The atypical RNA-binding protein Taf15 regulates dorsoanterior neural development through diverse mechanisms in *Xenopus tropicalis*

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

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend substantial revisions are needed prior to considering publication. In addition to some new experiments (including more morpholino experiments and better controls), the reviewers suggest that the manuscript could be better structured and written to help readers. If you are able to revise the manuscript along the lines suggested, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision.

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

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

This manuscript describes the characterisation of Xenopus loss of function of TAF15, a RNA splicing factor belonging to the FUS family. It combines MO and sgRNA approaches and provides a brief morphological description, some gene expression pattern at gastrula-neurula stage and an RNAseq datasets. Overall, the study brings some interesting elements together to give an idea of what TAF15 may do to transcripts in the embryos. However, the various elements are somehow giving a feeling of random pick of gene expression changes grabbing the interest of the authors but not really emerging from an unbiased rational analysis. The finding of intron retention is of great interest but somehow gets reduced to a specific case. My view is that the study needs to be reshaped in depth to present a more coherent and in depth understanding of these loss of function models.

Comments for the author

Specific points:
Fig. 1 needs more characterisation of the depleted animals than the general morphology of the late embryo.

The MO and sgRNA embryos look significantly different morphologically as the MO is presumed maternal and zygotic loss of function (M+Z). We also need to see the embryo for splice-blocking Z only MO. The impossibility of rescuing phenotypes by expressing the normal TAF15 makes it a bit tricky, lacking solid controls. Do we know how many TAF15 splice variants there are (at least four suggested by the western in Fig. 1)? Are there splice variant specific expression pattern? Is the “zygotic” splice MO affecting all splice variants? These Qs need to be answered to accurately evaluate the meaning of the experimental data.

Why was RNAseq not done with both M+Z MO and Z MO?

The RNAseq section is dramatically lacking of rigorous details. Are the 100 genes affected in both stages having up-regulated and/or down-regulated transcripts in the MO? What about analysing stage 10 alone as the earlier the best to identify direct events after loss of function.

Fig. 2C is a percentage, which can be interpreted in two ways: the maternal IR is more abundant than normal in the TAF KD, or the zygotic intron retention is less abundant than expected. No validation is done on embryos with intron probe.

Fig. 2D is not compelling as the M+Z qRT-PCR values are not reflecting relative abundance between the transcripts validated, found in the RNAseq (example: gpr110 is by far the most abundant in the RNAseq and is not detectable in the qRT-PCR).

Are the IR mostly in the first intron or is fgfr4 an exception? What is the overall data there? You have a chance to provide some mechanistic inside on intron retention due to TAF15 loss of function.

The increase of intron retention of fgfr4 is only significant in the M+Z MO. This is interpreted as a maternal versus zygotic difference. The same MO shows no difference in RNA level between zygotic and uninjected suggesting that the transcript level increases in M-Z MO but not significantly decrease in zygotic MO. The results with sgRNA is much clearer and yet this decrease in the sgRNA injected is seen while the mosaicism of greater and lowering of TAF15 across the embryos described as much less efficient. To really trust the conclusion, specific intron ISH needs to be done in MZ and Z embryos to compare with the fgfr4 probe in Fig 4.

The clear lateral expansion of the territories on the injected side in Fig 4 and to a lesser extent Fig 7 suggests a delay in convergence on that side, or a change in D/V gene regulation and point to an earlier effect of TAF15. If expansion was due to DV fate changes, it would rather suggest that losing TAF15 is reducing BMP contrary to the ventx results and the model shown in Fig 6. So, lateral expansion of the neural plate is likely to be due to delay in convergence. Is there any information from the stage 10 RNAseq suggesting such event?

In Figure 6, double in situ would really be needed to identify the cells where TAF and ventx are complementary or co-expressed. As it is presented now, it is really hard to say whether in the forebrain they truly overlap or ventx sits at the border of TAF (embryos compared are not exactly at the same stage as neurulation is extremely dynamic at this stage.

Although the direct or indirect regulation of vntx by TAF15 is clear, it does not explain the many aspects of the phenotypes described at stage 15 and seems a very random pick of one event amongst many revealed by the RNAseq.

Reviewer 2

Advance summary and potential significance to field

The manuscript describes the role of TAF15 in early development. The authors suggest that TAF15 regulates neural development by regulating the expression of fgfr4 and ventx2.1. They propose that maternal and zygotic TAF15 regulate gene expression via distinct mechanisms.
The work is one of the first describing a function for the RNA binding protein in development.

Comments for the author

The manuscript by deJong et al. entitled “The atypical RNA-binding protein TAF15 regulates dorsoanterior neural development through diverse mechanisms in Xenopus tropicalis” describes the role of TAF15 in early development. The authors suggest that TAF15 regulates neural development by regulating the expression of fgfr4 and ventx2.1. They propose that maternal and zygotic TAF15 regulate gene expression via distinct mechanisms but the evidence to support these conclusions are not very strong.

Major points
1) Much of the interpretation of the data centered on the assumption that the translation blocking morpholino depletes both maternal and zygotic TAF15 while the splice blocking morpholino will only target the zygotic version. While this assumption is reasonable, the differences in phenotypes could also be due to difference in morpholino efficiency. For such strong claims regarding the different properties of maternally-derived or zygotically-derived TAF15, they need to provide stronger evidence that the translation blocking morpholino significantly diminished TAF15 protein levels prior to zygotic genome activation. Also, from their own splicing analysis, they suggest that some maternally-derived RNA are spliced post-fertilization and that TAF15 is involved in that process (Figure 2). If true, then they need to demonstrate that the splice blocking morpholino has no impact on maternally-derived TAF15 RNA.

2) In most cases, the in situ hybridization patterns shown are different between the controls (mismatch MO vs. Cas9 only). Variation in in situ signal intensity often occurs when experiments are done on different days, but it makes it hard to interpret the validity of the differences claimed across experimental samples. In particular, it is difficult to determine if the taf15 sgRNA+Cas9 injected embryos show similar effects to the Z TAF15 depleted embryos.

3) Maternal transcripts should be fully processed, so the finding that maternal RNAs in M+Z depleted TAF15 embryos have splicing defects is somewhat unprecedented. If this finding is true, then these TAF15-regulated maternal RNAs should be unspliced earlier in development before zygotic genome activation, such as in 2-cell stage embryos. For the validated TAF15-regulated maternal RNAs (Figure 2D), this should be documented via RT-PCR or from analysis of published RNA-seq from pre-ZGA stages.

4) Morpholinos can have unwanted off-target effects. To validate the taf15 morpholino effects were not off-target, they used taf15 sgRNA+Cas9 injections as a second approach, yet key controls are missing for these experiments. First, the effectiveness of taf15 mutagenesis in taf15 sgRNA+Cas9 injected versus Cas9 only injected was not demonstrated. Second, the phenotype of taf15 sgRNA+Cas9 injected embryos was not shown in Figure 1 so it is unclear if it did phenocopy the effect of Z TAF15 depletion. Indeed, throughout the manuscript the data are not always consistent between the taf15 sgRNA+Cas9 and Z TAF15 depletion suggesting that one or both of the approaches result in additional off-target effects.

5) There is a general paucity of quantification throughout the manuscript. While it is difficult to quantify the broad and diffuse in situ hybridization patterns of taf15 or fgfr4, it is unclear even how heterogeneous the staining is within the analyzed embryos. At a minimum, the authors need to include the number embryos analyzed and the number that displayed a similar pattern to the representative image shown in each figure. For the more restricted pax2 expression pattern, additional quantification of the signal using image quantification should be include.

Specific figure comments

Figure 1. Image of the taf15 sgRNA + Cas9 injected embryos needs to be included. Also need to include what stage were the embryos used for western blot analysis in 1E.
Figure 3. In figure 3C, the statistical comparison shown is for M+Z TAF15 and ZTAF15 depleted, but the correct comparison should be each of those to uninjected and mismatch MO controls.
Figure 4. In figure 4F, western blot analysis of the taf15 sgRNA + Cas9 injected embryos is missing.
Figure 6. It is difficult to compare cross section images as they are not shown in the same way especially F' and K.

Reviewer 3

Advance summary and potential significance to field

This study hits on few significant issues: 1) Although basic mechanisms of RNA splicing are relatively well understood, mechanisms that regulate alternative splicing are still emerging and this is an active field of discovery; 2) How RNA splicing regulates development and differentiation is a critical issue that is even less understood than general splicing mechanisms; 3) TAF15 and related EWS and FUS are proteins that can uniquely function as regulators of both gene transcription and RNA splicing, but they have been studied mostly in the context of neurodegenerative diseases and cancer. Their normal biological functions and mechanisms are poorly understood. This paper advances all of these issues by first demonstrating that TAF15 is essential for dorso-anterior development of Xenopus embryos and they show that TAF15 acts mechanistically to regulate the splicing and transcription of hundreds of maternally and zygotically expressed genes. Although the authors identify hundreds of genes affected by TAF15, they focus attention on two major targets: fgfr4 and venbtx2.1. They show that TAF15 promotes maternal fgfr4 mRNA splicing and subsequent zygotic fgfr4 transcription. In contrast, TAF15 negatively regulates zygotic ventx2.1 expression but not mRNA splicing. This study advances general knowledge by showing that a FET family member has dual actions as regulator of transcription and RNA splicing, and that those actions are critical for embryonic development. The results advance specific knowledge of TAF15 gene targets and molecular actions. The normal biological functions of FET proteins are largely unknown, so the present study makes significant advances in understanding this novel gene family.

Comments for the author

The authors have used morpholino knockdown and CRISPR/Cas9 mutagenesis to demonstrate that TAF15 is essential for dorsoanterior development in Xenopus embryos as a regulator of maternal RNA splicing and zygotic transcription. TAF, EWS and FUS constitute a small family function in gene transcription and RNA splicing, and although they have been studied in disease contexts, there is little understanding of their normal biological functions. Here the authors have made a significant advance in understanding the molecular and biological/developmental roles of TAF15 by blocking its expression in Xenopus embryos to reveal its developmental actions and its gene targets. Loss of TAF15 causes abnormal dorso-anterior development, particularly in ectodermal tissues, and results

in aberrant splicing (intron retention) and altered levels of maternal and early zygotic transcripts. A unique subset of genes were perturbed transcriptionally and post-transcriptionally and among those, fgfr4 and ventx2.1 were evaluated in detail. The results strongly support the conclusions that TAF15 can regulate splicing of maternal transcripts such as fgfr4, and that after zygotic gene activation TAF15 can regulate zygotic transcript levels.

This study is technically well done and reveals significant new information on the function of TAF15 and the FET family of proteins, and it would be acceptable for publication after a few Figure and textual changes described below, and some optional data analysis or experiments (and be acceptable for publication).

Topic: taf15 expression and knockdown phenotypes The taf15 expression pattern and knockdown phenotype results (Fig 1) are straightforward but a few points should be addressed for better understanding.

