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YAP-TEAD signaling promotes Basal Cell Carcinoma development via a c-JUN/AP1 axis

Dejan Maglic, Karin Schlegelmilch, Antonella F.M. Dost, Riccardo Panero, Michael Dill, Raffaele A. Calogero, and Fernando D. Camargo

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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 22 December 2017

Thank you for the submission of your manuscript (EMBOJ-2017-98642) to The EMBO Journal. Your study has been sent to three referees, and we have received reports from all of them, which are enclosed below.

The referees acknowledge the potential interest and novelty of your work, although they also express a number of concerns that will have to be addressed before they can support publication of your manuscript in The EMBO Journal. While referee #1 is overall more positive, both referees #2 and #3 express reservations regarding your claims on a functional link between YAP and Jnk signaling, which in their view are not sufficiently well supported by the current data (ref#2, pt. 4; ref#3, pt.4). In addition, the referee point to the need for better characterization of potential redundancy between YAP and TAZ (ref#2, pt.1; ref#3, pt.6), and the tumorigenesis level affected by YAP loss-of-function (ref#3, pt.3). In addition, the referees point to a number of issues regarding technical controls, reporter lines utilized and purity of cell populations analyzed by expression profiling, which would need to be resolved to achieve the level of robustness required for The EMBO Journal.

I judge the comments of the referees to be generally reasonable, thus we invite you to revise your manuscript experimentally to address the referees' comments. Please note however, that we would need strong support from the referees on such a revised version of the manuscript to move towards publication.

Referee #1:

Maglic et al. showed that YAP is required for basal cell carcinoma development. The authors show

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by clonal analyses that Yap activity is required in dysplastic cells to be competitive and to be able to progress to invasive basal cell carcinoma. Mechanistically, the authors suggest that YAP is required in BCC tumors to activate JNK-JUN signalling to drive tumorigenesis.

This paper has interesting findings, it is well supported by experimental data and addresses an important question in cancer biology.

Importantly, the finding that YAP inactivation does not affect Hedgehog signalling has great clinical potential because it suggests that targeting YAP can overcome cancer cell resistance to Hedgehog inhibitors currently used in the clinic to treat basal cell carcinoma. In my opinion this paper can be publishes as is.

Referee #2:

The manuscript from Camargo and colleagues describes the role of YAP in the development of basal cell carcinoma (BCC). Here the authors take advantage of a number of mouse models as well as a few specimens from patient BCCs. This analysis shows that YAP displays a pronounced nuclear localization in the majority of analyzed tumors. In order to test the role of YAP in tumor development, the authors use a conditional knockout model allowing them to delete YAP completely or focally within the developing BCCs. In the absence of YAP, BCC development is significantly reduced. Although, neglected by the authors it is interesting that animals even in the absence of YAP develop a few BCCs. Based on in situ hybridization studies the authors argue that Yap is the only hippo effector operating in BCCs, and that it is pivotal for BCC formation, however, the experiments included in the manuscript could suggest that they need to also look carefully at TAZ. The authors show that proliferation is reduced in the YAP negative cells, and that these cells still display signs of active beta-catenin and hedgehog signaling suggesting that YAP is not responsible for activation of these pathways. Expression analysis of supposedly YAP deleted cells from BCCs identify very few differentially expressed genes, however, a number of these can be confirmed as TEAD1 and 4, the DNA binding co-factors of YAP, target genes. In line with previous findings (Zanconato et al., 2015, Nature Cell Biology) the authors identify AP1 sites in close proximity to the TEAD DNA binding elements suggesting that YAP and AP1 transcription factors could be working together during BCC formation. At the protein level the authors go on to show that Jnk activation and Jun phosphorylation potentially is deregulated in the KO and propose that this is the key mechanism for the YAP mediated effects in BCC formation.

Although the authors describe an interesting role for YAP in the development of BCCs, there are still a number of unresolved issues that the authors need to address.

