EMBO
reports

Loss of ANCO1 repression at AIB1/YAP targets drives breast cancer progression

Max H. Kushner, Virginie Ory, Garrett G. Graham, Ghada M. Sharif, William B. Kietzman, Sophia Thevissen, Meng Yuan, Marcel O. Schmidt, Anton Wellstein, Anna T. Riegel

Review timeline:

Submission date:	25 June 2019
Editorial Decision:	25 July 2019
Revision received:	23 September 2019
Editorial Decision:	22 October 2019
Revision received:	28 October 2019
Accepted:	12 November 2019

Editor: Achim Breiling

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 25 July 2019

Thank you for the transfer of your research manuscript to EMBO reports. We have now received reports from the three referees that were asked to evaluate your study, which can be found at the end of this email.

As you will see, all referees think that the findings are of interest, but they also have several comments, concerns and suggestions, indicating that a major revision of the manuscript is necessary to allow publication in EMBO reports. As the reports are below, I will not further detail them here.

Given the constructive referee comments, we would like to invite you to revise your manuscript with the understanding that all referee concerns must be addressed in the revised manuscript and in a detailed point-by-point response. Acceptance of your manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact me if a 3-months time frame is not sufficient so that we can discuss the revisions further.

When submitting your revised manuscript, please also carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision. When submitting your revised manuscript, we will require:

1) a .docx formatted version of the final manuscript text (including legends for main figures, EV figures and tables), but without the figures included. Please make sure that the changes are highlighted to be clearly visible. Figure legends should be compiled at the end of the manuscript text.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure), of main figures and EV figures. Please upload these as separate, individual files upon re-submission.

The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf labeled Appendix. The Appendix should have page numbers and needs to include a table of content on the first page (with page numbers) and legends for all content. Please follow the nomenclature Appendix Figure Sx, Appendix Table Sx etc. throughout the text, and also label the figures and tables according to this nomenclature.

For more details please refer to our guide to authors:
http://www.embopress.org/page/journal/14693178/authorguide#manuscriptpreparation

See also our guide for figure preparation:
http://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/EMBOPress_Figure_Guidelines_061115-1561436025777.pdf

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14693178/authorguide). Please insert page numbers in the checklist to indicate where the requested information can be found in the manuscript. The completed author checklist will also be part of the RPF.

Please also follow our guidelines for the use of living organisms, and the respective reporting guidelines: http://www.embopress.org/page/journal/14693178/authorguide#livingorganisms

5) that primary datasets produced in this study are deposited in an appropriate public database. See: http://embor.embopress.org/authorguide#datadeposition

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability " section (placed after Materials & Methods) that follows the model below. Please note that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

The datasets produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

*** Note - All links should resolve to a page where the data can be accessed. ***

Moreover, I have these editorial requests:

5) We strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire

© European Molecular Biology Organization

gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. If you want to provide source data, please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

6) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at: http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

7) Regarding data quantification and statistics, can you please specify, where applicable, the number "n" for how many independent experiments (biological replicates) were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. Please provide statistical testing where applicable, and also add a paragraph detailing this to the methods section. See:
http://www.embopress.org/page/journal/14693178/authorguide#statisticalanalysis

8) Please format the references according to our journal style. See:
http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

9) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript. Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines:
http://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

REFEREE REPORTS

Referee #1:

The authors originally find and mechanistically explain the oncogenic role of the YAP/TAZ/TEAD/AIB1 complex in breast cancer. Interestingly, AIB1 can co-activate some genes or co-repress some other genes together with YAP/TAZ and TEAD. This depends on the presence or not of the tumor suppressor ANCO1/ANKRD11, recruited on some TEAD targets by AIB1 itself. The loss of ANCO1 makes YAP/TEAD/AIB1 loosing their ability to repress some genes, among which those of the 1Q21.3 locus. These are amplified or overexpressed in cancer, while the complex aberrantly transactivates oncogenic loci. The work is original and very interesting, however it lacks some controls and some experiments which are required for full evaluation of the manuscript.
Listed below are the specific comments.
1. Simplify the abstract
2. In the text, replace figure S1, S2 with EV1, EV2
3. In figure EV1C, measure also endogenous YAP (not only that tagged with Myc)
4. Provide the list of genes regulated by YAP/TAZ and AIB1 summarized in the Venn diagram in figure 1A. Provide also the list of genes affected by AIB1 and YAP through interference experiments also in the absence of exogenously expressed YAP. Authors cannot use as control YAP S94A, because this YAP mutant protein is unable to interact with TEAD but can regulate genes in association with other transcription factors. It should be better to use as a control cells not overexpressing YAP. Same comment for figure 2C, 2E, 3A.
5. In figure EV1D, provide a blot also for TEAD
6. In the text, replace figure 2E and S2F with 1E and EV1F
7. Same comment for RE-CHIP. It is shown in figure 1F, not 2F.
8. In figure EV2A, indicate in which cell line the experiment has been performed. Why the authors

did not perform co-IP experiments also for ANCO1 to check whether it complexes with YAP, TAZ, TEAD and AIB1?
9. For figure 2B, provide a supplementary table with the list of genes affected by ANCO1 knockdown, AIB1 knockdown, YAP overxpression in the different cell lines
10. Authors state that ANCO1 knockdown does not affect the expression of CTGF and ANKRD1 that are activated by YAP and TAZ. However, it seems that their transcription is reduced. Moreover, authors should show by chip the absence of ANCO1 on CTGF and ANKRD1 promoter. Provide also a blot of YAP, TAZ and TEAD abundance upon ANCO1 and AIB1 knockdown to check for any reciprocal stabilization.
11. Provide a chip of the level of ANCO1 on repressed targets upon AIB1 knock down, in order to state that AIB1 recruits ANCO1 on repressed promoters.
12. In figure 3B, describe what defines an aberrant mammosphere compared to a normal one.
13. In figure EV3E, add also a blot showing the expression of ANCO1 protein upon YAP overexpression in MCF10A cells. Again, perform a WB of ANCO1 upon AIB1 interference or overexpression and conversely AIB1 blot upon ANCO1 knockdown or overexpression.
14. Analyze cell cycle markers and of the mTOR pathway in cells upon anco1 or AIB1 knockdown in order to dissect the role of AIB1 and ANCO1 in the regulation of cell cycle and in the regulation of cell size/cell growth.
15. Fore figure 3D and EV3G provide the list of genes differentially expressed in early and late stage xenografts in mice
16. In vivo data are not consistent with in vitro. In mammospheres, the authors state that ANCO1 loss increase the size of mammosphere, while AIB1 is responsible of the invasive phenotype. However, in vivo the authors show that anco1, is anticorrelated to the invasive phenotype. Authors should perform also the staining of the xenografted tumors with AIB1 antibody and look for any correlation with tumor invasiveness.
17. In figure 4C, provide the Kaplan Mayer also for AIB1.
18. Perform some simple functional experiments in cell lines to assess the oncogenic or oncosuppressive role of S100A7, S100A8, SPRR3, SPRR2E through the analysis of cell phenotype upon their manipulation (cell growth, cell cycle). Moreover, simplify the description of figure 4D and clarify its take home message. On the basis of figure 4D, how do the authors conclude that the loss of ANCO1 determines the loss of 1q221.3 repression? Reorganize the text of this last part of the results section.
19. Perform also some functional experiment of TEAD interference to demonstrate directly (not only through the mutant version of YAP) that TEAD is involved in the YAP/AIB1/ANCO1 axis in breast cancer.