- Provide a schematic indicating the location of the MO and crispr target sites in taf15
- Is the western blot upper band also TAF15? Does it jibe with predicted TAF15 isoforms?
- Was TAF protein quantification (Fig 1E) an average of more than one run?
- The TAF loss of function phenotypes are rather pleiotropic and can't be rescued by simple mRNA injection, and I agree that it is difficult to rescue splicing or other factors that have complex expression patterns or are very sensitive to dose. The fact that the production of TAF15 protein was assessed in the MO or crispr treatments is reasonable evidence that the intended target was knocked down.
- An alternative SUGGESTION is to attempt to rescue a "molecular phenotype" of TAF15 knockdown using an animal cap ectoderm assay, for example to score restoration of normal RNA splicing or transcript levels for fgfr4 or other target mRNAs.

TOPIC: Maternal TAF15 regulates splicing of developmental regulators (line 472)
It is very interesting and significant that RNA-seq identified over 1000 genes that exhibited intron retention in gastrula and neurula stage embryos when TAF15 was knocked down. This indicates a broad impact of TAF15 in embryonic cells and developmental processes.
Understanding this section was a bit difficult to follow and could be made more clear.

- I had to read these Results and consult the data in Fig 2 and the supplemental tables several times to get the logical flow and deduce how the large set of candidates were eventually narrowed down for validation tests by qRT-PCR. For example, the text between lines 496 through 500 (and the Fig 2B, C and D text) could be improved to better clarify how and why the 100 genes with stage-persistent DEU were reduced to just 11 for validation of intron retention. It took some contemplation of the data summaries in Fig 2B to realize that those 11 genes were selected out of the 100 because they parsed into the "Developmental Process" GO category, and that the 12 cases of intron retention noted in line 500 correspond 11 genes because dgka had two exons retained.
- The reader could benefit from a more detailed explanation of why fgfr4 was selected for in-in more detaildepth analysis. I surmise that it was selected mainly because fgfr4 was the top candidate based on the "p" and "adjusted p" values in Sup Table 1? Did the validation results in Fig 2D, or other reasons, factor into the choice?
- Advocating for those who don't regularly work with RNA-seq and next-gen statistics, the contents of Sup Table 1, which seemed to inform the decision to focus on fgfr4 could be explained in more detail to improve the reader's understanding. For instance, what was evaluated to determine statistical significance, i.e. what data were those p values derived from? What was the "adjustment" to the p value? Also, are the values in columns CTRL_ST10 and TAF15MO_ST10 the RPKM values or something else?
- In supp Table 3 what does inverse intron usage mean? Is it relevant? Why are some genes underlined?
- Can you include some more data (in the Supplement would be fine) so we can learn more about the intron retention behavior of genes (besides fgfr4) listed in Fig 2D that passed qRT-PCR validation? It was nice to get a visual depiction of the data showing intron retention dynamics of Isl1 with the Integrative Genome Viewer in Sup Figure 1. Can the RNA-seq read maps for the other validated genes be presented in the same way?
- Might it be possible to use retained intron sequences to glean an explanation or hypothesis about how TAF15 engages in intron removal? For instance, can compare the sequences of the retained introns? Might the retained introns of the "100 genes" with DEU (in response to M+Z TAF15

knockdown) share conserved sequences that are binding sites for splicing factors or RNA binding proteins that potentially interact with TAF15?

TOPIC: Depletion of TAF15 causes fgfr4 intron 1 retention (paragraph at 506)
The story of how TAF15 depletion affects fgfr4 is presented very clearly in Figures 3 and 4 and the ms text. A coherent set of data and a very plausible scenario are presented that argue for a dual action of TAF15, first on maternal fgfr4 splicing of intron 1, and subsequently on the transcriptional activity of zygotic fgfr4. The fgfr4 transcript dynamics in response to M+Z or zygotic TAF15 knockdown were reflected by the spatial expression of fgfr4 in embryos scored by in situ hybridization (Fig 4 A-E). The reduction in TAF15 and FGFR4 protein expression upon TAF15 knockdown nicely confirms that the retention of intron 1 interferes with FGFR4 protein synthesis even though transcript levels are elevated. It's an interesting finding. The authors also convincingly show that two FGFR4 target genes in the anterior neural region
(pax2 and engrailed 2) are reduced by TAF15 knockdown to produce an interesting multi-step regulatory.

Minor points:
- Note that in Figs 3 and 4, the TAF15 MOs or CRISPR/Cas9 were (presumably) injected into both cells of 2 cell stage embryos that were subsequently evaluated by qRT-PCR or Western blot, whereas embryos evaluated by in situ hybridization were injected with these reagents into one blastomere at the 2-cell stage.
- Was genomic DNA sequenced to assess the types of F0 CRISPR mutations and the level of mosaicism (% normal and % mutated DNA)?

TOPIC: Changes in gene expression and Ventx regulation by TAF15 (Figs 5 and 6):
The RNA-seq data gathered from TAF15 morphant neurulae were evaluated for differential expression (DESeq) and about 2,000 candidates were filtered by GO categories to capture genes with developmental and ectodermal expression (Fig 5A,B).
- The results were presented as a heatmap of up and down regulated genes (Fig 5C), but the figure legend or ms text do not specify what the columns in the heatmap represent. One might assume each column reflects individual embryo data, but please provide the information.

The ventx2.1 gene was a top hit from the DE results, and the embryonic expression patterns of taf15 and ventx2.1 are reasonably complementary, which is consistent with the possibility that TAF15 suppresses Ventx2 expression. This possibility is supported by results showing expanded ectodermal ventx2 expression in the neural border region upon knockdown of TAF15
(Fig 6). Panels 6E' and F' show reasonably distinct expression of taf15 in the neural and epidermal ectoderm, and ventx2 in lateral plate mesoderm, but there is uncertainty (due to slight blue staining) whether each gene has low expression in the adjacent germ layer or the stain simply diffused a bit. More importantly, the TAF15 knockdown embryos show expanded ventx2.1 expression in the MO-injected side (panels I and J) with significant ectopic expression of ventx2.1 in the injected neural plate (panel K).
- Although close inspection shows a thin boundary separation of the neural ectoderm and lateral plate mesoderm, an arrowhead or two pointing out that boundary would help the reader. Alternatively, make that image much larger.
- There seems to be a morphological abnormality in that injected region (thickened ectoderm and mesoderm). Is that part of the real phenotype or experimental aberration? Do you have adjacent cross-sections of that embryo or another that could be substituted or added to Supplemental data?
- Panel L is helpful to interpret the territory of TAF15 knockdown, but I think it would work better if this was paired with bigger images of I and J. The diagram of the model for TAF15 repression of the ventrolateral BMP/Vent circuit is not described in the legend or ms text.

The final data figure examines changes in Ventx2.1 expression levels and patterns in response to TAF15 M+Z knockdown. The results of RNA-seq read mapping and qRT-PCR are solid and clearly support the conclusion that ventx2.1 expression is elevated upon maternal or zygotic TAF15 inhibition.
- The authors state that the RNA-seq data show elevated expression of ventx2.1 at stage 15 but not at stage 10, but the number of reads mapped across ventx2.1 in the Genome Viewer at

stage 10 are, to my eye, higher in the MO-injected than in the uninjected embryo, making that data inconsistent with the qRT-PCR results at stage 10. Please revisit this point in the ms text.

- The embryonic in situ expression patterns in 7C-L bolster the results shown in Fig 6.

Altogether the data support the proposal that TAF15 represses ventx2.1, and since ventx2.1 derepression can be detected at stage 10, it seems quite probable (though not unequivocal) that TAF15 acts directly on vent2.1 transcription. CHiP-qPCR with TAF15 antibodies could resolve this, but I don't propose the authors test that. Instead, I suggest (if possible; not required) that qRT-PCR analysis of TAF15 morphant animal cap explants might more clearly resolve whether TAF15 represses ventx2.1 in the ectoderm. Also, the ability to assess vent2.1 expression at its earliest timepoints could better support whether this relationship is direct.
I realize that limited access to labs in this Covid-19 era may preclude any additional experiments, so any suggested here are just that, suggestions. Revision of the text and figures would be sufficient for resubmission, but ideally it would be great to also address any suggestions for non-wet lab data analysis.

First revision

Author response to reviewers' comments

Address to the Reviewers:

We thank the reviewers for taking the time and making the effort to share their insightful and thoughtful questions and comments; we believe their input has helped us clarify and improved the manuscript. We hope the reviewers agree that we have reasonably addressed their experimental concerns in light of the current laboratory constraints of the Sars-Cov2 pandemic and we thank them for their time and effort in suggesting changes and improvements to the manuscript.

Reviewer 1 Advance Summary and Potential Significance to Field:
This manuscript describes the characterisation of Xenopus loss of function of TAF15, a RNA splicing factor belonging to the FUS family. It combines MO and sgRNA approaches and provides a brief morphological description, some gene expression pattern at gastrula-neurula stage and an RNAseq datasets. Overall, the study brings some interesting elements together to give an idea of what TAF15 may do to transcripts in the embryos. However, the various elements are somehow giving a feeling of random pick of gene expression changes grabbing the interest of the authors but not really emerging from an unbiased rational analysis. The finding of intron retention is of great interest but somehow gets reduced to a specific case. My view is that the study needs to be reshaped in depth to present a more coherent and in depth understanding of these loss of function models.

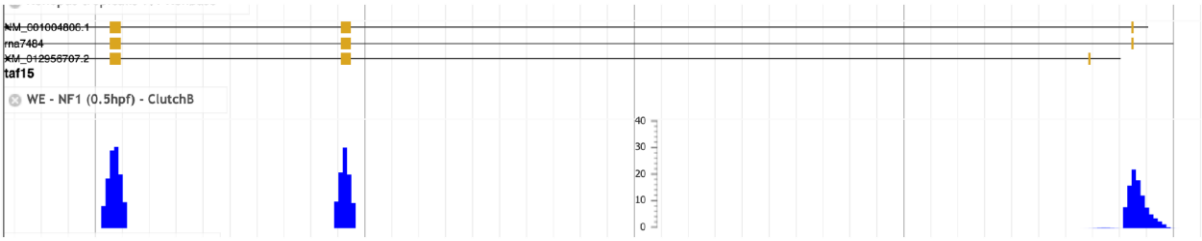
Reviewer 1

Comments for the Author:
Specific points:
Fig. 1 needs more characterisation of the depleted animals than the general morphology of the late embryo. The MO and sgRNA embryos look significantly different morphologically as the MO is presumed maternal and zygotic loss of function (M+Z). We also need to see the embryo for splice-blocking Z only MO.

We thank the reviewer for their interest in the morphology of the depleted embryos. We focused our study on st.32 embryos because while we are able to see molecular changes in earlier embryos following TAF15 depletion (see Figures 2-7), it was at this later stage that one could appreciate the developmental changes that occur following TAF15 depletion. We believe this later stage characterization is best way to visualize the phenotype associated with TAF15 depletion. This justification has been added to the manuscript on lines 450-454. As for the embryo with splice-blocking morpholino, we have included a picture of the ZTAF15-depleted morphant in Figure 1 to complete the comparison of TAF15 depletion by st.32.

The impossibility of rescuing phenotypes by expressing the normal TAF15 makes it a bit tricky, lacking solid controls. Do we know how many TAF15 splice variants there are (at least four suggested by the western in Fig. 1)? Are there splice variant specific expression pattern? Is the "zygotic" splice MO affecting all splice variants? These Qs need to be answered to accurately evaluate the meaning of the experimental data.