1) The authors conclude on page 9 that 'BCC establishment and tumor progression from dysplasia to an invasive phenotype' require YAP-TEAD interactions. If this is the case why do YAP null BCCs form in the first place? Is there redundancy between TAZ and Yap in this case? Obviously, YAP-TEAD interactions are important but based on the data displayed in the submitted manuscript it is impossible to conclude that this is necessary. The analysis of TAZ therefore needs to be enhanced by performing additional experiments both at the protein and RNA levels. This will also validate their conclusion that TAZ is not important.

2) It is unclear what the experiments using Cyr61-GFP and SmoM2-YFP adds to the manuscript. It is obviously an interesting reporter line, but is impossible to make any conclusion with regard to reporter activity in the developing BCCs as the antibody used recognize both GFP and YFP. The spectra from these chromophors can be resolved by confocal microscopy as well as by flow cytometry, which would give them the required resolution to actually make conclusion as to how many cells are active during normal homeostasis and how this changes during BCC development and in the established tumours. Surprisingly, a large fraction of cells display nuclear localization of YAP, but are negative for both YAP and GFP.

3) The provided expression data is very surprising as the authors detect only 98 genes differentially expressed gene (1.5 fold cutoff) and looking at this gene list there are no obvious genes involved in

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cell cycle progression. Moreover, a slightly more stringent cutoff (2 fold) illustrate that only 5 genes are detected as differentially expressed using a padj cut off of 0.05. Combined with the strategy for isolating these cells, it is questionable that this is a pure cell population from BCCs. In these experiments, the authors use SmoM2-YFP/Rosa26-lsl-tdTomato lines combined with YAPfl/fl in order to assess deletion based on Krt14CreER activation. Here tdTomato is used as a proxy for both SmoM2 and YAP deletion. As this reporter model is known recombine very easily upon cre activation, tdTomato expression is likely to be much more widespread than YAP deletion and SmoM2 activation. Importantly, no controls are provided to demonstrate that this indeed enable purification of cells from BCCs with YAP deleted. The authors should consequently have adopted a sorting strategy for YFP as this obviously is a measure for both BCCs and activation of Cre. The provided analysis showing differences in cell cycle genes is not convincing as analysis of the differentially expressed genes in the Panther database identify either none or cell adhesion as the biological process associated with the sorted population. This is an important point because the authors essentially exploit this data for the rest of the paper, and all the required controls are missing. Moreover, the observed functional characteristics described in the previous figures are missing completely missing in the differentially expressed gene list provided.

4) The link to AP1 is not novel (Zanconato et al., 2015, Nature Cell Biology). Moreover, the data related to AP1 binding elements remains purely descriptive will little additional results to back up the observation. Right now this is based on staining, a few Western blots and qPCR for some AP1 genes, where the authors describe only minor changes to gene expression. Is this really relevant in the context effects mediated via YAP? Is AP1 important in this context? Do they bind the same elements? Why is Jnk suddenly activated? Is this related to YAP? All of these questions and potentially more needs to be addressed in order to reach the conclusion that 'YAP-TEAD signaling promotes Basal Cell Carcinoma development via cJun/AP1 axis' (the title of the manuscript).

Minor concerns:
The authors state that YAP promotes growth independent of Wnt and Hedgehog signaling. YAP could however be an important mediator of the effects of Wnt and Hedgehog as suggested previously (Azzolin et al., 2014, Cell).

Figure 5 - gene names are listed but cannot be read.

The authors need to be consistent when describing genes differentially expressed. Is this 97 or 98 (Page 10).

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The authors suggest that YAP plays an essential role in development of BCC based on correlation of expression in tumor models as well as functional evidence with loss of function in an animal model. While these basic observations are not surprising given the extensive role for YAP previously described in skin tumorigenesis, the authors provide some significant new data to suggest that YAP exerts its effect through an interaction with the JNK pathway. There are several improvements that could be made to make the study stronger, as well as several outstanding questions not yet addressed by the data provided.

1, in fig 1C, it is difficult to see where the in situ signal is positive versus negative. this should be shown with a sense control side by side for both YAP and TAZ.

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3, in fig 2, the authors show the effect of loss of YAP in a BCC model. However, the authors do not show a temporal analysis of the effect, so it is not clear if the tumorigenesis is identical but just slower or if the process is affected in a more fundamental way. the quantification in C suggests that the pathology is not much different.