Referee #2:

This manuscript explores the role of a complex that involves AIB1, YAP1 and ANCO1 in early stage breast cancer. The authors show that YAP/TAZ regulate a set of genes in diploid, non-transformed cell lines and that a subset of these genes are influenced by AIB1. Specific clusters of gene targets are explored and CTGF is used as an example gene to define regulatory activity of these proteins. Protein complexes are confirmed using a variety of methodological approaches and a specific focus on the 1q21 locus is explored. AIB1 is shown to be an important mediator of TEAD-ANCO1 complex formation and the effect of modulating these components on cellular phenotype is explored. To provide clinical support for these findings, in vivo analysis of AIB1 is conducted and correlations between ANCO1 and known AIB1 targets, with clinical outcome is provided.

This is an interesting paper on a biologically important and clinically relevant topic. The data are, for the most part, convincing and clear. However, several issues need to be addressed.

- Are YAP levels (or the protein levels of known associated and regulatory proteins) altered with AIB1 modulation, potentially explaining the changes in the target genes?
- The use of the CTGF promoter is OK, assuming that the promoter is the regulatory element used by AIB1 and YAP. Does the ChIP-seq data show that there is predominant binding at the promoter and not further upstream or downstream? Given that AIB1 typically doesn't associated with promoters, this is an important point.
- The big missing experiment, which is essential, is the AIB1 ChIP-seq. Without this the authors are

inferring about the generality of the AIB1/YAP interactions and to get to the heart of the mechanistic interplay, this needs to be conducted.
- Some of the ChIP-enrichments are very low and as described in the point above, this might be because the bone fide regulatory elements are not known and the authors are guessing that the promoters are the main regulatory domains.
- Is TEAD (based on the published data) binding enriched at the CTGF promoter?
- Are the TEAD ChIP-seq peaks and the target genes enriched near each other, more than would be expected by chance?
- The authors claim "AIB1-YAP coactivated genes appear to contribute directly to the malignant progression and the proliferation of the MCF10A cells whereas the ANCO1-AIB1-YAP corepressed gene targets impact overall sphere size". Where is the evidence for this, beyond correlative expression at these different cellular stages? I can't see any functional evidence linking those genes as the mediators of the altered phenotypic endpoints.
- The authors claim that the AIB1-YAP coactivated genes are significantly enriched in the xenografts. Is this statistically enriched, more than expected by chance?
- Does AIB1 have prognostic value in the patient cohorts? Not a couple of target genes, but AIB1 itself?
- Is there a way of quantifying the PLA spots to show a score of the different conditions?
- Some of the figure legends in the text don't match the actual figures.

Referee #3:

This manuscript describes physical and functional interactions between the AIB1 (aka NCOA3 or SRC3) nuclear receptor coactivator and the Yap/TEAD complex in the context of an invasive breast cancer model with cell lines and a mouse model. AIB1 is shown to bind to Yap1 and to be required for a subset of "Yap1 activated" and "Yap1 repressed" mRNA targets in breast cancer cell lines. With regard to the AIB1-Yap1 'co-activated' targets, depletion of TEAD blocks the effect of AIB1, indicating that AIB1-Yap1 cooperativity is mediated through TEAD binding to its DNA motif in target enhancers. The authors then go on to present evidence that the 'co-repressed' targets are repressed by the AIB1 associated repressor Ankrd11 (aka ANCO1), which this group had previously shown to bind AIB1. These molecular data are nicely complemented by effects of AIB1/Yap1 in cell lines and oraganoids, and further supported by the finding that Ankrd11 loss is a prognostic marker in breast cancer.

Overall the paper is quite strong, but could be strengthened further by a bit more mechanistic insight.
1. For example, what domain/motif within AIB1 binds Yap1?
2. Is the interaction mediated through the WW domains of Yap1?
3. Does AIB1 contain PY motifs, which are classic WW-interaction motifs present in many Yap1 interactors?
4. Does AIB1 also interact with the Yap1 paralog Taz?
5. There is evidence that Yap1 hyperactivity may be involved in the transition to hormone-independent prostate cancer (Kuser-Abali, Nature Comm, 2015). Could the AIB1-Yap1 interaction be involved in a similar transition in breast cancer.
6. Does AIB1 also recruit an NHR into this complex?

Importantly, the authors should credit two prior papers that document very similar physical and functional interactions between Drosophila homologs of AIB1 and Yap1 (Taiman and Yorkie) in fly cancer models: Zhang, 2015, Dev Cell, and Wang, 2016, Cell Discovery.

1st Revision - authors' response 23 September 2019

Referee #1

The authors originally find and mechanistically explain the oncogenic role of the YAP/TAZ/TEAD/AIB1 complex in breast cancer. Interestingly, AIB1 can co-activate some genes

© European Molecular Biology Organization

or co-repress some other genes together with YAP/TAZ and TEAD. This depends on the presence or not of the tumor suppressor ANCO1/ANKRD11, recruited on some TEAD targets by AIB1 itself. The loss of ANCO1 makes YAP/TEAD/AIB1 losing their ability to repress some genes, among which those of the 1Q21.3 locus. These are amplified or overexpressed in cancer, while the complex aberrantly transactivates oncogenic loci. The work is original and very interesting; however, it lacks some controls and some experiments which are required for full evaluation of the manuscript.