We know from JBrowse (Version 10) on www.xenbase.org that there are two *taf15* isoforms; one isoform expressed off of the canonical transcription start site (TSS)(NM_001004806.1), and another expressed off of a downstream TSS(XM_012956707.2). When comparing the levels of expression throughout development (NF1 through NF10; screenshot below of NF1 for illustration; the 5' end is on the right) we find them to be expressed at very different levels. We are not always able to find evidence of transcripts from the downstream TSS but when we do, the downstream TSS composes (on average) 1% of the total *taf15* transcript.



We cannot say if there are splice variant specific expression patterns having not conducted an ISH for transcripts off of the downstream TSS. None of the molecular tools used to deplete TAF15 target the 1% of transcripts from the downstream TSS(XM_012956707.2); they are all targeted to deplete the 99% of transcripts from the canonical TSS(NM_001004806.1). However, given that the downstream TSS isoform makes up an average 1% of the total *taf15* expression we are confident that we are targeting the vast majority (99%) of *taf15* transcripts. We propose that the different TAF15 bands observed in the Western blot are possibly due to post-translational modifications as reduction in their expression is also observed following TAF15 depletion (Fig1E and Fig4F); suggesting that the TAF15 that corresponds to these bands are affected by our depletion tools. We have added this clarification to lines 478-484 of the manuscript.

Why was RNAseq not done with both M+Z MO and Z MO?

We observed a consistently greater TAF15 depletion (as indicated by WB of stage 15 embryo lysates) following M+Z TAF15 depletion compared to Z-only TAF15 depletion. Figure 1F indicates TAF15 expression reduced to 30% following M+Z TAF15 depletion versus 46% following Z-only TAF15 depletion. We did observe a comparable TAF15 depletion reduced to ~20% (Fig4F) following M+Z or Z-only TAF15 depletion but, more generally, TAF15 was consistently more reduced following M+Z TAF15 depletion. As such, we chose to perform RNAseq analysis on the embryos that indicated a more consistently significant TAF15 depletion. This justification has been added to lines 491-494 of the manuscript.

The RNAseq section is dramatically lacking of rigorous details. Are the 100 genes affected in both stages having up-regulated and/or down-regulated transcripts in the MO?

We apologize for not making this clearer. The 100 genes affected in both stages ("stage-persistent") are those that exhibit intron retention (differential exon usage (DEU)); this list does not focus on genes that have an increase or decrease in expression level, but instead, highlights changes in intron/exon retention. We have clarified this distinction in the text. This clarification was added to lines 510-513 of the manuscript.

What about analysing stage 10 alone as the earlier the best to identify direct events after loss of function.

Thank you for making this suggestion. We chose to focus on DEU genes that were affected in both stages 10 and 15 to generate a list of genes whose intron usage was most robustly affected throughout development. At your suggestion, we have compared the top 100 DEU genes (as sorted by adjusted P value) at stage 10 to the 100 stage- persistent DEU genes and we found that 53% of

the stage-persistent genes are also found within the top 100 DEU genes at stage 10; evenly distributed throughout. This finding suggests that our method for generating a list of genes whose exon usage is affected throughout development was sound. We have included this finding, that 53% of the stage-persistent DEU genes are found within the top 100 DEU at st.10, to the manuscript on lines 520-524.

Fig. 2C is a percentage, which can be interpreted in two ways: the maternal IR is more abundant than normal in the TAF KD, or the zygotic intron retention is less abundant than expected. No validation is done on embryos with intron probe.

Thank you for suggesting this alternative hypothesis. We were not able to perform validation of maternal intron- retentions using intron-specific ISH probes. We have tried to detect the *fgfr4* intron by qRT-PCR (in the following embryos: uninjected, M+ZTAF15 MO, mismatchMO) in pre-ZGA. We could detect some *fgfr4* intron 1 in M+Z TAF15 depleted embryos in stage 2-8 but the Cq values for *fgfr4* intron 1 were >35 making them hard to trust. We have proposed the alternative hypothesis that the zygotic retention is less abundant than expected at lines 517-518 of the manuscript.

Fig. 2D is not compelling as the M+Z qRT-PCR values are not reflecting relative abundance between the transcripts validated, found in the RNAseq (example: gpr110 is by far the most abundant in the RNAseq and is not detectable in the qRT-PCR).

We apologize for not making this clearer. The purpose of the qRT-PCR was to validate the RNAseq results. In doing this, we found that 3/12 of the RNAseq candidates were not able to be validated by a second method (qRT-PCR).
The remaining 9/12 candidates were clearly validated by RNAseq, indicating these targets were real and worth pursuing further. This clarification has been added to lines 524-530 of the manuscript.

Are the IR mostly in the first intron or is fgfr4 an exception? What is the overall data there? You have a chance to provide some mechanistic inside on intron retention due to TAF15 loss of function.

We examined the top 10 DEU at stage 10 and 15 (as sorted by adjusted P value) and find retained introns throughout the transcripts (see excel file included below). FGFR4 is not the exception in retaining the first intron, we see six other transcripts with this signature in this cohort. The most common intron retention signature is a single intron retention affecting 13/20 transcripts in this cohort. We have included this table as well as more examples of transcripts with affected DEU (using Integrative Genome Viewer; supplemental figures 2 and 3) to illustrate the different characteristics. (Supplemental Table 7). This elaboration has been added to lines 604-612 of the manuscript.

The increase of intron retention of fgfr4 is only significant in the M+Z MO. This is interpreted as a maternal versus zygotic difference. The same MO shows no difference in RNA level between zygotic and uninjected, suggesting that the transcript level increases in M-Z MO but not significantly decrease in zygotic MO. The results with sgRNA is much clearer and yet this decrease in the sgRNA injected is seen while the mosaicism of greater and lowering of TAF15 across the embryos described as much less efficient. To really trust the conclusion, specific intron ISH needs to be done in MZ and Z embryos to compare with the fgfr4 probe in Fig 4.

Figure 3B is measuring intron retention in *fgfr4*, across all depletion conditions. To be sure we are assaying intron retention specifically, we normalized to the total expression of the transcript. As a result, in 3B we find the only condition with intron retention is with M+Z TAF15 depletion. There is not a significant difference in intron retention in the other conditions. In Figure 3C we are measuring total transcripts only, not looking at intron retention. In this case, we see relatively similar fgfr4 transcript levels in all conditions, with a trend towards more fgfr4 transcript in the M-Z TAF15 depleted embryos and a trend towards decreased fgfr4 expression in the Z TAF15 depleted animals. We believe the trend towards increased total *fgfr4* transcript level with M+Z TAF15 depletion is due to failure of the transcripts with a retained intron to undergo degradation in this condition (interestingly, this finding is why we realized we needed to normalize the

retained intron to total transcript to avoid artificially inflating the amount of measured retained intron). To be clear, we believe that even though there are differences in the total transcript levels seen with the different depletion conditions, because we have normalized intron retention to total transcript we can be confident in our conclusion of the specific intron retention we see with M-Z TAF15 depletion. While we agree that a specific intron ISH is one way to observe intron retention between TAF15 depletion conditions, we respectfully disagree that this is required to interpret our results. The qRT-PCR primers are specific to the retained intron and serve as a sound additional method for measuring intron retention across depletion conditions once corrected for by total *fgfr4* expression. For these reasons we feel we have sufficient evidence to indicate the specificity of intron retention in the M+ZTAF15 depletion condition.
With Z TAF15 depletion (either by MO or CRISPR Cas9) we observed a decrease in total *fgfr4* transcript levels; albeit with a statistically significant depletion seen in the sgRNA treatment (Figure 3C). The average *fgfr4* levels are very similar between the Z TAF15 MO and sgRNA treatment but there is more variation observed with Z TAF15 MO making this condition not statistically significant with the current number of embryos. Total transcript qRT-PCR findings are validated by ISH in Figure 4 A-E. The trend in transcript levels holds true in two independent methods of Z TAF15 depletion, and is confirmed by ISH. This explanation has been added throughout section "Depletion of M+Z TAF15 leads to intron retention in *fgfr4*" of the manuscript.

The clear lateral expansion of the territories on the injected side in Fig 4 and to a lesser extent Fig 7 suggests a delay in convergence on that side, or a change in D/V gene regulation and point to an earlier effect of TAF15. If expansion was due to DV fate changes, it would rather suggest that losing TAF15 is reducing BMP, contrary to the ventx results and the model shown in Fig 6. So, lateral expansion of the neural plate is likely tobe due to delay in convergence. Is there any information from the stage 10 RNAseq suggesting such event?

We agree that these ISH results are suggestive of a delay in convergence but we also observe that the neural tube does close, suggesting that the delay must be modest. This explanation has been added to the manuscript on lines 587-589.

In Figure 6, double in situ would really be needed to identify the cells where TAF and ventx are complementary or co-expressed. As it is presented now, it is really hard to say whether in the forebrain they truly overlap or ventx sits at the border of TAF (embryos compared are not exactly at the same stage as neurulation is extremely dynamic at this stage.

While we agree that a double *in situ* would be one way to decipher if *taf15* and *ventx2.1* are complementary or co- expressed, the single ISH data we currently have is highly consistent and reproducible (>12 embryos from varied clutches). We interpret the early ISH findings in Figure 6 C,D to clearly illustrate complementary *taf15* and *ventx2.1* expression patterns between the future dorsal and ventral sides of the gastrulating embryo. Looking later during neurulation, the ISH in Figure 6 E',F' suggests the possibility of both overlapping (within the neural "n" tissue) and complementary expression (lateral plate "lp" vs overlying ectoderm "e"). Taken together, these results suggest that whether *taf15* and *ventx2.1* are expressed in an overlapping and complementary pattern to one another is dependent on both the timing and placement of expression throughout development. This explanation has been added to the manuscript on lines 669-679.

Although the direct or indirect regulation of vntx by TAF15 is clear, it does not explain the many aspects of the phenotypes described at stage 15 and seems a very random pick of one event amongst many revealed by the RNAseq.

We agree that there are many aspects of the TAF15 depletion phenotype that cannot be explained by changes in *ventx2.1* expression alone. In an effort to focus on a few candidate genes that exhibit differential expression following TAF15 depletion, we employed PANTHER GO term analysis. Based on the ectodermal expression pattern of *taf15* (Figure 1,6) we looked closer at changes to genes involved in both developmental process and ectoderm development (Figure 5B). Within this cohort we found members of the *ventx* family of genes affected (increased) following TAF15 depletion. Because *taf15* is expressed in the future dorsal domain of the gastrula, we considered that a gene family (*vent*) that is known to play a role in ventral tissue development was interesting. In addition to the *ventx* family of genes we also screened other candidates by ISH

for changes in expression pattern following TAF15 depletion. The expression patterns of *ventx2.1* specifically (not true for all the *ventx* candidates) and *taf15* appeared to be complementary or overlapping throughout development which suggested to us that they could be closely linked. Our data suggests a relationship (indirect or direct) between *ventx2.1* and *taf15* that merited further investigation but by no means provides the full picture of the role TAF15 has on *Xenopus* development. We have included the full lists of affected genes in supplementary tables (differential expression and differential exon usage). This explanation has been added to the manuscript on lines 622-646.