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5, the link to Jnk signaling is interesting, could the authors demonstrate this functionally? if a transgenic method is beyond the scope, how about a topical small molecule stimulator or inhibitor?

6, the cell line work in EV2 is interesting, what happens when YAP and TAZ are downregulated? are these two hippo factors compensatory? could double downregulation lead to synthetic lethality in cancer model?

1st Revision - authors' response 12 April 2018

(begins on next page)

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2nd Editorial Decision 1 May 2018

Thank you for submitting your revised manuscript for consideration by The EMBO Journal. Your revised study was sent back to referees #2 and #3 for re-evaluation, and we have received comments from both of them, which I enclose below. As you will see the referees find that their concerns have been sufficiently addressed and they are now broadly favour of publication.

Thus, we are pleased to inform you that your manuscript has been accepted in principle for publication in The EMBO Journal, pending some minor issues regarding material and methods and formatting as outlined below, which need to be adjusted at re-submission.

Please contact me if you have any questions, or need further input.

Thank you again for giving us the chance to consider your manuscript for The EMBO Journal, I look forward to your final revision.

Again, please contact me at any time if you need any help or have further questions.

Referee #2:

The authors have carefully addressed all of my concerns and I find that the manuscript is now appropriate for publication in EMBO J.

Referee #3:

the authors have addressed most of the concerns raised by the reviewers, and mostly with new data. the manuscript is improved as a result, and the conclusions are sound. the study is strong, and while some of its conclusions confirm a previous report in NCB, it does serve as a very strong confirmation and provides more mechanistic insight into that previous work.

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1) The authors conclude on page 9 that 'BCC establishment and tumor progression from dysplasia to an invasive phenotype' require YAP-TEAD interactions. If this is the case why do YAP null BCCs form in the first place? Is there redundancy between TAZ and Yap in this case? Obviously, YAP-TEAD interactions are important but based on the data displayed in the submitted manuscript it is impossible to conclude that this is necessary. The analysis of TAZ therefore needs to be enhanced by performing additional experiments both at the protein and RNA levels. This will also validate their conclusion that TAZ is not important.

We greatly appreciate the reviewer's point that we need to correctly reflect our data in the text. We have now made changes on page 9 and elsewhere in the manuscript to indicate that a loss of YAP-TEAD interaction significantly impairs BCC initiation and progression. In the present work and published studies, BCC initiation is defined when dysplastic clones appear in the basal epidermis (~ 4 weeks post SmoM2 expression). We quantified the number of dysplastic BCC clones (4 weeks post tamoxifen) in *Yap*$^{+/+}$ and *Yap*$^{fl/fl}$ backgrounds, which indicated that YAP loss impairs BCC initiation (Fig. EV2A). However, few YAP-negative dysplastic clones establish (6 weeks post tamoxifen) but are lost as the tumors progress to more invasive phenotype (22 weeks post tamoxifen) (Fig. EV2B). Additionally, we utilized the YAP^{S79A} knock-in allele to assess *in vivo* role of YAP-TEAD interaction in BCC development. By quantifying BCC tumor burden in *Yap*$^{+/+}$, *Yap*$^{fl/fl}$ (differentiating YAP-positive and YAP-negative by IHC), and *Yap*$^{fl/S79A}$ backgrounds, we observe striking phenotypic overlap between YAP-negative and YAP^{S79A}-expressing BCC tumors (Fig. 2E and 2F). Thus, we conclude that YAP-TEAD interaction significantly contributes to BCC growth.

We agree with the reviewer that additional genetic evidence was necessary to determine TAZ contribution in YAP-negative BCC. To further strengthen our observation that TAZ is not compensating in YAP-negative BCCs, we compared BCC initiation and progression in *Yap*$^{fl/fl}$ and *Yap*$^{fl/fl}$ *Taz*$^{fl/fl}$ backgrounds (Fig S2DE). Indeed, we confirm that Yap/Taz loss has no additive decrease in tumor burden or growth and thus, further confirms that TAZ does not compensate for YAP loss in BCC tumors.

