EMBO reports

BAZ2A-mediated repression via H3K14ac-marked enhancers promotes prostate cancer stem cells

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DOI: 10.15252/embr.202153014

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Review Timeline:

Submission Date:	6th Apr 21
Editorial Decision:	12th May 21
Revision Received:	18th Jun 21
Editorial Decision:	26th Jul 21
Revision Received:	29th Jul 21
Accepted:	2nd Aug 21

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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 12th May 2021

Dear Prof. Santoro,

Thank you for the submission of your research manuscript to EMBO reports. We have now received the 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, the referees think that these findings are of interest. However, they have several comments, concerns and suggestions, indicating that a major revision of the manuscript is necessary to allow publication of the study in EMBO reports. As the reports are below, and all their points need to be addressed, I will not 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 or in the 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 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. We are aware that many laboratories cannot function at full efficiency during the current COVID-19/SARS-CoV-2 pandemic and we have therefore extended our 'scooping protection policy' to cover the period required for full revision. Please contact me to discuss the revision should you need additional time, and also if you see a paper with related content published elsewhere.

When submitting your revised manuscript, please also carefully review the instructions that follow below.

PLEASE NOTE THAT upon resubmission revised manuscripts are subjected to an initial quality control prior to exposition to re-review. Upon failure in the initial quality control, the manuscripts are sent back to the authors, which may lead to delays. Frequent reasons for such a failure are the lack of the data availability section (please see below) and the presence of statistics based on n=2 (the authors are then asked to present scatter plots or provide more data points).

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 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 file 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 (e.g. RNA-seq, ChIP-seq, structural and array data) are deposited in an appropriate public database. If no primary datasets have been deposited, please also state this a dedicated section (e.g. 'No primary datasets have been generated and deposited'), see below.

See also: 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. This is now mandatory (like the COI statement). 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:

6) 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 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.

7) 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

8) Regarding data quantification and statistics, please make sure that, where applicable, the number "n" for how many independent experiments were performed and the type of replicate (biological or technical), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated 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

9) Please note our new reference format: http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

10) For microscopic images, please add scale bars of similar style and thickness to all the microscopic images, using clearly visible black or white bars (depending on the background). Please place these in the lower right corner of the images. Please do not write on or near the bars in the image but define the size in the respective figure legend.

11) Please add up to 5 key words to the title page (below the abstract).

12) Please add a conflict-of-interest statement and a section explaining the authors contributions to the manuscript text (nest to the acknowledgements). Please order the manuscript sections like this: Title page - Abstract - Key words - Introduction - Results - Discussion - Materials and Methods -Data availability section - Acknowledgements - Author contributions - Conflict of interest statement - References - Figure legends - Expanded View Figure legends.

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.

Yours sincerely,

Achim Breiling
Editor

EMBO Reports

Referee #1:

In the manuscript from Pena-Hernandez et al the authors show how BAZ2A, through the recognition of H3K14ac on specific inactive enhancer regions, is able to silence genes involved in development and differentiation that are also commonly downregulated in aggressive PCa. In the characterization of the mechanism of action of BAZ2A the authors suggest also that EP300 is required to acetylate H3K14ac that is then recognize by the BRD of BAZ2A.

In the second part of the paper the authors focus on defining a potential role of BAZ2A in the transition of PC3 cells to a more cancer-stem like state. Suggesting that the inhibition of BAZ2A could be of relevance from a clinical point of view in targeting specific subpopulation of the tumor bulk with cancer stem-like features.

Major:
The authors specifically use the term "transition into a cancer stem like state", is there a reason why they use the term transition rather than selection or enrichment? The use of the term transition suggests plasticity, the experiments reported tackle selection/enrichment not plasticity of cell transition. To address this the authors should design different and appropriate experimentations.

In the experiments reported in figure 4C and D it is not clear when was the inhibitor added. Was the inhibitor added at the beginning of the culture in low adhesion plate or once the tumor-spheres were formed? Depending on when the inhibitor was added one could be affecting tumor-spheres formation or proliferation/survival. Again, the experiment designed is confusing and opens more questions then resolving them.