Listed below are the specific comments.

1. Simplify the abstract
We have simplified and focused the abstract, specifically reorganizing the structure to better mirror the flow and content of the manuscript. We have also clarified the text.

2. In the text, replace figure S1, S2 with EV1, EV2
We apologize for this, and we have corrected the "extended view" nomenclature and numbering.

3. In figure EV1C, measure also endogenous YAP (not only that tagged with Myc)
We have provided the blot as requested, probed with an antibody against endogenous YAP. The blot can be found in Figure EV1B.

4. Provide the list of genes regulated by YAP/TAZ and AIB1 summarized in the Venn diagram in figure 1A. Provide also the list of genes affected by AIB1 and YAP through interference experiments also in the absence of exogenously expressed YAP. Authors cannot use as control YAP S94A, because this YAP mutant protein is unable to interact with TEAD but can regulate genes in association with other transcription factors. It should be better to use as a control cells not overexpressing YAP. Same comment for figure 2C, 2E, 3A.
We have provided the list of genes described as the Venn Diagrams in Supplementary Table 1.

The YAP S94A was chosen in discussion with our YAP collaborators, in order to best control for the YAP-TEAD interaction, and to fully understand how AIB1 and YAP converge specifically on TEAD, as opposed to the potential non-TEAD, YAP-induced effects that the reviewer notes. By using the S94A, we used a control that cleanly attenuated the direct TEAD binding effects, which was the aim of our study. However, in order to address the reviewer's concerns regarding the YAP S94A, we have made a new supplementary figure (EV2) with a series of experiments and bioinformatic analyses described below:

- **EV2A: We show minimal protein stabilization differences between MCF10A, MCF10A-S94A, and MCF10A-YAP cells. YAP activity modestly downregulates TEAD and TAZ levels, which has been described previously (Moroishi et al, 2015. *Gene Dev.* doi: 10.1101/gad.262816.115.).**
- **EV2B: We show the YAP S94A mutant does not interact with endogenous TEADs by co-immunoprecipitation.**
- **EV2C: We analyzed RNA-seq of YAP v Vector control MCF10A cells (GSE70506) and show that differentially regulated genes in this set (using the same cutoffs as Figure 1A-B) do not significantly differ from our YAP vs YAP/S94A comparison. Further, these data sets are significantly and highly correlated.**
- **EV2D: We analyzed RNA-seq of YAP v Vector control MCF10A cells (GSE70506), specifically examining the YAP *de novo* signature we describe in Figure 1A-B. We show that there is no significant difference between RNA-seq of YAP v Vector control MCF10A cells and RNA-seq of YAP v S94A MCF10A cells.**

Additionally, as requested, we repeated the results in Figures 2C by creating cell lines expressing vector control to show that 1q21.3 genes are repressed by YAP when compared to vector as measured by RT-qPCR (Now displayed in a new figure, Figure EV3L). We also analyzed RNA-seq of YAP v Vector control MCF10A cells (GSE70506) to show by gene set enrichment analysis that 1q21.3 genes are suppressed (Now displayed in a new figure, Figure EV3J-K). We also added vector control data to the quantification of sphere assays in Figures 3A-C.

5. In figure EV1D, provide a blot also for TEAD

Reviewers 1& 2 asked questions related to reciprocal stability of the proteins discussed in the manuscript. To address these concerns, we have provided western blots of AIB1, YAP, TAZ, and TEADs for all experimental approaches. The only induced changes in expression of these proteins that we observed were noted previously in response to Point 4 above i.e. that YAP has been previously shown by others to downregulate levels of TAZ and TEAD. These blots can be found in panels EV2A, EV2E.

6. In the text, replace figure 2E and S2F with 1E and EV1F

We apologize for this, and we have corrected the "extended view" nomenclature and numbering.

7. Same comment for RE-CHIP. It is shown in figure 1F, not 2F.

We apologize for this, and we have corrected the "extended view" nomenclature and numbering.

8. In figure EV2A, indicate in which cell line the experiment has been performed. Why the authors did not perform co-IP experiments also for ANCO1 to check whether it complexes with YAP, TAZ, TEAD and AIB1?

EV3A-B (formerly EV2A) was performed in MCF10A shGFP cells, matching the corresponding panels in Figure 2A; this has been noted in the resubmitted manuscript.

The endogenous ANCO1/ANKRD11 IP is not efficient with current commercially available antibodies. They are however highly efficient for IHC/IF, which is why we chose to perform proximity ligation assays (which also give an idea of the cellular location of the interaction). In addition, we have attempt immunoprecipitation with tagged ANCO1 during the revision period, however, we were not confident in the results of the experimental controls. We have provided additional data, as suggested by Reviewer 1, in Point 11, that we feel strengthens our manuscript and addresses this point: reChIP of AIB1-TEAD shows significant enrichment at 1q21.3 enhancer (Figure 2H), and ANCO1 recruitment to these sites is significantly reduced upon AIB1 knockdown (Figure 2I).

9. For figure 2B, provide a supplementary table with the list of genes affected by ANCO1 knockdown, AIB1 knockdown, YAP overexpression in the different cell lines

We have provided the list of genes affected by ANCO1 knockdown in Supplementary Table 2, in addition to genes affected by YAP overexpression and AIB1 knockdown (see Reviewer 1, Point 4).

10. Authors state that ANCO1 knockdown does not affect the expression of CTGF and ANKRD1 that are activated by YAP and TAZ. However, it seems that their transcription is reduced. Moreover, authors should show by chip the absence of ANCO1 on CTGF and ANKRD1 promoter. Provide also a blot of YAP, TAZ and TEAD abundance upon ANCO1 and AIB1 knockdown to check for any reciprocal stabilization.