2) It is unclear what the experiments using Cyr61-GFP and SmoM2-YFP adds to the manuscript. It is obviously an interesting reporter line, but is impossible to make any conclusion with regard to reporter activity in the developing BCCs as the antibody used recognize both GFP and YFP. The spectra from these chromophors can be resolved by confocal microscopy as well as by flow cytometry, which would give them the required resolution to actually make conclusion as to how many cells are active during normal homeostasis and how this changes during BCC development and in the established tumours. Surprisingly, a large fraction of cells display nuclear localization of YAP, but are negative for both YAP and GFP.

We believe that the Cyr61 reporter ($Cyr61^{eGFP}$) experiments are useful because YAP nuclear localization is not a reliable marker of its transcriptional activity. For example, in this manuscript we show that YAP is highly nuclear in a large number of IFE basal keratinocytes. However, using $Cyr61^{eGFP}$ reporter as a measure of YAP transcriptional activity, we observe that GFP signal marks a small number of basal keratinocytes, in addition to sebaceous gland (Fig. 1D). Our observation that $Cyr61^{eGFP}$ infrequently marks basal keratinocytes suggests that YAP may not be transcriptionally active in the majority of wildtype epidermis. However, in BCC tumors, $Cyr61^{eGFP}$ is significantly upregulated and predominantly marks basally located tumor cells. Using ZEN software, we generated pixel intensity histograms of $R26^{SmoM2YFP}$ and $R26^{SmoM2YFP}$/$Cyr61^{eGFP}$ that demonstrate a significant increase in eGFP signal (Fig. 1D, EV1C). Additionally, we generated tumors in the $Yap^{fl/fl}$ $R26^{SmoM2YFP}$ $Cyr61^{eGFP}$ background and show that YAP-negative clones or cells have baseline YFP/eGFP expression (Fig. S1A). Thus, while we agree that the readout eGFP might not be ideal given the background YFP expression, our data demonstrate significant upregulation of eGFP activity in a subset of BCCs, which is YAP dependent.

The provided expression data is very surprising as the authors detect only 98 genes differentially expressed gene (1.5 fold cutoff) and looking at this gene list there are no obvious genes involved in cell cycle progression. Moreover, a slightly more stringent cutoff (2 fold) illustrate that only 5 genes are detected as differentially expressed using a padj cut off of 0.05. Combined with the strategy for isolating these cells, it is questionable that this is a pure cell population from BCCs. In these experiments, the authors use SmoM2-YFP/Rosa26-lsl-tdTomato lines combined with YAPfl/fl in order to assess deletion based on Krt14CreER activation. Here tdTomato is used as a proxy for both SmoM2 and YAP deletion. As this reporter model is known recombine very easily upon cre activation, tdTomato expression is likely to be much more widespread than YAP deletion and SmoM2 activation. Importantly, no controls are provided to demonstrate that this indeed enable purification of cells from BCCs with YAP deleted. The authors should consequently have adopted a sorting strategy for YFP as this obviously is a measure for both BCCs and activation of Cre. The provided analysis showing differences in cell cycle genes is not convincing as analysis of the differentially expressed genes in the Panther database identify either none or cell adhesion as the biological process associated with the sorted population. This is an important point because the authors essentially exploit this data for the rest of the paper,

and all the required controls are missing. Moreover, the observed functional characteristics described in the previous figures are missing completely missing in the differentially expressed gene list provided.

We greatly appreciate the reviewer's input and believe that addressing the raised questions will significantly improve our manuscript. Initially, we attempted to FACS purify SmoM2-YFP positive cells based on the YFP expression alone but found that this fluorophore was extremely dim. This FACS strategy detected unexpectedly low numbers of SmoM2-YFP positive BCC cells in the epidermis with high tumor burden, and therefore we would have not been able to purify sufficient cells for molecular analysis. With this observation we concluded that YFP was an insufficient marker to purify all SmoM2-YFP positive BCC cells. Hence, we resorted to using tdTomato (*R26*$^{LSL\text{-}SmoM2YFP/LSL\text{-}tdTomato}$) as a more reliable marker for BCC cell purification. In order to address whether tdTomato expression in *R26*$^{LSL\text{-}SmoM2YFP/LSL\text{-}tdTomato}$ background could be used as a surrogate for SmoM2-YFP expression, we performed SmoM2-YFP IF co-staining with tdTomato in the ear BCC tissues used for RNAseq analyses. We detected approximately 96% overlap between tdTomato and SmoM2-YFP expression (Fig. S3A). Although the *R26*$^{LSL\text{-}tdTomato}$ allele has been reported as easily activated upon Cre, it seems that similar efficiencies are obtained within the *R26*$^{LSL\text{-}SmoM2}$ allele. Additionally, SmoM2-YFP/tdTomato double positive cells will have selective advantage to expand over tdTomato alone expressing cells, so while we might sort a few tdTomato+/SmoM2 - cells, we believe that these will be a small number of the total sorted cells.