Reviewer 2 Advance Summary and Potential Significance to Field:
The manuscript describes the role of TAF15 in early development. The authors suggest that TAF15 regulates neural development by regulating the expression of fgfr4 and ventx2.1. They propose that maternal and zygotic TAF15 regulate gene expression via distinct mechanisms. The work is one of the first describing a function for the RNA binding protein in development.

Reviewer 2 Comments for the Author:
The manuscript by deJong et al. entitled "The atypical RNA-binding protein TAF15 regulates dorsoanterior neural development through diverse mechanisms in Xenopus tropicalis" describes the role of TAF15 in early development. The authors suggest that TAF15 regulates neural development by regulating the expression of fgfr4 and ventx2.1. They propose that maternal and zygotic TAF15 regulate gene expression via distinct mechanisms but the evidence to support these conclusions are not very strong.

Major points
1) Much of the interpretation of the data centered on the assumption that the translation blocking morpholino depletes both maternal and zygotic TAF15 while the splice blocking morpholino will only target the zygotic version. While this assumption is reasonable, the differences in phenotypes could also be due to difference in morpholino efficiency. For such strong claims regarding the different properties of maternally-derived or zygotically-derived TAF15, they need to provide stronger evidence that the translation blocking morpholino significantly diminished TAF15 protein levels prior to zygotic genome activation.
Also, from their own splicing analysis, they suggest that some maternally-derived RNA are spliced post- fertilization and that TAF15 is involved in that process (Figure 2). If true, then they need to demonstrate that the splice blocking morpholino has no impact on maternally-derived TAF15 RNA.

We thank the reviewer for suggesting this control. To address the reviewers comments we re-ordered Genetools "hold backs" of the splice-blocking and translation-blocking TAF15 morpholinos for revision experiments. We have performed a Western blot including 2-cell stage through zygotic genome activation into early gastrulation (stage 11/12) and were able to confirm that embryos injected with the re-ordered splice-blocking morpholino do not reduce their TAF15 expression until after zygotic genome activation (and therefore do not affect maternal TAF15).

Further, we demonstrate by WB that TAF15 expression levels vary stochastically pre-ZGA (Supplemental Figure 1). This is observed both in the uninjected control embryos as well as the mismatch morphants where there is stage-to- stage variation in TAF15 detection pre-ZGA; importantly in the absence of the splice-blocking (Z) TAF15 morpholino. In the ZTAF15-depleted embryos we do see a dip in TAF15 expression in the 8-cell embryos but based on the variation we observer in pre-ZGA stages in the uninjected and mismatch morphant blots, we do not interpret this to be indicative of ZTAF15 morpholino-specific depletion. We do, however, interpret the post-ZGA reduction in TAF15 expression to be specific to the splice-blocking morpholino as TAF15 expression at these stages appears more stable across conditions (by Western blot); specifically stages 9, 10, and 11.

Unfortunately, when we performed the same depletion time course using the re-ordered translation-blocking TAF15 morpholino we experienced technical difficulties with this batch of the morpholino as it proved to be ineffective at depleting TAF15 (as assayed by Western blot) and toxic (embryos exogastrulated); results inconsistent with previous batches of this same MO. Due to this technical difficulty, we are only able to provide data supporting that the splice- blocking

TAF15 morpholino only affects zygotic TAF15 and not maternal TAF15 (Supplemental figure 1). This additional information has been added to lines 534-537 of the manuscript.

2) In most cases, the in situ hybridization patterns shown are different between the controls (mismatch MO vs. Cas9 only). Variation in in situ signal intensity often occurs when experiments are done on different days, but it makes it hard to interpret the validity of the differences claimed across experimental samples. In particular, it is difficult to determine if the taf15 sgRNA+Cas9 injected embryos show similar effects to the Z TAF15 depleted embryos.

We agree with the reviewer that the injected sides (as indicated by *) in Figure 4B and D do have a differing *fgfr4* expression pattern between the two depletion conditions. The discrepancy in expression pattern for this TAF15 target (*fgfr4*), could possibly be due to differences in TAF15 depletion efficiency as well as some variable delay in development we sometimes observed with the ZTaf15 MO (as the injected side of the embryo in Fig4B looks delayed in addition to having reduced *fgfr4*); Explanation added to lines 587-589 of the manuscript. In looking at the qRT-PCR data measuring *fgfr4* (Fig 3C), which is a more objective and quantifiable method for measuring transcript levels, we do see consistent reduction in *fgfr4* expression between the ZTAF15 morphant and taf15 sgRNA+Cas9 depleted embryos. Looking at additional markers (*pax2*, *en2* (Figure 4), *ventx2.1* (Figure 6)) either by ISH or qRT-PCR, we find the results to be consistent between the ZTAF15 morphant and taf15 sgRNA+Cas9 depleted embryos, suggesting similar effects between the two methods aimed at depleting zygotic TAF15.

3) Maternal transcripts should be fully processed, so the finding that maternal RNAs in M+Z depleted TAF15 embryos have splicing defects is somewhat unprecedented. If this finding is true, then these TAF15-regulated maternal RNAs should be unspliced earlier in development before zygotic genome activation, such as in 2-cell stage embryos. For the validated TAF15-regulated maternal RNAs (Figure 2D), this should be documented via RT-PCR or from analysis of published RNA-seq from pre-ZGA stages.

We agree with the reviewer that the finding that maternally deposited RNAs exhibit splicing defects in M+Z depleted TAF15 embryos is surprising and interesting. We appreciated the reviewer's suggestions on how to validate these findings. However, there are a number of issues that preclude these methods from being conclusive tests for this finding. First, *fgfr4* intron 1 expression in stage 2-8 embryos is below the limit of detection for this method. In fact, we have attempted to detect *fgfr4* intron 1 in early embryos in uninjected, M+Z TAF15 depleted embryos, and mismatch MO-injected embryos. The only condition where we could detect some *fgfr4* intron 1 by qRT-PCR was in the M+Z TAF15 depleted embryos but the Cq values were >35 making any findings not trustworthy. Second, given the low expression of *fgfr4* intron 1 in these early embryos, we examined various RNAseq data of pre-ZGA stages (available on Xenbase) but did not find libraries with detectable *fgfr4* intron 1 transcripts. We believe this is likely due to a lack of sequencing depth of the RNAseq libraries needed to find the *fgfr4* intron 1 transcript at these pre-ZGA stages. We suspect RNAseq libraries specifically from M+Z TAF15 morphants would be required to detect *fgfr4* intron 1 at pre- ZGA stages. Given these issues, we do not feel that there is presently an immediately accessible method to confirm the findings proposed in our paper. That being said, we have shown a significant enrichment in differential exon usage for maternally deposited genes (within the stage-persistent cohort, Figure 2C) and these data very clearly support our conclusion that more RNAs that are known to be maternally deposited exhibit affected intron retention following M+Z TAF15 depletion than zygotically transcribed genes. While consistent with this conclusion, we agree with the reviewer that there are alternative hypotheses that could explain these findings: maternal TAF15 could be affecting the DEU of zygotically transcribed targets that just happen to also be maternally deposited (e.g. *fgfr4*) (added to lines 805-806 of the discussion) or that fewer zygotic genes have splicing defect than expected (versus an enrichment for maternally deposited genes; added to lines 517-518 of the manuscript).

4) Morpholinos can have unwanted off-target effects. To validate the taf15 morpholino effects were not off-target, they used taf15 sgRNA+Cas9 injections as a second approach, yet key controls are missing for these experiments. First, the effectiveness of taf15 mutagenesis in taf15 sgRNA+Cas9 injected versus Cas9 only injected was not demonstrated.

The reviewer is correct that we did not evaluate the effectiveness of *taf15* mutagenesis in the *taf15* sgRNA+Cas9 embryos. Instead, we decided to evaluate the efficiency of TAF15 depletion across all of our conditions (morphant and CRISPR) by Western blot as well as changes in TAF15 targets (by ISH and qRT-PCR). Our data indicate consistency in changes to TAF15 targets across the various depletion methods, suggesting that even if there are off- target effects, the on-target effects which we discuss are specific to TAF15 depletion. To thoroughly demonstrate a lack of off target mutagenesis in the *taf15* sgRNA+Cas9 condition, we would need to sequence the embryos and then validate that any mutations did have specific effects on development; this was beyond the scope of our study.

Second, the phenotype of taf15 sgRNA+Cas9 injected embryos was not shown in Figure 1 so it is unclear if it did phenocopy the effect of Z TAF15 depletion.

We do include an image of the taf15 sgRNA+Cas9 injected embryos but did not have a ZTAF15 MO embryo, that has now been added (Figure 1D).

Indeed, throughout the manuscript the data are not always consistent between the taf15 sgRNA+Cas9 and Z TAF15 depletion suggesting that one or both of the approaches result in additional off-target effects.

We agree with the reviewer that the results for the *fgfr4* ISH are not always consistent between the taf15 sgRNA+Cas9 and Z Taf15 depletion, however we do observe consistent results between the two methods throughout the rest of the paper. There are differences in TAF15 depletion efficiency between these two techniques (WB in Fig 1E) therefore it is possible these *fgfr4* ISH differences are due to a more complete depletion (and possibly increased delay) with the ZTAF15 morpholino. We address the differences in *fgfr4* expression between conditions in lines 576- 578 and 587-589 of the manuscript.

5) There is a general paucity of quantification throughout the manuscript. While it is difficult to quantify the broad and diffuse in situ hybridization patterns of taf15 or fgfr4, it is unclear even how heterogeneous the staining is within the analyzed embryos. At a minimum, the authors need to include the number embryos analyzed and the number that displayed a similar pattern to the representative image shown in each figure. For the more restricted pax2 expression pattern, additional quantification of the signal using image quantification should be include.

Fortunately, the images that have been included in the manuscript are all representative of consistently reproducible results. We agree with the reviewer and have added to the figure legends that the images are of representative embryos as well as the number of embryos represented.

Specific figure comments
Figure 1. Image of the taf15 sgRNA + Cas9 injected embryos needs to be included.

We agree with the reviewer that it is important to compare the morphology of the ZTAF15 MO with the taf15 sgRNA+Cas9. Therefore, we include an image of the taf15 sgRNA+Cas9 injected and have added an image of the ZTAF15 MO embryo Figure 1D.

Also need to include what stage were the embryos used for western blot analysis in 1E.

Thank you for pointing this out. We have now included the embryonic stage for the Western blot in the Figure legend; stage 15.

Figure 3. In figure 3C, the statistical comparison shown is for M+Z TAF15 and ZTAF15 depleted, but the correct comparison should be each of those to uninjected and mismatch MO controls.

We apologize for not being clearer. We did run these analyses. As we described in the legend, all means were compared to one another by one-way ANOVA and a Tukey post-hoc analysis. In this instance, it was only the M+ZTAF15 and ZTAF15 depletion conditions that were significantly

different from one another. All other comparisons were not statistically significant. Even though the *fgfr4* expression only trends lower in the ZTAF15 depleted embryos, as measured by qRT-PCR, we know that FGFR4 levels are affected in ZTAF15 depleted embryos as measured by Western blot and ISH of downstream targets.