The discrepancy between the effect on PC3 proliferation using the siRNA for BAZ2A or chemical inhibitors specific for BAZ2A-BRD could be due to a different efficiency in inhibiting BAZ2A expression/activity, did the authors check the status of known target of BAZ2A after the treatment with the inhibitor in the specific conditions analyzed?

In figure 4F and G the authors show that BAZ2A inhibitors affect the morphology of prostate organoids in which Pten is downregulated, although the change in morphology is clear a viability/proliferation assay should be showed, indeed also for ki67 staining in figure 4H a quantification of the staining in terms of percentage of ki67 positive nuclei should be added.

Moreover, after infection with shRNA PTEN cells have a more compact structure but still few translucent organoids are present, do these organoids have more PTEN compared to the compact ones? Is organoid density affected?

In a previous work (Pietrzak et al PNAS 2020) the authors show that BAZ2A(TIP5) is critical for the initiation of PCa of luminal origin mediated by Pten-loss but dispensable once Pten-loss mediated transformation is established. In figure 4F cells were first depleted of Pten and then treated with the inhibitors, according to previous result one would expect no effect of the inhibitors instead the inhibitors seem to work, the authors should address this discrepancy.

Finally, the authors should rethink the title, there is not sufficient evidence in their data that

demonstrated transition of of prostate cancer cells into a cancer stem-like state

Minor:
(1)In Figure 4C in the schematic describing the experimental design I would change TIP5-BRDi with BAZ2A-BRDi.

Referee #2:

The manuscript of Pena-Hernandez and colleagues examines the role of the epigenetic regulator BAZ2A in prostate cancer. They use a prostate cancer cell model and perform ChIP-seq and biochemical analyses to show that the BAZ2A bromodomain binds to H3K14ac-regions. Interestingly, BAZ2A-H3K14ac seems to mark a special class of repressive enhancers that silence developmental and differentiation genes. They use a tumorsphere assay and expression analyses to show that BAZ2A is necessary for cancer cells to transition to a stem cell like state. As the knock-downs of BAZ2A, pharmacological inhibition of the BAZ2A bromodomain with small molecules reduced the ability to form stem-cell like structures / tumorspheres offering a potential druggable strategy along this axis.

On the technical side, this manuscript is very well done. Apart from a couple of minor things (see below), I could not find any major controls missing. Experiments are replicated in sufficient amounts and well described. The bioinformatics is according to the state-of-the art and thoroughly explored, the raw data provided on GEO is complete. I envision the ChIP-seq profiles and RNA-seq datasets to be a valuable resource for the field and they may stimulate many follow up studies.

The biology of prostate cancer cells is not among my expertise, so I am unable to make an in-depth judgement of this part of the work. Though, I had no problems following the logic being a reader outside the field and this seemed well done, too.

In my view, the paper should be published and I would be happy to read a revised version where the following points are addressed:
Fig. 1 - The ChIP-seq has been generated with a plasmid-based approach, where HA-BAZ2A and mutant are expressed from. From Fig. S1D it looks like these proteins are highly overexpressed compared to endogenous BAZ2A. Since the authors provide a mutant and many other validations, this is fine in my view, but could the authors comment on that in the manuscript? Particularly, since Baz2a is overexpressed in cancer - how do these levels (Baz2a-Plasmid // Baz2a-Cancer // Baz2a-non-Cancer) relate?

Fig 1. F/G Could the authors create a metaplot instead of a boxplot? This would be more informative, since, it would show whether the pattern / local enrichment at these bound regions changes too.

Fig. 1A shows n=96351 peaks. This is probably the total number of called peaks? However, in Fig. 2 and associated text, 527 genes with Baz2a at the promoter are mentioned. If this is 11% (Fig. 2B), that would relate to approximately 5000 peaks? Could the authors clarify these numbers and relationship between genes and peaks (maybe include a n= in Fig. 2B/C).

p.5 / L.24 - This should probably read Phosphorylation of H3S10.