In our model, we demonstrate that YAP-positive BCC cells have selective advantage and eventually outgrow YAP-negative cells. Hence, we had to identify the time point when we observe the highest *Yap* knockout efficiency in BCC tumors. In figure S3B, we show an example of 6-week time point post tamoxifen in ear and tail to demonstrate that ear epidermis has the highest frequency of YAP-negative BCC cells. We quantitated the *Yap* knockout efficiency at around ~70% in SmoM2-YFP positive cells (6 weeks post high-dose tamoxifen) (Fig. S3C). Hence, we proceeded to use 6-week post high-dose tamoxifen time point for the RNAseq analysis. We acknowledge that the population used for the RNAseq was not pure, and accounting for all the caveats of cell isolation, probably the YAP-negative cells were more prone to dying during our cell purification protocol. This is likely a reason that the fold changes were not very high, but we believe that this dataset still allowed us to make important conclusions about the role of YAP in BCCs.

In the figure EV4A, we provided GSEA analyses of YAP-positive versus YAP-negative BCC tumors. The input data used for the GSEA analyses were normalized RNAseq counts without any thresholds (i.e., fold change). We agree with the reviewer that discovering change in cell cycle does not provide additional value to our manuscript. We have removed GSEA analysis from the manuscript. However, our Ingenuity Pathway Analysis uses

differentially expressed genes with indicated thresholds (fold change 1.5 and p-val adjusted <0.05) to identify reduction in JNK signaling.

4) The link to AP1 is not novel (Zanconato et al., 2015, Nature Cell Biology). Moreover, the data related to AP1 binding elements remains purely descriptive will little additional results to back up the observation. Right now this is based on staining, a few Western blots and qPCR for some AP1 genes, where the authors describe only minor changes to gene expression. Is this really relevant in the context effects mediated via YAP? Is AP1 important in this context? Do they bind the same elements? Why is Jnk suddenly activated? Is this related to YAP? All of these questions and potentially more needs to be addressed in order to reach the conclusion that 'YAP-TEAD signaling promotes Basal Cell Carcinoma development via cJun/AP1 axis' (the title of the manuscript).

We value the reviewer's input regarding the connection between YAP and AP1 signaling. We agree that our ChIPseq data confirm the previous report by Zanconato, F., *et al*. that YAP and AP1 bind to overleaping promoters/enhancers to modulate gene signatures. We did not pursue chromatin studies to further demonstrate this, given that this has already been shown. Our work instead focuses on the previously unreported finding that YAP increases overall c-JUN phosphorylation, c-JUN protein, and c-JUN activity. Using *in vitro* and *in vivo* models, we show that YAP modulation significantly affects phosphorylation and total protein level of c-JUN (Fig 6). Specifically, we have added *in vivo* YAP overexpression data, which shows induction of BCC-like lesions in epidermis and significant increases in JNK and c-JUN phosphorylation (Fig. EV6C). Conversely, JNK inhibition *in vitro* completely impaired BCC cell line proliferation, which was concomitant with a decrease in c-JUN phosphorylation (Figure EV6A).

Minor concerns:

The authors state that YAP promotes growth independent of Wnt and Hedgehog signaling. YAP could however be an important mediator of the effects of Wnt and Hedgehog as suggested previously (Azzolin et al., 2014, Cell).