We have infected and recreated shANCO1 cell lines in MCF10A and MCFDCIS cells and assayed these in addition to previous cell lines. In aggregate, we saw no significant regulation of *CTGF* or *CYR61* by ANCO1, with the exception of small yet statistically significant changes in *CTGF* in MCFDCIS cells (Figure EV3M). It is worth noting that these changes are orders of magnitude smaller than the changes that we observe with differential up regulation of 1q21.3 genes we observe with ANCO1 knockdown (Figure 2D-E). We did observe modest enrichment of ANCO1 at the CTGF and CYR61 promoter in MCF10A cells (Figure EV3N), however, we do not believe it is having a functional repressive effect, as their gene expression is unaltered by ANCO1 shRNA. Further, this enrichment is much smaller in value as compared to the enrichment at enhancer regions throughout 1q21.3, where we observe is a functional effect. We have added comment on this now in the results section.

We have provided blots showing there are no changes in reciprocal stabilization of YAP, TAZ, or TEAD following ANCO1 knockdown in Figure EV3E. We did see a slight reduction in AIB1 levels following ANCO1 knockdown (Figure EV3E). Despite this slight reduction in AIB1 levels, upregulation of 1q21.3 gene levels are so dramatic with ANCO1 shRNA

highlighting the critical role of these proteins in regulating expression. Also, of note is that the AIB1 decrease with ANCO1 shRNA is not sufficient to modulate AIB1-YAP coactivated targets (Figure EV3M). Finally, we show that knockdown of AIB1 and overexpression of YAP/S94A does not alter the stabilization of ANCO1 (Figure EV3H-I).

11. Provide a chip of the level of ANCO1 on repressed targets upon AIB1 knock down, in order to state that AIB1 recruits ANCO1 on repressed promoters.
We thank the reviewer for this suggestion, as we feel it has meaningfully increased the experimental evidence of sequential recruitment. We have shown that AIB1 knockdown significantly reduces levels of ANCO1 at 1q21.3 enhancer sites (Figure 2I). These same sites are where AIB1-TEAD are co-enriched (Figure 2H). Knockdown of AIB1 does not affect the levels of ANCO1 protein (Figure EV3I).

12. In figure 3B, describe what defines an aberrant mammosphere compared to a normal one.
We have recreated previously published phenotypes (shown previously in Overholtzer et al. 2006. *PNAS.* doi:10.1073/pnas.0605579103), where YAP overexpressing MCF10A cells form aberrant mammospheres as lacking circularity and defined borders, and express EMT markers. We are the first to report that AIB1 is required to sustain these invasive phenotypes. These spheres were quantified with ImageJ. We have clarified these details in the results and methods sections in the resubmitted manuscript.

13. In figure EV3E, add also a blot showing the expression of ANCO1 protein upon YAP overexpression in MCF10A cells. Again, perform a WB of ANCO1 upon AIB1 interference or overexpression and conversely AIB1 blot upon ANCO1 knockdown or overexpression.
We have provided blots showing there are no changes in reciprocal stabilization of YAP, TAZ, or TEAD following ANCO1 knockdown in Figure EV3E. We note that there is a slight decrease of AIB1 following ANCO1 knockdown (see Reviewer 1, Point 10 above), however, it is not sufficient to modulate AIB1-YAP coactivated targets. Further, we show that knockdown of AIB1 and overexpression of YAP/S94A does not alter the stabilization of ANCO1 (Figure EV3H-I).

14. Analyze cell cycle markers and of the mTOR pathway in cells upon anco1 or AIB1 knockdown in order to dissect the role of AIB1 and ANCO1 in the regulation of cell cycle and in the regulation of cell size/cell growth.
As the reviewer accurately notes, previous reports have demonstrated that YAP regulates cell size upstream of the mTOR pathway (Tumaneng et al. 2012. *Nature Cell Biol.* doi: 10.1038/ncb2615). We note in Figure EV4F that we indeed observe increased mTOR activity following AIB1 or ANCO1 knockdown, as the reviewer suggested. These effects may be downstream of *S100A* and *SPRR* gene de-repression, as has been shown in the literature previously. We have further commented on this in the discussion as follows:

"Consistent with this, recent reports have demonstrated the role of YAP in modulating cell size and proliferation through distinct pathways such as upstream regulation of MTOR (Csibi et al. 2012. *Nature Cell Biol.* doi: 10.1038/ncb2634 ; Tumaneng et al. 2012. *Nature Cell Biol.* doi: 10.1038/ncb2615; Mugahid et al. 2018. *BioRxiv*. doi: 10.1101/482836). Interestingly, the S100A proteins that we find regulated by the ANCO1/AIB1/YAP complex in our normal breast and breast cancer models have previously been implicated in upregulation of MTOR signaling (Leclerc and Vetter, 2015. *Biochem Biphys Acta.* dio: 10.1016/j.bbadis.2015.09.022; Kuperappa et al. 2016. *J Clin Diagn Res.* doi: 10.7860/JCDR/2016/17949.8022; Brenner and Bruserud. 2018. *Neoplasia*. doi: 10.1016/j.neo.2018.09.007). Taken together with the published work, our data suggest that de-repression of YAP followed by increased S100A proteins could contribute to the upregulation of canonical MTOR signaling (Figure EV4F) in normal and early stage breast cancer cells, resulting in the increased cell size that we observe with knockdown of AIB1 or ANCO1."

15. For figure 3D and EV3G provide the list of genes differentially expressed in early and late stage xenografts in mice
We have provided the list of genes differentially regulated in early and late stage xenografts in Supplementary Table 3.

16. In vivo data are not consistent with in vitro. In mammospheres, the authors state that ANCO1 loss increase the size of mammosphere, while AIB1 is responsible of the invasive phenotype. However, in vivo the authors show that anco1, is anticorrelated to the invasive phenotype. Authors should perform also the staining of the xenografted tumors with AIB1 antibody and look for any correlation with tumor invasiveness.

We feel that the *in vitro* and *in vivo* data are highly consistent. The sphere data shows that ANCO1 knockdown increases the size of aberrant spheres (Figure 3A-C), and matches with the *in vivo* data, which shows that during the invasive transition, the MCFDCIS xenografts lose ANCO1.

As we noted in the introduction, we have previously described the role and localization of AIB1 in the MCFDCIS xenografts in Ory et al (*Oncogene*, doi: 10.1038/onc.2013.263). We showed that AIB1 levels increase during the invasive transition, and loss of AIB1 slows proliferation and tumor growth.

