 Advance Summary and Potential Significance to Field...

see previous

Reviewer 1 Comments for the Author...

The authors' response and the incorporated new data have addressed all my concerns. I am satisfied with the revisions made on the manuscript. I believe that this manuscript is a novel and important contribution to the field with obvious potential applications to address clinical needs.

Minor Comments:

1) Some of the figure labels are very small and/or difficult to read.

Figures 1-4 were revised after reviewing Development figure guidelines, and font sizes were increased where necessary. We hope this adds clarity to our data presentation.

2) Line 186-198. The increase in col2a1/col1a1 ratio in DBZ treated fish is an important finding. It would be good to have some explanation as to the choice of this markers and the ratio. The readers may not know that col2a1 and col1a1 are chondrocyte and osteoblast markers, respectively.

Thank you for this good point. We have changed lines 186-190 to directly and concisely state the rationale and interpretation of the new in situ hybridization data.

Reviewer 2

Reviewer 2 Advance Summary and Potential Significance to Field...

The authors have satisfactorily addressed the issues raised during the review, and I support acceptance of the article.

Reviewer 2 Comments for the Author...

The authors have satisfactorily addressed the issues raised during the review, and I support acceptance of the article.

Concluding Remarks

Thank you to the Reviewers for your contribution to the improvement of this manuscript.

Third decision letter

MS ID#: DEVELOP/2021/199995

MS TITLE: Notch Signaling Enhances Bone Regeneration in the Zebrafish Mandible

AUTHORS: Jessica M. Kraus, Dion Giovannone, Renata Rydzik, Jeremy L. Balsbaugh, Isaac L. Moss, Jennifer L. Schwedler, Julien Y. Bertrand, David Traver, Kurt D. Hankenson, Gage Crump, and Daniel W. Youngstrom
ARTICLE TYPE: Research Report

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