Figure 4. In figure 4F, western blot analysis of the taf15 sgRNA + Cas9 injected embryos is missing.

We do not have Western blot analysis for FGFR4 of taf15 sgRNA+Cas9 embryos. We do have Western blot analysis showing TAF15 depletion (Figure 1), as well as qRT-PCR and ISH showing depleted *fgfr4* (Figures 3 and 4) as well as reduced expression of FGFR4 targets (*pax2* and *en2*). These ISH and qRT-PCR results are consistent with the M+ZTAF15 and ZTAF15 morphant embryos (which do show depleted FGFR4 by Western blot). We believe, given these findings, that there is clear evidence supporting that the taf15 sgRNA+CAS9 treatment are behaving similarly to our morpholino treatments.

Figure 6. It is difficult to compare cross section images as they are not shown in the same way, especially F' and K.

We apologize for not being clearer. To compare changes in *ventx2.1* expression (following TAF15 depletion), the reader should look at the uninjected side of the embryo within the same panel (K). This has been clarified in the figure legend stating "All injections for downstream RNA ISH were into one cell of 2-cell stage embryos; uninjected side serves as internal control.".

Reviewer 3 Advance Summary and Potential Significance to Field:
This study hits on few significant issues: 1) Although basic mechanisms of RNA splicing are relatively well understood, mechanisms that regulate alternative splicing are still emerging and this is an active field of discovery; 2) How RNA splicing regulates development and differentiation is a critical issue that is even less understood than general splicing mechanisms; 3) TAF15 and related EWS and FUS are proteins that can uniquely function as regulators of both gene transcription and RNA splicing, but they have been studied mostly in the context of neurodegenerative diseases and cancer. Their normal biological functions and mechanisms are poorly understood. This paper advances all of these issues by first demonstrating that TAF15 is essential for dorso-anterior development of Xenopus embryos, and they show that TAF15 acts mechanistically to regulate the splicing and transcription of hundreds of maternally and zygotically expressed genes. Although the authors identify hundreds of genes affected by TAF15, they focus attention on two major targets: fgfr4 and venbtx2.1. They show that TAF15 promotes maternal fgfr4 mRNA splicing and subsequent zygotic fgfr4 transcription. In contrast, TAF15 negatively regulates zygotic ventx2.1 expression but not mRNA splicing. This study advances general knowledge by showing that a FET family member has dual actions as regulator of transcription and RNA splicing, and that those actions are critical for embryonic development. The results advance specific knowledge of TAF15 gene targets and molecular actions. The normal biological functions of FET proteins are largely unknown, so the present study makes significant advances in understanding this novel gene family.

Reviewer 3 Comments for the Author:
The authors have used morpholino knockdown and CRISPR/Cas9 mutagenesis to demonstrate that TAF15 is essential for dorsoanterior development in Xenopus embryos as a regulator of maternal RNA splicing and zygotic transcription. TAF, EWS and FUS constitute a small family function in gene transcription and RNA splicing, and although they have been studied in disease contexts, there is little understanding of their normal biological functions. Here the authors have made a significant advance in understanding the molecular and biological/developmental roles of TAF15 by blocking its expression in Xenopus embryos to reveal its developmental actions and its gene targets. Loss of TAF15 causes abnormal dorso- anterior development, particularly in ectodermal tissues, and results in aberrant splicing (intron retention) and altered levels of maternal and early zygotic transcripts. A unique subset of genes were perturbed transcriptionally and post-transcriptionally and among those, fgfr4 and ventx2.1 were evaluated in detail. The results strongly support the conclusions that TAF15 can regulate splicing of maternal transcripts such as fgfr4, and that after zygotic gene activation TAF15 can regulate zygotic transcript levels.

This study is technically well done and reveals significant new information on the function of TAF15

and the FET family of proteins, and it would be acceptable for publication after a few Figure and textual changes described below, and some optional data analysis or experiments (and be acceptable for publication).

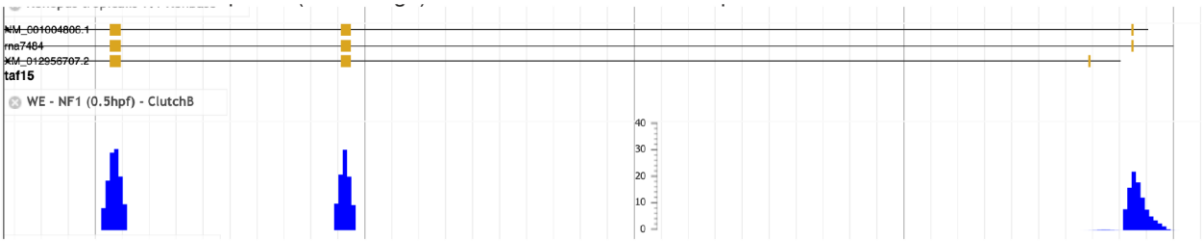
Topic: taf15 expression and knockdown phenotypes
The taf15 expression pattern and knockdown phenotype results (Fig 1) are straightforward but a few points should be addressed for better understanding.

- **Provide a schematic indicating the location of the MO and crispr target sites in taf15**

Thank you for this suggestion, we have made this schematic and have added it to Figure 1 (panel C).

•Is the western blot upper band also TAF15? Does it jibe with predicted TAF15 isoforms?

We know from JBrowse (Version 10) on www.xenbase.org that there are two *taf15* isoforms; one isoform expressed off of the canonical transcription start site (TSS)(NM_001004806.1), and another expressed off of a downstream TSS(XM_012956707.2). When comparing the levels of expression throughout development (NF1 through NF10; screenshot below of NF1 for illustration; the 5' end is on the right) we find them to be expressed at very different levels. We are not always able to find evidence of transcripts from the downstream TSS but when we do, the downstream TSS composes (on average) 1% of the total *taf15* transcript.



We cannot say if there are splice variant specific expression patterns having not conducted an ISH for transcripts off of the downstream TSS. None of the molecular tools used to deplete TAF15 target the 1% of transcripts from the downstream TSS(XM_012956707.2); they are all targeted to deplete the 99% of transcripts from the canonical TSS(NM_001004806.1). However, given that the downstream TSS isoform makes up an average 1% of the total *taf15* expression we are confident that we are targeting the vast majority (99%) of *taf15* transcripts. We propose that the different TAF15 bands observed in the Western blot are possibly due to post-translational modifications as reduction in their expression is also observed following TAF15 depletion (Fig1E and Fig4F); suggesting that the TAF15 that corresponds to these bands are affected by our depletion tools. We have added this clarification to lines 478-484 of the manuscript.

•Was TAF protein quantification (Fig 1E) an average of more than one run?

The quantification in Figure 1E is of the Western displayed. This particular blot was chosen as a representative blot; displaying a typical amount of TAF15 depletion. This western blot was repeated in >3 independent experiments. We have added this quantification detail and the number of independent experiments to the figure legend.

•The TAF loss of function phenotypes are rather pleiotropic and can't be rescued by simple mRNA injection, and I agree that it is difficult to rescue splicing or other factors that have complex expression patterns or are very sensitive to dose. The fact that the production of TAF15 protein was assessed in the MO or crispr treatments is reasonable evidence that the intended target was knocked down.
•An alternative SUGGESTION is to attempt to rescue a "molecular phenotype" of TAF15 knockdown using an animal cap ectoderm assay, for example to score restoration of normal RNA splicing or transcript levels for fgfr4 or other target mRNAs.

We agree with the reviewer that this is a very reasonable way to attempt a rescue and are very interested in trying it out. Unfortunately, due to COVID restrictions, and problems with our X. tropicalis colony, we will not be able to attempt this experiment in time for re-submission.
TOPIC: Maternal TAF15 regulates splicing of developmental regulators (line 472)
It is very interesting and significant that RNA-seq identified over 1000 genes that exhibited intron retention in gastrula and neurula stage embryos when TAF15 was knocked down. This indicates a broad impact of TAF15 in embryonic cells and developmental processes. Understanding this section was a bit difficult to follow and could be made more clear.

•I had to read these Results and consult the data in Fig 2 and the supplemental tables several times to get the logical flow and deduce how the large set of candidates were eventually narrowed down for validation tests by qRT-PCR. For example, the text between lines 496 through 500 (and the Fig 2B, C and D text) could be improved to better clarify how and why the 100 genes with stage-persistent DEU were reduced to just 11 for validation of intron retention. It took some contemplation of the data summaries in Fig 2B to realize that those 11 genes were selected out of the 100 because they parsed into the "Developmental Process" GO category, and that the 12 cases of intron retention noted in line 500 correspond 11 genes because dgka had two exons retained.

We apologize for not being clearer and thank the reviewer for taking the time and effort to bring this to our attention. We have made changes throughout the section, "Maternal TAF15 regulates splicing of developmental regulators" and hope it is now easier for the reader to understand.

•The reader could benefit from a more detailed explanation of why fgfr4 was selected for in-in more detail depth analysis. I surmise that it was selected mainly because fgfr4 was the top candidate based on the "p" and "adjusted p" values in Sup Table 1? Did the validation results in Fig 2D, or other reasons, factor into the choice?

We generated our list of possible candidate genes to further investigate for differential exon usage (DEU) first based on where they ranked by their adjusted P-value. Next, we examined the RNAseq alignments using DEXseq DEU models and Integrative Genome Viewer (IGV) to visualize the DEU's. We then selected a number of genes whose DEU could robustly visualize in DEXseq gene models and IGV and validated their DEU by qRT-PCR. Through each of these steps FGFR4 remained the most significantly affected candidate. Additionally, FGFR4 was an intriguing candidate because it is known that FGFRs are alternatively spliced. We have added this justification to the section "Depletion of M+Z TAF15 leads to intron retention in *fgfr4*" of the manuscript.

•Advocating for those who don't regularly work with RNA-seq and next-gen statistics, the contents of Sup Table 1, which seemed to inform the decision to focus on fgfr4 could be explained in more detail to improve the reader's understanding. For instance, what was evaluated to determine statistical significance, i.e. what data were those p values derived from?

The p-values are the result of the statistical modeling peformed by DESeq2/DEXSeq and indicates to which degree the difference in expression of a given gene or exon is significant, i.e. is expression of a specific gene or exon higher or lower in morphants compared to controls.

The modeling that goes into this is somewhat complicated, but in essence assumes a negative binomial distribution (allowing for a wider dispersion/"noisiness" of the data, since gene expression data are like that) of the normalized counts (normalized by number of reads per sample to adjust for one sample simply being sequenced deeper and that would cause all genes to appear "upregulated") and assumes a model where lowly expressed genes are penalized due to them being more likely to display large fold-changes by chance (if a given gene has almost zero or few read counts a few random changes would produce large fold changes). We have added this clarification to the methods section now called "RNA sequencing Library preparation and Analysis"; lines 295-301 of the manuscript.

What was the "adjustment" to the p value?