17. In figure 4C, provide the Kaplan Mayer also for AIB1.

We have run this analysis and see that high AIB1 levels are a poor prognostic marker in basal breast cancer (Figure EV4I). This corroborates much of the AIB1 literature (IE-see Zhao et al. 2003. *Cancer*. doi:10.1002/cncr.11482 ; Harigopal et al. 2009. *Breast Cancer Research and Treatment.* doi:10.1007/s10549- 008-0063-9; Hudelist et al. 2003. *Breast Cancer Research and Treatment.* doi:10.1023/A:1022930710850; Alkner et al. 2017. *Breast Cancer Research and Treatment.* doi: 10.1007/s10549-017-4416-0; Lee et al. 2011. *World J Surgical Oncology.* doi: 10.1186/1477-7819-9-139).

18. Perform some simple functional experiments in cell lines to assess the oncogenic or oncosuppressive role of S100A7, S100A8, SPRR3, SPRR2E through the analysis of cell phenotype upon their manipulation (cell growth, cell cycle). Moreover, simplify the description of figure 4D and clarify its take home message. On the basis of figure 4D, how do the authors conclude that the loss of ANCO1 determines the loss of 1q221.3 repression? Reorganize the text of this last part of the results section.

The emphasis of this study was to define a novel protein complex mediated by AIB1 – we have discussed this with the editor, and he, and we, feel that validating the role of each individually regulated protein, or potentially their combinatorial actions on disease progression is outside the scope of this manuscript, but is an important area of future research.

In this regard, the reviewer correctly notes, many of the *S100A* and *SPRR* genes have been individually implicated in oncogenesis and disease progression. We cite many of these published studies defining these roles throughout the manuscript, especially the works that have been done in early stage breast cancer. Apart from the four genes that we chose to validate by qPCR as proof of concept, we observed more than 25 genes located at 1q21.3 were upregulated during DCIS progression (See figure EV4H).

We have simplified Figure 4D and hope that the key message is clear: i.e. in patients, levels of AIB1-YAP coactivated genes and AIB1-YAP repression of 1q21.3 genes are inversely correlated in the presence of ANCO1. These clinical findings reflect our *in vitro* and *in vivo* findings.

19. Perform also some functional experiment of TEAD interference to demonstrate directly (not only through the mutant version of YAP) that TEAD is involved in the YAP/AIB1/ANCO1 axis in breast cancer.

In using the YAP S94A in our experiment series as a control, we inherently controlled for non-TEAD activities in our data analysis (see Reviewer 1, Point 4). The reviewer was thoughtful in addressing this distinction, and we have shown that non-TEAD gene expression changes are insignificant through informatic analysis and repeating experiments using a vector control as opposed to S94A. Therefore, through our experimental design and additional analysis of Vector vs. S94A, we conclude that the gene expression and phenotypic changes we observe are through TEAD family members.

© European Molecular Biology Organization

Referee #2

This manuscript explores the role of a complex that involves AIB1, YAP1 and ANCO1 in early stage breast cancer. The authors show that YAP/TAZ regulate a set of genes in diploid, non-transformed cell lines and that a subset of these genes are influenced by AIB1. Specific clusters of gene targets are explored and CTGF is used as an example gene to define regulatory activity of these proteins. Protein complexes are confirmed using a variety of methodological approaches and a specific focus on the 1q21 locus is explored. AIB1 is shown to be an important mediator of TEAD-ANCO1 complex formation and the effect of modulating these components on cellular phenotype is explored. To provide clinical support for these findings, in vivo analysis of AIB1 is conducted and correlations between ANCO1 and known AIB1 targets, with clinical outcome is provided.

This is an interesting paper on a biologically important and clinically relevant topic. The data are, for the most part, convincing and clear. However, several issues need to be addressed.

1. Are YAP levels (or the protein levels of known associated and regulatory proteins) altered with AIB1 modulation,
potentially explaining the changes in the target genes?
To address these concerns, we have provided blots of ANCO1, AIB1, YAP, TAZ, and TEADs for all experimental paradigms (YAP overexpression; comparative overexpression of YAP and S94A to parental; AIB1 knockdown; ANCO1 knockdown). The only differences we observed were noted previously and do not impact our conclusions (See Reviewer 1, Points 3, 5, 10, and 13), and we also note that that YAP activity modestly downregulates TEAD and TAZ levels, which has been described in Moroishi et al, 2015 (*Gene Dev.* doi: 10.1101/gad.262816.115). These blots can be found in panels EV2A, EV2E, EV3E, EV3H-I.

2. The use of the CTGF promoter is OK, assuming that the promoter is the regulatory element used by AIB1 and YAP. Does the ChIP-seq data show that there is predominant binding at the promoter and not further upstream or downstream? Given that AIB1 typically doesn't associated with promoters, this is an important point.
3. The big missing experiment, which is essential, is the AIB1 ChIP-seq. Without this the authors are inferring about the generality of the AIB1/YAP interactions and to get to the heart of the mechanistic interplay, this needs to be conducted.
4. Some of the ChIP-enrichments are very low and as described in the point above, this might be because the bone fide regulatory elements are not known and the authors are guessing that the promoters are the main regulatory domains.

Response to Points 2-4: The reviewer is raising many important points and addressing them has improved the impact of our work. First, specifically in terms of CTGF and CYR61, two different publications show that these are a direct YAP-TEAD target, where the proteins bind at the promoter and regulate transcription (Zanconato et al. 2015. *Nature Cell Biology*. doi: 10.1038/ncb3216; Stein et al. 2015. *PLOS Genetics*. doi:10.1371/journal.pgen.1005465). In fact, the primer sequences for *CTGF* and *CYR61* promoters were taken from these publications, which overlaid ChIP-seq, Hi-C, and additional regulatory elements and histone marks. Further, the Genehancer regulatory element scoring algorithm (Fishilevich et al. 2017. *Database*. doi: 10.1093/database/bax028) ranks the *CTGF* promoter as the key regulatory element for that specific gene. We felt that the promoter ChIP, for these genes, was sufficient as a regulatory binding event. Further, the CTGF promoter is sufficient to drive transcription and is a regulatory event, as seen by our luciferase reporter assay, which has the endogenous promoter (without enhancer) cloned upstream of a firefly luciferase (Figure 1G). Globally, the reviewer is asking a crucial question about promoter versus enhancer enrichment, and how we can best understand the mechanistic interplay resulting in gene regulation. The two publications noted above noted that YAP and TEAD are most enriched on enhancer regions. As the reviewer accurately noted, AIB1 has been shown to be enriched predominantly on

enhancer regions as well (Zwart et al. 2011. *EMBO*. doi: 10.1038/emboj.2011.368.). We indeed observe globally enrichment of TEAD and AIB1 on enhancers and promoters (Figure 1D-E), and YAP, TEAD, AIB1, and ANCO1 engaged on specific enhancer regions throughout 1q21.3 that were annotated previously (Figure 2G-I).