We agree with the reviewer that our data can not completely exclude that YAP mediates a particular Wnt or Hedgehog phenotypes; however, we used specifically established markers for these pathways to determine if the canonical signaling had been affected. In the case of Wnt signaling, Blanpain lab has demonstrated that Wnt signaling drives embryonic hair follicle phenotype (EHFP) in BCC tumors. We show that none of the EHFP markers (LHX2, CUX1, LEF1, PCDH) are changed in the YAP-negative BCC (Fig. EV3A). We also show that canonical Wnt markers (Lgr5 and Axin2) are not changed in YAP-negative BCC (Fig. EV3B). Similarly, we show that canonical Hedgehog markers

(Gli1 and Ptch1) are also not changed in YAP-negative BCC. Overall, we concluded that YAP-loss has no impact on canonical Wnt or Hedgehog signaling output in BCC.

Figure 5 - gene names are listed but cannot be read.

We have increased the text size for Figure 5D.

The authors need to be consistent when describing genes differentially expressed. Is this 97 or 98 (Page 10).

We appreciate the reviewer for pointing out this discrepancy. We have changed differentially expressed genes to 97.

Controls are missing for the ChIPseq analysis

We included Input ChIPseq tracks for all genes in Figure 5E.

Referee #3:

The authors suggest that YAP plays an essential role in development of BCC based on correlation of expression in tumor models as well as functional evidence with loss of function in an animal model. While these basic observations are not surprising given the extensive role for YAP previously described in skin tumorigenesis, the authors provide some significant new data to suggest that YAP exerts its effect through an interaction with the JNK pathway. There are several improvements that could be made to make the study stronger, as well as several outstanding questions not yet addressed by the data provided.

1, in fig 1C, it is difficult to see where the in situ signal is positive versus negative. this should be shown with a sense control side by side for both YAP and TAZ.

For the *in situ* hybridization, the approach we use is RNAscope, a commercial single molecule *in situ* hybridization platform. The gene specific probes in this platform are composed up to 20 oligos that are complementary to the gene of interest, which increases specificity and sensitivity, and therefore no antisense control is needed. In figure EV1B, we show a magnified view of Yap and Taz mRNA detection, which shows non-overlapping expression pattern in tissue.

2, in fig 1D the authors use a YAP reporter, but do not show the activity in normal skin, so it is not clear how to interpret the signal in the Smo induced skin.

We agree with the reviewer that Cyr61^{eGFP} reporter activity was missing for wildtype epidermis. We have included these data, which demonstrate that most Cyr61^{eGFP} activity is

localized in sebaceous gland and infrequent basal keratinocytes (Fig. 1D, upper panels). Additionally, we have generated BCC tumors with *Yap*$^{fl/fl}$ *R26*^{SmoM2YFP} *Cyr61*^{eGFP} background to show reporter specificity for YAP activity. The YAP-negative clones or cells have baseline YFP/eGFP expression compared to YAP-positive clones with high mosaic YFP/eGFP expression (Fig. S1A).

3, in fig 2, the authors show the effect of loss of YAP in a BCC model. However, the authors do not show a temporal analysis of the effect, so it is not clear if the tumorigenesis is identical but just slower or if the process is affected in a more fundamental way. the quantification in C suggests that the pathology is not much different.

We appreciate the reviewer's suggestion to clarify whether tumor initiation or progression is impacted by YAP loss. We have now quantified the number of dysplastic BCC clones emerging early (4 weeks post tamoxifen) in *Yap*$^{+/+}$ versus *Yap*$^{fl/fl}$ backgrounds. Indeed, we detect significant reduction in the number of dysplastic clones in *Yap*$^{fl/fl}$ ear and tail epidermis at this time point (Fig. EV2A). However, when we study the percentage of YAP-positive versus YAP-negative tumors between 6- and 22- week time points, we see mainly outgrowth of YAP-positive tumors. Our Figure 3 addresses growth and survival of YAP-negative BCC clones over time. Overall, our data support the conclusion that YAP regulates BCC initiation and progression.

4, I fig 5, the authors perform a molecular analysis in an attempt to understand how loss of YAP affects tumorigenesis. It si surprising that that only 150 or so genes were affected by loss of YAP, though perhaps this is explained by the fact that the phenotype is not too dramatic. How many genes are affected when YAP is deleted from normal epidermis?