The p-values are then adjusted for false discovery rate (also performed by DESeq2/DEXSeq) for the number of features since, by chance, false positives calls are a problem sampling a large number of features (genes or exons) as we do in gene expression studies. Illustrated in https://xkcd.com/882/.
There are many statistical packages that test for differential gene expression. Different methods were used when gene expression gained traction, but has now converged to a use methods that are very similar to those implemented in DESeq2. We have added a section called "RNAseq analysis" to the Methods section; lines 295-301of the manuscript.

Also, are the values in columns CTRL_ST10 and TAF15MO_ST10 the RPKM values or something else? The values are read counts normalized to library size per feature. As such they are similar to RPKM but not normalized to a fixed feature length but the length of the feature. Since a given feature length is fixed it can be compared across samples. We have added a section called "RNAseq analysis" to the Methods section; lines 295- 301of the manuscript.

•In supp Table 3 what does inverse intron usage mean? Is it relevant? Why are some genes underlined?

We apologize for choosing terminology that was not clear. "Inverse intron" comes from when we were using DEXseq DEU models. By this visualization of the gene models, when an intron was significantly retained it would look like the inverse of itself in the normal spliced gene model. I will change this column to "Retained intron as visualized by DEXseq DEU model". I have also changed the column titled "Increased intron" to "Increased levels of intron detected as visualized by DEXseq DEU model"; these intron retention levels do not reach the same "inverse" threshold and are thus not "Retained introns". I have removed the underlines as this is a vestige of a code for myself that I was using to organize the candidates.

•Can you include some more data (in the Supplement would be fine) so we can learn more about the intron retention behavior of genes (besides fgfr4) listed in Fig 2D that passed qRT-PCR validation? It was nice to get a visual depiction of the data showing intron retention dynamics of Isl1 with the Integrative Genome Viewer in Sup Figure 1. Can the RNA-seq read maps for the other validated genes be presented in the same way?

At the reviewer's suggestion (as well one of the other reviewers) we have included more data on the characteristics of intron retention; including number of retained introns as well as location throughout the transcript. For this, we chose to focus on the top 10 DEU genes as sorted by adjusted P value for stages 10 and 15 (Supplemental figures 2 and 3). In addition to using IGV to visualize the transcripts, we also generated a table describing the DEU characteristics of the top 10 genes exhibiting differential exon usage (DEU) at stages 10 and 15 (Supplemental table 7). This additional DEU characterization and visualization has been included in the manuscript on lines 545-550 and 604-612.

•Might it be possible to use retained intron sequences to glean an explanation or hypothesis about how TAF15 engages in intron removal? For instance, can compare the sequences of the retained introns? Might the retained introns of the "100 genes" with DEU (in response to M+Z TAF15 knockdown) share conserved sequences that are binding sites for splicing factors or RNA binding proteins that potentially interact with TAF15?

We agree with the reviewer that this is a very interesting question and could help us understand the mechanism of how TAF15 plays a role in splicing. An initial analysis did not show any obvious candidate sequences, though this has not been pursued, since any claims would need to be tested by further experiments beyond the scope of this work.

TOPIC: Depletion of TAF15 causes fgfr4 intron 1 retention (paragraph at 506)
The story of how TAF15 depletion affects fgfr4 is presented very clearly in Figures 3 and 4 and the ms text. A coherent set of data and a very plausible scenario are presented that argue for a dual action of TAF15, first on maternal fgfr4 splicing of intron 1, and subsequently on the transcriptional activity of zygotic fgfr4. The fgfr4 transcript dynamics in response to M+Z or zygotic

TAF15 knockdown were reflected by the spatial expression of fgfr4 in embryos scored by in situ hybridization (Fig 4 A-E). The reduction in TAF15 and FGFR4 protein expression upon TAF15 knockdown nicely confirms that the retention of intron 1 interferes with FGFR4 protein synthesis even though transcript levels are elevated. It's an interesting finding. The authors also convincingly show that two FGFR4 target genes in the anterior neural region (pax2 and engrailed 2) are reduced by TAF15 knockdown to produce an interesting multi-step regulatory.

Minor points:
•Note that in Figs 3 and 4, the TAF15 MOs or CRISPR/Cas9 were (presumably) injected into both cells of 2 cell stage embryos that were subsequently evaluated by qRT-PCR or Western blot, whereas embryos evaluated by in situ hybridization were injected with these reagents into one blastomere at the 2-cell stage.

We apologize for not being clearer. The reviewer is correct, embryos collected for Western blot and qRT-PCR were injected 2/2 cell, whereas embryos collected for ISH were injected ½ cell for use of the uninjected side as an internal control. We have clarified this in the figure legends.

•Was genomic DNA sequenced to assess the types of F0 CRISPR mutations and the level of mosaicism (% normal and % mutated DNA)?

We did not perform genomic DNA sequencing to assess the F0 CRISPR mutations and level of mosaicism, we chose instead to infer functional depletion of TAF15 following sgRNA taf15+CAS9 injection.

TOPIC: Changes in gene expression and Ventx regulation by TAF15 (Figs 5 and 6):
The RNA-seq data gathered from TAF15 morphant neurulae were evaluated for differential expression (DESeq) and about 2,000 candidates were filtered by GO categories to capture genes with developmental and ectodermal expression (Fig 5A,B).

•The results were presented as a heatmap of up and down regulated genes (Fig 5C), but the figure legend or ms text do not specify what the columns in the heatmap represent. One might assume each column reflects individual embryo data, but please provide the information.

We apologize for not being clearer, the reviewer is correct in assuming that each column represents an individual embryo. We have clarified this in the figure legend.

The ventx2.1 gene was a top hit from the DE results, and the embryonic expression patterns of taf15 and ventx2.1 are reasonably complementary, which is consistent with the possibility that TAF15 suppresses Ventx2 expression. This possibility is supported by results showing expanded ectodermal ventx2 expression in the neural border region upon knockdown of TAF15 (Fig 6). Panels 6E' and F' show reasonably distinct expression of taf15 in the neural and epidermal ectoderm, and ventx2 in lateral plate mesoderm, but there is uncertainty (due to slight blue staining) whether each gene has low expression in the adjacent germ layer or the stain simply diffused a bit. More importantly, the TAF15 knockdown embryos show expanded ventx2.1 expression in the MO-injected side (panels I and J) with significant ectopic
expression of ventx2.1 in the injected neural plate (panel K).

•Although close inspection shows a thin boundary separation of the neural ectoderm and lateral plate mesoderm, an arrowhead or two pointing out that boundary would help the reader. Alternatively, make that image much larger.

We apologize for not being clearer. In Figure 6 E' and F' the white dotted lines mark the thin boundary between the neural ectoderm (n) and lateral plate mesoderm (lp). We have now included in the figure legend the meaning of these dotted lines.

•There seems to be a morphological abnormality in that injected region (thickened ectoderm and mesoderm). Is that part of the real phenotype or experimental aberration? Do you have adjacent cross-sections of that embryo or another that could be substituted or added to Supplemental data?

I do have a more posterior cross-section from the same embryo that shows similar results. Unfortunately I no longer have additional embryos to go back to.

•Panel L is helpful to interpret the territory of TAF15 knockdown, but I think it would work better if this was paired with bigger images of I and J.

We apologize for the confusion. We have guided the reader to compare more panels from Figure 6 to help with interpreting expression *ventx* changes following TAF15 depletion. There are now additional references to Figure 6 throughout lines 669-679 of the manuscript.

The diagram of the model for TAF15 repression of the ventrolateral BMP/Vent circuit is not described in the legend or ms text.

Thank you for bringing this to our attention, we have added it to the legend and referenced within the manuscript. The final data figure examines changes in Ventx2.1 expression levels and patterns in response to TAF15 M+Z knockdown. The results of RNA-seq read mapping and qRT-PCR are solid and clearly support the conclusion that ventx2.1 expression is elevated upon maternal or zygotic TAF15 inhibition.

•The authors state that the RNA-seq data show elevated expression of ventx2.1 at stage 15 but not at stage 10, but the number of reads mapped across ventx2.1 in the Genome Viewer at stage 10 are, to my eye, higher in the MO-injected than in the uninjected embryo, making that data inconsistent with the qRT-PCR results at stage 10. Please revisit this point in the ms text.

We agree with the reviewer that, by eye, the RNAseq data shows elevated *ventx2.1* expression (compared to UC) at both stages 10 and 15. A 2-fold expression cutoff was applied to the RNAseq data and at st.10, *ventx2.1* expression was not increased by 2-fold; the same was observed by qRT-PCR at this stage. The reviewer is correct that there is observable increase in *ventx2.1* transcript at st.10, but it does not make the 2-fold threshold. We have added this detail to line 684 of the manuscript.

•The embryonic in situ expression patterns in 7C-L bolster the results shown in Fig 6. Altogether the data support the proposal that TAF15 represses ventx2.1, and since ventx2.1 derepression can be detected at stage 10, it seems quite probable (though not unequivocal) that TAF15 acts directly on vent2.1 transcription. CHiP-qPCR with TAF15 antibodies could resolve this, but I don't propose the authors test that. Instead, I suggest (if possible; not required) that qRT-PCR analysis of TAF15 morphant animal cap explants might more clearly resolve whether TAF15 represses ventx2.1 in the ectoderm. Also, the ability to assess vent2.1 expression at its earliest timepoints could better support whether this relationship is direct.

We agree with the reviewer that both the ChIP-qPCR and animal cap experiments they've proposed would help address the molecular relationship between TAF15 and *ventx2.1*. Unfortunately, with our COVID restrictions and unreliability of embryo production, we have not been able to complete such experiments.

I realize that limited access to labs in this Covid-19 era may preclude any additional experiments, so any suggested here are just that, suggestions. Revision of the text and figures would be sufficient for resubmission, but ideally it would be great to also address any suggestions for non-wet lab data analysis.

Second decision letter

MS ID#: DEVELOP/2020/191619

MS TITLE: The atypical RNA-binding protein TAF15 regulates dorsoanterior neural development through diverse mechanisms in Xenopus tropicalis.

AUTHORS: Caitlin S DeJong, Darwin S Dichmann, Cameron R.T. Exner, Yuxiao Xu, and Richard M Harland

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 come to different conclusions regarding suitability for publication of this revisedversionof your manuscript. Reviewer 3 is positive, reviewer 2 has specific concerns about yourinterpretationof some aspects of the data and reviewer 1 considers that more work is needed to validate your data and ensure conclusions are robust. Some of their concerns relate to validation of data, but they also thinkthatsome of your most interesting observations deserve more attention. I doubt that you will be willing/able to fully address all the issues raised in this review but I think your conclusions could bestrengthened if you can provide further data relating to the issues raised.

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

The revised version is still not satisfactory. See comments below

Comments for the author

The explanations provided by the authors clarify the results obtained but do not alleviate the uncertainties regarding their interpretations. Additional experiments were required and not provided.