The reviewer's suggestion of AIB1 ChIP-seq is a logical direction to take. We have taken a multipronged approach to address this:

1) We have performed ChIP-seq in our MCF10A cells against TEAD4 and AIB1. We observed significant co-occupancy between the factors, comprising about 25% of all AIB1 peaks. We further expanded this by performing *de novo* motif enrichment on the AIB1 peaks, to find a significant ($p<1e-111$) enrichment of TEAD motifs within AIB1 peaks. These data can be found in a new series of panels, Figures 1D-F.

2) We have now utilized available AIB1 and TEAD4 ChIP-seq data from the ENCODE consortium to examine the co-enrichment of AIB1 and TEAD in the MCF7 breast cancer cell line. From this analysis we have identified significant overlap of a majority of AIB1 ChIP-seq peaks with TEAD4 also in this cell line. Further, we examined overlapping peaks and matched them with histone marks ChIP-seq from a published database to show that, as the reviewer notes, AIB1 and TEAD are coenriched on both promoters and enhancers. Finally, with HOMER *de novo* motif analysis, we have identified a statistically significant enrichment of the TEAD binding motif within AIB1 ChIP-seq ($p<1e-61$), independently validating the coincidence of AIB1 and pan-TEAD. These data can be found in a new series of panels, Figures 1D-F.

3) Further, to address the reviewer's comments, we have assayed co-enrichment by performing sequential ChIP-reChIP at identified enhancers and promoters of the coregulated genes we describe in Figures 1-2. Specifically, we see high levels of enrichment (100+ Fold) following sequential ChIP within our coactivated and corepressed promoters and enhancers (Figures 1H-I and 2H). These are regulatory elements that have been described in previous publications to contribute to gene expression.

5. Is TEAD (based on the published data) binding enriched at the CTGF promoter?

Yes, this has been previously published (Zanconato et al. 2015. *Nature Cell Biology*. doi: 10.1038/ncb3216; Stein et al. 2015. *PLOS Genetics*. doi:10.1371/journal.pgen.1005465), and we used the published primers for ChIP-qPCR. Further, the Genehancer regulatory element scoring algorithm (Fishilevich et al. 2017. *Database*. doi: 10.1093/database/bax028) ranks the *CTGF* promoter as the key regulatory element for that specific gene. We also see TEAD motif enrichment in our TEAD ChiP seq analysis in MCF10A cells (Figure 1D-H) .

6. Are the TEAD ChIP-seq peaks and the target genes enriched near each other, more than would be expected by chance?

YAP-TEAD target genes have been well defined not only by ChIP-seq, but through a series of ChIP-seq, RNA-seq, and Hi-C experiments. The most robust and comprehensive analysis has been done in triple negative breast cancer, and the targets are widely used and validated (Zanconato et al. 2015. *Nature Cell Biology*. doi: 10.1038/ncb3216). This analysis, as well as others (Liu et al. 2016. *Cell Rep.* doi: 10.1016/j.celrep.2015.12.104), show that TEAD target genes are significantly associated with TEAD ChIP-seq peaks, and there are a set of core target genes associated with specific peaks across many cell lines. Further, we have shown that our described *de novo* YAP signature (Figure 1A-B), has significantly more TEAD binding sites by HOMER *de novo* motif discovery (Figure EV1D), which uses a scrambled background to account for chance occurrence.

7. The authors claim "AIB1-YAP coactivated genes appear to contribute directly to the malignant progression and the proliferation of the MCF10A cells whereas the ANCO1-AIB1-YAP corepressed gene targets impact overall sphere size". Where is the evidence for this, beyond correlative expression at these different cellular stages? I can't see any functional evidence linking those genes as the mediators of the altered phenotypic endpoints.
We have clarified this statement within resubmission, specifically saying that the phenotypes we observe are downstream of ANCO1-AIB1-YAP regulation, but not necessarily the direct targets. Our manuscript describes a novel transcription repression complex and elucidates the dual role of AIB1 within YAP-TEAD signaling. We observe that the phenotypic effects of ANCO1 loss are increased cell size (Figure EV4A-B) and increased spheroid size (Figure EV4D-E), which corresponds with our *in vivo* data (Figure 3D-F). However, as we noted in response to Reviewer #1 in Point #18, many genes within 1q21.3 were modulated by ANCO1/AIB1/YAP/TEAD. Further, many genes were differentially regulated by AIB1/YAP/TEAD independent of ANCO1.

8. The authors claim that the AIB1-YAP coactivated genes are significantly enriched in the xenografts. Is this statistically enriched, more than expected by chance?
All RNA-seq and array data was filtered for statistical significance with a p-value of $p<0.05$. We have included a GSEA plot of AIB1-YAP coactivated genes in Figure 3D corresponding to the volcano plot in Figure EV4H. The coding packages and statistical analyses used are detailed in the methods section of the manuscript.

9. Does AIB1 have prognostic value in the patient cohorts? Not a couple of target genes, but AIB1 itself?
We have run this analysis and see that high AIB1 levels are a poor prognostic marker in basal breast cancer (Figure EV4I). This corroborates much of the AIB1 literature (IE-see Zhao et al. 2003. *Cancer*. doi:10.1002/cncr.11482; Harigopal et al. 2009. *Breast Cancer Research and Treatment.* doi:10.1007/s10549- 008-0063-9; Hudelist et al. 2003. *Breast Cancer Research and Treatment.* doi:10.1023/A:1022930710850; Alkner et al. 2017. *Breast Cancer Research and Treatment.* doi: 10.1007/s10549-017-4416-0; Lee et al. 2011. *World J Surgical Oncology.* doi: 10.1186/1477-7819-9-139). (See Referee #1, Point 17)

10. Is there a way of quantifying the PLA spots to show a score of the different conditions?
We have quantified the PLA foci in both control and knockdown conditions and show a statistically significant decrease in foci following AIB1 knockdown (Figure 2A and EV3A-C).