We believe that one explanation for the low number of differentially expressed genes recovered has to do with the incomplete deletion of the *Yap* alleles. Thus, even though we show data now that up ~70% BCC cells have *Yap* deleted in ear BCC tumors (6 weeks post high dose tamoxifen, Fig S3C), we believe that our RNAseq was contaminated with BCC cells that did not fully delete *Yap*. Additionally, our YAP reporter line (*Cyr61*^{eGFP}) indicates that YAP is transcriptionally active only in subset of BCC tumors cells (~ 40 – 50%) at any time. This would suggest that, additionally, cells with low YAP activity dampen RNAseq detection of differently expressed genes. Still, even though our approach is not the most ideal, we are able to identify a number of informative differentially expressed genes.

Since we see no phenotypic impact on normal epidermis upon YAP loss, and our reporter (*Cyr61*^{eGFP}) shows few YAP active basal keratinocytes, we conclude that YAP is not transcriptional active in the majority of these cells. In this case, we opted out from doing RNAseq in *Yap*-null epidermis, which would likely show very few differentially expressed genes.

5, the link to Jnk signaling is interesting, could the authors demonstrate this functionally? if a transgenic method is beyond the scope, how about a topical small molecule stimulator or inhibitor?

We agree with the reviewer that the JNK connection to BCC growth is very interesting and should be pursued further as a potential therapeutic option. In fact, we tested whether BCC cell lines were sensitive to JNK inhibitor (SP600125) and showed significant impairment in cell proliferation. The decrease in proliferation was concomitant with decrease in c-JUN phosphorylation (Fig EV6A).

Additionally, we assessed whether YAP-induced IFE thickening, which resemble BCC-like lesions, is associated with increased JNK and JUN activity. When compared to wildtype IFE, YAP induced lesions show robust c-JUN (S63 and S73) and milder JNK phosphorylation (Fig. EV6C).

Although we demonstrated that JNK activity was necessary for BCC growth *in vitro*, in response to the reviewer's comments we attempted to study its function *in vivo*. BCC tumors were induced in $Yap^{+/+}$ background and allowed to develop for 8 weeks post tamoxifen. The mice were randomized to receive daily intraperitoneal injections of vehicle or two JNK inhibitor doses (30mg/kg or 50mg/kg/ per day). The doses chosen were based on the previously published studies using the SP600125 compound (Takahashi, R.*, et al*. Cancer Science 2013). Seven days after starting daily SP600125 administration, animals were euthanized for tissue analyses. However, we found that animals receiving even the higher SP600125 (50mg/kg) dose had no decrease in pJNK1/2, pJUN S63, and pJUN S73 compared to vehicle treated animals (not shown). Hence, our experimental approach was unable to answer whether JNK inhibition would impair BCC growth *in vivo*.

6, the cell line work in EV2 is interesting, what happens when YAP and TAZ are downregulated? are these two hippo factors compensatory? could double downregulation lead to synthetic lethality in cancer model?

We agree with the reviewer that additional genetic data was needed to assess TAZ contribution in BCC growth. We have included data describing the effect of Yap or Yap/Taz knockdown on BCC cell line proliferation *in vitro*. We observed that Yap/Taz depletion had a much stronger effect on BCC proliferation data, arguing for functional overlap (Fig. EV2C). We then tested *in vivo* Taz contribution in YAP-negative BCC via genetic approach. For this, we analyzed BCC growth in $Yap^{fl/fl}$ and $Yap^{fl/fl}$ $Taz^{fl/fl}$ backgrounds and demonstrated that *Yap/Taz* knockout is no different than *Yap* knockout alone (Fig. S2D-E). Thus, our data strongly suggest that TAZ activity does not significantly contribute to tumor growth *in vivo*, whereas it does *in vitro*. We still do not understand the basis for these differences. We also find that there is no compensatory Taz mRNA increase

following YAP knockdown (EV2C). Hence, our work highlights discrepancies between *in vitro* and *in vivo* dependency of cancer cells on the Hippo effectors, YAP and TAZ.