- I still find the phenotypic analysis of the loss of function embryos very rudimentary.
- I would still need to see validation of the RNAseq results by ISH using intronic probes.
- I am also still puzzled by the contradiction between the expansion of territories in injected embryos in Fig 4 and 7 contradicting the ventx results, especially now that the authors tell us that delay in convergence and extension is modest, therefore suggesting expansion is fate related therefore suggesting losing TAF is reducing BMP activity. Although a direct or indirect regulation of vntx by TAF15 is clear, it does not explain the many aspects of the phenotypes and therefore is a very random pick of one event amongst many revealed by the RNAseq.
- The finding of intron retention in embryos lacking TAF15 is of great interest but instead of relating the 100 IR events to biological processes and specific phenotypes, it gets reduced to a specific case. My view is still that the study needs to be reshaped in depth to present a more coherent and unbiased understanding of the function of TAF15 during development.
- The third point made by Reviewer 2 regarding the very unusual and more importantly unexplained finding of splicing defects induced by loss of maternal TAF is still unexplained and required more investigation too.

Reviewer 2

Advance summary and potential significance to field

The manuscript describes the role of TAF15 in early development. The authors suggest that TAF15 regulates neural development by regulating the expression of fgfr4 and ventx2.1. They propose that maternal and zygotic TAF15 regulate gene expression via distinct mechanisms. The work is one of the first describing a function for the RNA binding protein in development.

Comments for the author

The conclusion that maternal TAF15 is regulating the splicing of maternally-deposited pre-mRNA is not well-supported by the presented data (itemized below). Rather, the data support a role of maternal TAF15 in regulating the splicing of nascent zygotically transcribed pre-mRNA. The conclusion must be re-evaluated and re-written. If so desired, the authors could propose TAF15 regulation of maternal pre-mRNA as an alternative hypothesis, but without further evidence to support such RNAs exist, it cannot be the main interpretation of the results.

Rationale that M TAF15 regulates splicing of zygotic NOT maternal pre-mRNA
1) Current convention holds that maternally-deposited RNA are completely spliced mRNA. Substantive data would need to be provided to counter this convention. The authors could not find evidence for intron retention in their re-analysis of published pre-ZGA RNA-seq data. They argue a limit to detection, but the more likely conclusion is that there isn't any intron retention to detect in maternal transcripts.

2) The authors could not detect intron retention in fgfr4 at pre-ZGA stages even in the M+Z taf15 MO, although this was readily detected by stage 15. As the level of maternal fgfr4 do not go up post-ZGA, the most likely explanation is that TAF15 acts on zygotically transcribed fgfr4.

3) The argument that M TAF15 is acting on maternal RNA based on prior annotation of maternal vs. zygotic RNA. Many (or perhaps all) of the RNA annotated in that category are also zygotically transcribed (ex. fgfr4). The RNA-seq experiments were performed at post-ZGA stages. Thus, the conclusion of which RNA species is acted on by M TAF15 is conjecture without the experimental validation that it is on the maternal version of the transcript and not the zygotic version.

Other comments
1) The text from 503-537 is still quite confusing. For example, it is unclear what genes were used in the analysis for Figure 2C, which database was used to make the maternal vs zygotic annotations, are these categories exclusive, what do you do with genes that are both maternal and zygoti, and how was the "Expected" calculation made. Also the frequencies in the Observed and Expected categories are provided, but it is unclear the number of genes/splicing events used in the calculations of observed or expected.

2) For the western blots, please clarify which TAF15 bands were used for quantification.

Reviewer 3

Advance summary and potential significance to field

This paper demonstrates a critical requirement for TAF15, a protein that can bind RNA and TATA-associated proteins, in early Xenopus anterior development. The study demonstrates that TAF15 regulates hundreds of genes at the splicing and/or transcriptional levels at different stages of development. Detailed analysis of two highly affected target genes, fgfr4 and ventx2,1, revealed that TAF15 regulates fgfr4 transcription and post-transcriptional splicing, whereas TAF15 represses ventx2.1 transcription. Although TAF15 is related to FUS and EWS proteins, TAF15 and FUS function non-redundantly in development (EWS functions are unknown). Overall, the study highlights how different FET family of proteins can regulate development at transcriptional and post-

transcriptional levels, and suggests mechanisms for human FET members that are mutated in a variety of diseases.

Comments for the author

The authors have provided quite suitable responses to the many and varied reviewer comments. My concerns have been satisfactorily addressed, and from my reading the concerns of the other reviewers appear to have been addressed as well. The manuscript is acceptable.

Second revision

Author response to reviewers' comments

The additions of experiments to the manuscript were hindered by the first author having relocated, but mostly to the effects of the pandemic, which hindered the ability to do experiments, but also affected the quality of our frog colonies. The revision experiments had to be done in a collaborator's lab at UCSF, and had we had full access to our own functional facilities it would have been possible to do more of the experiments requested. However, with these experiments, and using the helpful comments of the reviewers to rewrite and clarify the manuscript, we believe we have supported the conclusions that we have made.

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

The revised version is still not satisfactory. See comments below

Reviewer 1 Comments for the Author...

The explanations provided by the authors clarify the results obtained but do not alleviate the uncertainties regarding their interpretations. Additional experiments were required and not provided.

-I still find the phenotypic analysis of the loss of function embryos very rudimentary.
We appreciate the reviewer's interest in the phenotypic analysis of embryos following TAF15 loss of function. As we stated in our original response to this concern, we selected the later stages for gross phenotypic analysis as this later stage clearly highlights the clear developmental changes following TAF15 depletion that can be appreciated at a gross level; importantly, we did not observe significant gross developmental differences at earlier stages and as such pictures of phenotype are not particularly informative. However, we have addressed the reviewer's concern, by showing the molecular phenotypes of a number of target genes (pictures of RNA ISH, qRT-PCR, and/or WB throughout Figures 4,6,&7) on younger stage embryos (gastrula st.10 and/or mid-neurula st.15). So there are ample molecular phenotypes that result from TAF15 depletion and that can be linked to the gross phenotypes observed at the later st.32 tailbud stage (e.g. reduced dorso-anterior head structures). We have added some text addressing this concern in the "*taf15*/TAF15 depletion leads to gross morphological defects." Section (lines 449-451).

-I would still need to see validation of the RNAseq results by ISH using intronic probes.

We appreciate that RNA ISH targeting a retained intron candidate (e.g. intron 1 of *fgfr4*) would reveal the location(s) within the embryo where intron retention is affected and would be an interesting contribution. However, we have found such relatively short intron probes to be challenging for detection of unspliced RNAs for such moderately expressed genes. Importantly, we have validated the RNAseq results using qRT-PCR to detect the retained introns (normalizing to total expression) and show this method sufficiently and quantitatively validates the RNAseq candidates (Figure 2D).

-I am also still puzzled by the contradiction between the expansion of territories in injected embryos in Fig 4 and 7 contradicting the ventx results, especially now that the authors tell us

that delay in convergence and extension is modest, therefore suggesting expansion is fate related therefore suggesting losing TAF is reducing BMP activity.

We apologize to the reviewer that we did not make our conclusions sufficiently clear in figures 4 and 7. We have reworded these sections to make our conclusions clearer. The reviewer views the expansion of the territory of expression as being an expansion of the neural plate, but we do not conclude that, and clarified the wording (line 589-590, 603-605). In figure 4 we are examining *fgfr4* expression, which marks not just the neural plate, but the domain just lateral to the neural crest region (which itself expresses less *fgfr4*). After TAF15 depletion, the domain of *fgfr4* expression becomes more diffuse, and at a higher level, but this marker alone does not tell us whether it is the more lateral domain that expands (which we favor based on the later results), or the closing neural tube. We do not conclude that these embryos show an expanded neural plate. Indeed the examination of neural markers shows a reduction in amount, if not so much the extent, of neural markers such as *pax2*, consistent with the increase in *ventx* gene expression shown later, and with increased BMP activity.

In figure 7, we examine the increased *ventx* gene expression after TAF15 depletion. Again, *ventx* expression marks tissue just below the outer edge of the neural plate and adjacent ectoderm. In the whole mounts, we see a considerable increase of the amount of *ventx* in this domain, consistent with the ultimate ventralized phenotype. The increase in *ventx* around the closing neural plate is particularly clear in the sections shown in figure 6, but again, we do not conclude that there is any substantial change in the size of the neural plate. We have clarified this in the text.(684-695).

Although a direct or indirect regulation of vntx by TAF15 is clear, it does not explain the many aspects of the phenotypes and therefore is a very random pick of one event amongst many revealed by the RNAseq.

We agree with the reviewer that there are pleiotropic effects to development following TAF15 depletion. Our *taf15* ISH results (Figs 1&6) indicate that *taf15* is expressed in ectodermal and dorsal tissues. In an effort to focus and pare down the gene candidates for analysis, we were interested in looking at the effect on genes involved in ectodermal development ("Ectodermal Development GO term"; Fig.5B&C), as we know *taf15* is expressed in the ectoderm. While there are many candidate genes within this category that can be investigated further, we were struck by the increase in expression of the *ventx* family of genes within this ectoderm development Go term category following *taf15* depletion as these genes are known to be expressed in ventral tissues. Upon further investigation, the expression patterns of *taf15* and *ventx2.1* appear largely complementary which we found to be an intriguing relationship between two genes to investigate further. Dysfunction to *ventx2.1* regulation following TAF15 depletion is also consistent with our gross phenotypic findings of affected dorsal head structures; though *ventx2.1* dysregulation does not alone explain this gross phenotype. For these reasons we decided to focus closely on TAF15 and *ventx2.1*, highlighting one example of how TAF15 affects the domain and differential expression (DE) of its target(s). We include the entire list of DE genes in supplemental tables (Supplemental Tables 4,5,&6), and show additional examples of the intron retention in supplemental figures 2,3, and 4.

We have included some clarifying text within the last paragraph of section "Measuring changes in gene expression following TAF15 depletion".

"Interestingly, *ventx* genes act in a positive feedback loop with the bone morphogenetic proteins (BMP), which specify the ventral domain of the Xenopus embryo (Onichtchouk, Glinka, & Niehrs, 1998; Sander et al., 2007). Because *taf15* is expressed in the future dorsal domain of the gastrula (Figure 1A), we were intrigued to find a gene family (*vent*) that functions in ventral tissue development, and is upregulated upon TAF15 depletion. This suggests an early and lasting regulatory relationship between *taf15* and *ventx*. Of the four *ventx* paralogs upregulated following M+Z TAF15 depletion, *ventx2.1* acts upstream of *ventx1.1, ventx2.2, and ventx3.1* therefore we investigate the relationship between *ventx2.1* and *taf15* further (Schuler-Metz, Knöchel, Kaufmann, & Knöchel, 2000)."

-The finding of intron retention in embryos lacking TAF15 is of great interest but instead of relating the 100 IR events to biological processes and specific phenotypes, it gets reduced to a

specific case. My view is still that the study needs to be reshaped in depth to present a more coherent and unbiased understanding of the function of TAF15 during development.

We agree that relating each of the 100 stage-persistent IR events to specific phenotypes would be of great value. Unfortunately, it is beyond the scope of our study to do this for the 99 additional genes with stage-persistent differential exon usage (DEU). We do run Panther-Go term analysis on the gene candidates highlighting the biological processes with which the DEU genes are involved; looking separately at stages 10 and 15 and then at the stage-persistent DEU candidates (Figure 2B). As illustrated by the supplemental IGV plots of the top 10 named genes as sorted by their adjusted P-values at stages 10 and 15 (Supplemental Figure 2&3), and from the qRT-PCR validation data (Figure 2D), FGFR4 intron 1 is the candidate with the most robustly affected differential exon usage; consistently throughout development at stage 10 and 15. Considering these consistent FGFR4 intron 1 findings following M+Z TAF15 depletion we wanted to take a closer look at this TAF15 target. As a result of looking closer, we uncovered the differential mechanisms by which maternal or zygotic TAF15 regulate FGFR4, a novel finding which we pursued further in depth and did relate back to the TAF15 depletion phenotype. As it was beyond the scope of this study to perform this depth of analysis for the remaining 99 stage-persistent genes with DEU, we have included the remaining candidates in Supplemental tables 1,2,&3.