11. Some of the figure legends in the text don't match the actual figures
We apologize for this, and we have corrected the "extended view" nomenclature and numbering.

Referee #3

This manuscript describes physical and functional interactions between the AIB1 (aka NCOA3 or SRC3) nuclear receptor coactivator and the Yap/TEAD complex in the context of an invasive breast cancer model with cell lines and a mouse model. AIB1 is shown to bind to Yap1 and to be required for a subset of "Yap1 activated" and "Yap1 repressed" mRNA targets in breast cancer cell lines. With regard to the AIB1-Yap1 'co-activated' targets, depletion of TEAD blocks the effect of AIB1, indicating that AIB1-Yap1 cooperativity is mediated through TEAD binding to its DNA motif in target enhancers. The authors then go on to present evidence that the 'co-repressed' targets are repressed by the AIB1 associated repressor Ankrd11 (aka ANCO1), which this group had previously shown to bind AIB1. These molecular data are nicely complemented by effects of AIB1/Yap1 in cell lines and oraganoids, and further supported by the finding that Ankrd11 loss is a prognostic marker in breast cancer.

Overall the paper is quite strong, but could be strengthened further by a bit more mechanistic insight.
1. For example, what domain/motif within AIB1 binds Yap1?
2. Is the interaction mediated through the WW domains of Yap1?

© European Molecular Biology Organization

3. Does AIB1 contain PY motifs, which are classic WW-interaction motifs present in many Yap1 interactors?

Response to Points 1-3: We do not state that there is a direct interaction between AIB1 and YAP, but rather believe there is convergent activity between the two coactivators. The reviewer correctly notes that the Drosophila homolog of AIB1 is Taiman, and that Taiman has a PY motif/PPxY motif that can interact with the Yki (Drosophila homolog of YAP) through the WW domain. However, during its evolution, AIB1 in humans no longer has the PPxY motif that canonically interacts with WW domains. The reviewer correctly notes that other publications have shown that NCOA6 (Oh et al. 2014. *Cell Reports.* doi:10.1016/j.celrep.2014.06.017), a different and distinct nuclear coactivator (not in the p160/NCOA/SRC coactivator family which AIB1 is a part of) does have PPxY motif and has been shown to interact with WW domains; however, AIB1 does not have the canonical PPxY domains.

4. Does AIB1 also interact with the Yap1 paralog Taz?
Yes, AIB1 can interact in complex with TAZ as well as YAP. We show this via endogenous IP in Figure EV1A.

5. There is evidence that Yap1 hyperactivity may be involved in the transition to hormone-independent prostate cancer (Kuser-Abali, Nature Comm, 2015). Could the AIB1-Yap1 interaction be involved in a similar transition in breast cancer.
We think this is a critical point of our study and underscores the role AIB1 may be playing in many hormone receptor negative disease. Our introduction and discussion in the resubmission emphasizes this point, in part we note "The extent to which the oncogenic activity of AIB1 can be attributed to interaction with TEADs and YAP/TAZ is not known". In addition, we have further emphasized this in the discussion:

"In fact, it is still unclear whether AIB1 interacts with YAP directly or indirectly convergently through TEADs, and how binding kinetics impact ANCO1 recruitment to specific loci. Although Drosophila homologues of YAP and AIB1 (Yki and Tai, respectively) have been shown to directly interact through WW-PPxY interaction, this domain has been lost in NCOA/SRC/p160 family during evolution (Wang et al. 2016. *Cell Disc.* Doi: 10.1038/celldisc.2016.6; Xu et al. 2003. *Mol Endocrinology*. doi: 10.1210/me.2003-0116 ; Zhang et al. 2015. *Dev Cell*. 10.1016/j.devcel.2015.05.010; Oh et al. 2014. *Cell Rep*. doi: 10.1016/j.celrep.2014.06.017) and cannot explain AIB1-YAP interactions in human cells.."

6. Does AIB1 also recruit an NHR into this complex?
It is interesting to consider what other proteins may be recruited to this complex by AIB1. New publications have highlighted the importance of YAP-TEAD-NHR activity (i.e. – Zhu et al. 2019. *Mol Cell*. doi:10.1016/j.molcel.2019.06.010) , and is an exciting area for future study. We have commented on this in the discussion in the resubmission.

2nd Editorial Decision — 22 October 2019

Thank you for the submission of your revised manuscript to our editorial offices. We have now received the reports from the three referees that were asked to re-evaluate your study, you will find below. As you will see, referees #1 and #2 have some remaining questions and suggestions to improve the manuscript I ask you to address in a final revised version.

Further, I have these editorial requests:

- We would like to publish, as you also indicated upon submission, the paper as Scientific Report. For a Scientific Report we require that results and discussion sections are combined in a single chapter called "Results & Discussion". Please do that for your manuscript. For more details please refer to our guide to authors:
http://www.embopress.org/page/journal/14693178/authorguide#researcharticleguide

- The supplementary tables are incorrectly named. Tables 1-4 should be named 'Dataset EV#' (these are too large to be shown as tables in the online version of the manuscript), starting with 'Dataset EV1', and Tables 5-8 should be named 'Table EV#', starting with 'Table EV1'. Then, please update all the callouts in the manuscript text. Please call out these tables or dataset individually and using the specific name (not just using 'see supplemental tables'). Please also provide a legend for all these datasets/tables on the first TAB of the individual excel sheets.

- Please add scale bars to the microscopic images in Fig. 3E and 4B. Please refrain from any writing indicating their size directly on the bars in images. Please indicate the size only in the respective figure legend.

- Please remove the reviewer tokens from the data availability section, and make sure the data will get public upon publication of the study.

- The contrast/exposure of the Western blots in the source data differs partly from the final figure panels (in particular in Fig. EV1A). Please provide the source data as similar exposures as in the manuscript figures. If you show different exposures, please also provide individual source data for these.

- It seems the source data for Fig. EV1B YAP does not match to the respective figure panel. Please check.

- Please supply an ORCID ID for the corresponding author. Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our author guidelines: http://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines

- Finally, please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text, and some queries, we ask you to address. Please provide your final manuscript file with track changes, in order that we can see the modifications done.

In addition I would need from you:
- a short, two-sentence summary of the manuscript
- two to three bullet points highlighting the key findings of your study
- a schematic summary figure (in jpeg or tiff format with the exact width of 550 pixels and a height of not more than 400 pixels) that can be used as a visual synopsis on our website. This can be based on Fig. 5.

I look forward to seeing the final revised version of your manuscript when it is ready. Please let me know if you have questions regarding the revision.

REFEREE REPORTS

Referee #1:

The authors have adequately addressed to the comments raised by this reviewer in the previous round of revision. The listed below minor comment needs to be clarified before final acceptance.
Minor point
In Figure EV2 C, the authors aim to demonstrate that mutant YAP S94A is a better control compared to YAP WT, more than the empty vector. I agree with this, however, I do not understand the difference between the left and the right panels. Why in the left panel the number of genes is 469 and in the right one is 295? Please, explain it better.

Referee #2:

The authors have addressed my concerns. The new data has filled many of the gap. Why are example peaks from the AIB1 and TEAD1 data not shown? Only showing the processed numbers provides no confidence about the quality or success of the ChIP-seq. The authors need to include example tracks.

Referee #3:

The prior reviews identified some significant data gaps in the data presented, esp ChiP experiments to further validate the authors model of AIB1-TEAD4 co-occupancy. The author's have been very responsive to these critiques and added quite a bit new data extending the AIB1-TEAD data to additional TEADs. Some experiments could not be added due to technical limits of available reagents, but these are not significant omissions. Overall, the paper has been significantly strengthened by these changes. The biological impact of the findings are quite high, as they imply crosstalk between AIB1-Ankrd11 and Yap-TEAD on shared target promoters. Because of the high impact and the good quality of the data in the original submission, I was in favor of provisional acceptance of the first version of the paper. Given the improvements in this revision, I am now more strongly in favor of acceptance.

2nd Revision - authors' response 28 October 2019

Referee #1:

The authors have adequately addressed to the comments raised by this reviewer in the previous round of revision. The listed below minor comment needs to be clarified before final acceptance.

Minor point:
In Figure EV2 C, the authors aim to demonstrate that mutant YAP S94A is a better control compared to YAP WT, more than the empty vector. I agree with this, however, I do not understand the difference between the left and the right panels. Why in the left panel the number of genes is 469 and in the right one is 295? Please, explain it better.

We have updated our description of the two panels in the text, specifically stating:

"We therefore examined our RNA-seq data from YAP S94A overexpressing MCF10A cells compared to RNA-seq expression data from the vector control (GSE70506). We analyzed these data using the same cutoffs for our RNA-seq analysis, we did not observe a significant difference in gene regulation when comparing this independent data set to our YAP/S94A comparison and saw significant correlation between both groups (Figure EV2C). Further, we repeated this analysis after removing our YAP signature genes (Figure 1A) to ensure that well described YAP target genes were not masking a significant off target effect of the S94A mutant. In doing so, we effectively compared the YAP S94A gene expression to the vector control, and we did not observe a significant difference in gene regulation, suggesting the YAP S94A mutant is functionally similar to a vector control (Figure EV2C). Finally, genes within the YAP signature (Figure 1A) did not behave significantly different in the YAP/Vector data set (Figure EV2D) suggesting that almost all of the YAP effect is mediated by TEAD in these cells."

The number of genes originally in the panel was a number of genes from the publicly available data set that was considered significant and regulated by the cutoffs we used on our data in Figure 1. We elected to remove the number of genes from panel EV2C because it caused confusion.

Referee #2:

The authors have addressed my concerns. The new data has filled many of the gap. Why are

example peaks from the AIB1 and TEAD1 data not shown? Only showing the processed numbers provides no confidence about the quality or success of the ChIP-seq. The authors need to include example tracks.

We have updated Figure 1D to include tracks from our ChIP-seq in MCF10A cells at *CTGF* and *CYR61*. We have also added a new panel, Figure EV3P, to show AIB1 and TEAD4 peaks at the 1q21.3 superenhancer sites by ChIP-seq.

© European Molecular Biology Organization

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ➜

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Anna Tate Riegel
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2019-48741-V3

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo
http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title
http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun
http://datadryad.org
http://figshare.com
http://www.ncbi.nlm.nih.gov/gap
http://www.ebi.ac.uk/ega
http://biomodels.net/
http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ➜ (Do not worry if you cannot see all your text once you press return)

Question	Answer
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	For animal experiments, sample size/statistical power determination was done before experimentation. For all in vitro experiments, all experiments were performed in three biological replicates and technical triplicates unless otherwise notes. Details are included in the methods, figures, and figure legends.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Group size was calculated based on the penetrance and effect size on the basis of a previous pilot experiment.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	All animals used were female athymic nude mice from Envigo, between 6-8 weeks old. Females only were used because this study assessed female breast cancer.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Animals were randomized to cages and to time points for euthanasia.
For animal studies, include a statement about randomization even if no randomization was used.	N/A
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Animals were randomized by computer to select groups. Quantification of staining was done computationally with preset algorithm.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Quantification of IHC was done by computer algorithm (ImageJ, described in Methods)
5. For every figure, are statistical tests justified as appropriate?	Statistical tests of every panel are described in the Methods. When a non-parametric test was used, the violated assumption of the parametric counterpart is described in the methods. For single comparisons, unpaired t-test were used if the assumptions were met. For group comparisons, ANOVA was used if the assumptions were met; post-hoc Tukey test was performed if the assumptions were met.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	If any assumptions were violated, the violated assumptions and resulting non-parametric test used is described in the Figure Legend.

Is there an estimate of variation within each group of data?	Yes, and SD or SEM is provided as an error bar (described in legends and methods).
Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Details on antibodies used in the study, including catalog number and RRID number, are listed in the methods and supplementary tables.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Sources of cell lines, either from repositories or external labs, are described in the Methods. Cell lines were routinely (every 6 months) tested for mycoplasma.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Female Athymic nude mice (Hsd:Athymic Nude-Foxn1nu); 6-8 week old mice; provided by Envigo (formerly Harlan). Animals were housed at Georgetown University's animal facility and animal experiments were conducted in accordance with procedures approved by the Institutional Animal Care and Use Committee.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Studies in mice were reviewed and approved by the Georgetown University Animal Care and Use Committee
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We confirm compliance

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	RNA-seq data: GSE132475 ; ChIP-seq data: GSE137284
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No