- The third point made by Reviewer 2 regarding the very unusual and more importantly unexplained finding of splicing defects induced by loss of maternal TAF is still unexplained and required more investigation too.

We apologize and agree with the reviewer and have modified the text to re-evaluate and re-write our conclusions to more closely reflect our data. We make clear that we do not find any evidence for unspliced or incompletely spliced RNAs in maternal or cleavage stage datasets, supporting the conclusion that the observed splicing defects are in nascent zygotic transcripts.

The following modifications can be found throughout the text.

Deleted from the introduction: …possibly acting to regulate expression of maternally deposited transcripts before the zygotic genome is activated.

Deleted from the end of the introduction: Here, we describe our findings as an example in *Xenopus* where the gene product TAF15: 1) a gene product, TAF15, uses distinct molecular mechanisms to regulate the expression of the same gene target depending on the time of development in which it is expressed (maternal versus zygotic) and 2) ensures proper dorsoanterior neural development through two different molecular pathways , and 2) a mechanism by which the expression of maternally deposited transcripts could be regulated in the absence of zygotic genome activation.

Deleted from the discussion: It is tempting to hypothesize that during the developmental stages that precede zygotic genome activation, maternal TAF15 might associate with splicing co-factors and regulate the translation of maternally deposited transcripts (e.g. *fgfr4*; though we have not found conclusive evidence for specific maternally deposited transcripts with single intron retentions using standard qRT-PCR and RNAseq approaches; possibly due to the low levels of expression). When the translation of a given mRNA is required, the intron could be quickly excised through the RNA binding activity of TAF15 and (presently unidentified) splicing cofactors for the mRNA to be translated (Figure 8). Thus, in this environment, where there is no active transcription but only maternally deposited mRNAs, this mechanism might temporally and spatially control the translation of specific transcripts.Alternatively, maternal TAF15 could be affecting the DEU of zygotically transcribed targets that just happen to also be maternally deposited (e.g. *fgfr4*).

Deleted from the discussion: There is, however, the unresolved question of why we observe a significant enrichment for genes with maternal expression within the stage-persistent DEU target genes following M+Z TAF15 depletion (though as we state previously this could be due to a lower-than-expected incidence of DEU in exclusively zygotically expressed genes). Although future studies will be required to either support or refute a provacative alternative hypothesis such as this (as convention holds that maternally deposited mRNAs are fully processed), one possibility is that during the developmental stages that precede zygotic genome activation, maternal TAF15 could associate with splicing co-factors and regulate the translation of maternally deposited transcripts

(e.g. *fgfr4*). When the translation of a given mRNA is required, the intron could be quickly excised through the RNA binding activity of TAF15 and (presently unidentified) splicing cofactors for the mRNA to be translated (Figure 8). Thus, in this environment, where there is no active transcription but only maternally deposited mRNAs, this mechanism might temporally and spatially control the translation of specific maternally deposited transcripts. To address this alternative hypothesis, future studies sequencing the RNA from M+Z TAF15 depleted embryos during the developmental stages prior to ZGA will be needed to determine if maternal TAF15 is involved in the post-transcriptional regulation of both maternal and zygotic transcripts or exclusively zygotic pre-mRNAs.
Added to the discussion: Our RNAseq data, generated from post-ZGA embryos (stages 10 and 15) support a model whereby translation of maternally deposited *taf15* is required for the proper post-transcriptional splicing of nascent zygotic pre-mRNA transcripts during the time of zygotic genome activation. Indeed, in looking at early RNA seq datasets (Xenbase) we find no evidence that any of the maternal transcript (e.g. *fgfr4*) is incompletely spliced.

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

The manuscript describes the role of TAF15 in early development. The authors suggest that TAF15 regulates neural development by regulating the expression of fgfr4 and ventx2.1. They propose that maternal and zygotic TAF15 regulate gene expression via distinct mechanisms. The work is one of the first describing a function for the RNA binding protein in development.

Reviewer 2 Comments for the Author...

The conclusion that maternal TAF15 is regulating the splicing of maternally-deposited pre-mRNA is not well-supported by the presented data (itemized below). Rather, the data support a role of maternal TAF15 in regulating the splicing of nascent zygotically transcribed pre-mRNA. The conclusion must be re-evaluated and re-written. If so desired, the authors could propose TAF15 regulation of maternal pre-mRNA as an alternative hypothesis, but without further evidence to support such RNAs exist, it cannot be the main interpretation of the results.

Rationale that M TAF15 regulates splicing of zygotic NOT maternal pre-mRNA
1) Current convention holds that maternally-deposited RNA are completely spliced mRNA. Substantive data would need to be provided to counter this convention. The authors could not find evidence for intron retention in their re-analysis of published pre-ZGA RNA-seq data. They argue a limit to detection, but the more likely conclusion is that there isn't any intron retention to detect in maternal transcripts.

2) The authors could not detect intron retention in fgfr4 at pre-ZGA stages, even in the M+Z taf15 MO, although this was readily detected by stage 15. As the level of maternal fgfr4 do not go up post-ZGA, the most likely explanation is that TAF15 acts on zygotically transcribed fgfr4.

3) The argument that M TAF15 is acting on maternal RNA based on prior annotation of maternal vs. zygotic RNA. Many (or perhaps all) of the RNA annotated in that category are also zygotically transcribed (ex. fgfr4). The RNA-seq experiments were performed at post-ZGA stages. Thus, the conclusion of which RNA species is acted on by M TAF15 is conjecture without the experimental validation that it is on the maternal version of the transcript and not the zygotic version.

We apologize and agree with the reviewer and have modified the text to re-evaluate and re-write our conclusions to more closely reflect our data. We make clear that we do not find any evidence for unspliced or incompletely spliced RNAs in maternal or cleavage stage datasets, supporting the conclusion that the observed splicing defects are in nascent zygotic transcripts.

The following modifications can be found throughout the text.

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Added to the discussion: Our RNAseq data, generated from post-ZGA embryos (stages 10 and 15) support a model whereby translation of maternally deposited *taf15* is required for the proper post-transcriptional splicing of nascent zygotic pre-mRNA transcripts during the time of zygotic genome activation. Indeed, in looking at early RNA seq datasets (Xenbase) we find no evidence that any of the maternal transcript (e.g. *fgfr4*) is incompletely spliced.

Other comments
1) The text from 503-537 is still quite confusing. For example, it is unclear what genes were used in the analysis for Figure 2C, which database was used to make the maternal vs zygotic annotations, are these categories exclusive, what do you do with genes that are both maternal and zygoti, and how was the "Expected" calculation made. Also the frequencies in the Observed and Expected categories are provided, but it is unclear the number of genes/splicing events used in the calculations of observed or expected.

We apologize that this section of the manuscript is still confusing. We have included the following modification:

Using a database designed to differentiate transcripts that are 1) "maternal", meaning both maternally deposited and zygotically transcribed (present in the egg and expressed upon ZGA) (e.g. *fgfr4*) from 2) "zygotic", transcripts that are exclusively zygotically transcribed (e.g. *isl1*), we were

able to compare the percentage of observed DEU target genes that have maternal expression to the percentage of all *X. tropicalis* genes with maternal expression (database provided by the Rokhsar lab at U.C. Berkeley; not shown). The “observed” category is comprised of the 100 stage-persistent genes that show differential exon usage (Supplemental Table 3) and whether they have maternal and zygotic (“maternal”) or exclusively zygotic (“zygotic”) transcript expression. Here we observe 92% of these DEU target genes as transcripts known to have both maternal and zygotic expression; with the remaining 8% composed of exclusively zygotically expressed genes. The “expected” category represents the percentage of all annotated *X. tropicalis* that have maternal and zygotic (“maternal”) or exclusively zygotic (“zygotic”) transcript expression. Here we observe 65% of the *X. tropicalis* transcripts as known to have both maternal and zygotic expression with the remaining 35% known to be exclusively zygotically expressed (Figure 2C). We were surprised to find 92% of the stage-persistent genes to be transcripts that are both maternally deposited and zygotically transcribed. These data suggest a preference for splice regulation of transcripts that are present throughout development (from egg through ZGA) in this M+Z TAF15 depletion condition, since only 65% of annotated *X. tropicalis* transcripts are both maternally deposited and zygotically transcribed (Figure 2C).

2) For the western blots, please clarify which TAF15 bands were used for quantification.

The following has been added to the legend of Figures 1 panel F: “Protein quantification is of the imaged blot using the most consistently expressed TAF15 band marked by an arrow, normalized to the corresponding ACTIN band; percent expression is relative to the uninjected condition; >3 blots were analyzed.”

The following has been added to the legend of Figures 4 panel F: “Protein quantification is of the imaged blot using the most consistently expressed TAF15 or FGFR4 band marked by an arrow, normalized to the corresponding ACTIN band; percent expression is relative to the uninjected condition; >3 blots were analyzed.”

Reviewer 3 Advance Summary and Potential Significance to Field...

This paper demonstrates a critical requirement for TAF15, a protein that can bind RNA and TATA-associated proteins, in early Xenopus anterior development. The study demonstrates that TAF15 regulates hundreds of genes at the splicing and/or transcriptional levels at different stages of development. Detailed analysis of two highly affected target genes, fgfr4 and ventx2,1, revealed that TAF15 regulates fgfr4 transcription and post-transcriptional splicing, whereas TAF15 represses ventx2.1 transcription. Although TAF15 is related to FUS and EWS proteins, TAF15 and FUS function non-redundantly in development (EWS functions are unknown). Overall, the study highlights how different FET family of proteins can regulate development at transcriptional and post-transcriptional levels, and suggests mechanisms for human FET members that are mutated in a variety of diseases.

Reviewer 3 Comments for the Author...

The authors have provided quite suitable responses to the many and varied reviewer comments. My concerns have been satisfactorily addressed, and from my reading, the concerns of the other reviewers appear to have been addressed as well. The manuscript is acceptable.

Third decision letter

MS ID#: DEVELOP/2020/191619

MS TITLE: The atypical RNA-binding protein TAF15 regulates dorsoanterior neural development through diverse mechanisms in Xenopus tropicalis.

AUTHORS: Caitlin S DeJong, Darwin S Dichmann, Cameron R.T. Exner, Yuxiao Xu, and Richard M Harland

ARTICLE TYPE: Research Article

Apologies for the delay in considering the revised version of your manuscript. I sent the revision back to one of the referees who is happy with the changes that you have made and consequently I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks. The referee report is appended below.

Reviewer 2

Advance summary and potential significance to field

The paper describes distinct roles of TAF15 in early vs. later development.

Comments for the author

All concerns have been satisfactorily addressed.