Fig. S2E - the y-axis (-log10(p-value)) seems to display very high values. Can the authors verify these extremely high values are correct? Also, was the p-value or the padj-value plotted (the corrected, padj/FDR would be accurate in my view).

p. 7 / L.7 - P<0.05 or Padj/FDR<0.05?

Fig. 2C - some numbers seem randomly floating at the plot (e.g. a 0 at the legend/scale). Or would this be a 10? And a 30?

p.7/L9 - Since this is an RNA-seq experiment, that informs on stady-state expression, rather than transcription per se, I would rephrase this sentence to:
Among the 527 genes with a BAZ2A peak at their promoter region, only a minority was affected upon BAZ2A depletion (30 up- and 25 downregulated).

Fig. 3C: Why are both datapoints for the PC3 at 1? Was there no variation in the PC3 condition among the replicates or were these datapoints mathematically set to 1?

Fig 3: How do BAZ2A and BAZ2B expression levels change in the tumorsphere RNA-seq data? Could this be provided as a Supp. panel?

Related to Fig. 4: Do bulk H3K14ac levels and BAZ2A levels change upon treatment with the BRD inhibitors in PC3 cells? Can the authors provide a Western Blot or an immunofluorescence (or another experiment, that addresses this point).

Fig. S4E - no replicates for the shRNA control? The legend I think also misses the information on replicates (Values ares from n=? experiments).

Almost all of the imaging panels are missing scale bars.

The manuscript is well written. Though, I had sometimes a hard time finding my way with all the abbreviations that were used (I am aware of the issue with the maximum word count). Could the authors improve on that aspect (for example BRD could be written as bromodomain, so that it is not confused with bromodomain proteins such as BRD4)?

The authors only provide 2 replicates of their RNA-seq. I think this is fine, because a similar dataset has been previously published (which is acknowledged and cited by the authors, Gu et al., 2015). Since there are only 2 Reps, could the authors though provide some information on how well their dataset overlaps with the Gu et al. paper?

In the GEO upload, I noticed that the authors also generated ChIP-seq profiles from spheres. Why was this data not included in the manuscript?

Referee #3:

The manuscript by Pena-Hernandez et al "BAZ2A association with H3K14ac is required for the transition of prostate cancer cells into a cancer stem-like state" reports on a key role of the bromodomain containing protein BAZ2A in regulating cell state transition of prostate cancer cells into a stem-like state. The authors show that BAZ2A associates with inactive enhancers that are

characterized by H3K14ac marks.

Bromodomain dependent recruitment to these enhancers results in repression of genes that are frequently silences in prostate cancer. Mutation, knockdown as well as treatment with BAZ2A-BRD inhibitors BAZ2-ICR or GSK2801 affected the growth of prostate cancer cell lines in 3D and impaired a Pten-loss mediated transformed phenotype.

This is a very interesting and comprehensive report with new data on the role of the BAZ2A bromodomain and consequences of its pharmacological inhibition. The study has been designed well and data are appropriately discussed and documented. I have therefore only minor suggestions and recommend publication with high priority.

My only concern is that studies are "only" cell lines based and study have been carried out often with ectopically expressed protein. Prostate cancer is certainly not an easy model. However, recent reports suggest that patient derived organoid cultures can be established that may allow studies in the future on tumor tissue and in the context of the prostate microenvironment. This should not be a requirement for the current study.

The authors should however comment on potential roles of BAZ2B as inhibitors target both bromodomains and these proteins may have overlapping functions.

Response to the reviewers
We thank the reviewers for the positive comments. In this revised version, we modified the text to clarify points that were not sufficiently clear and included new data according to the reviewers' suggestions. All the new data support the conclusions of our work.

List of changes
Modifications in the text were highlighted in red.
The new data are listed here below.

	Reviewer	**Old manuscript**	**Revised version**
BAZ2A peak annotation	**Rev. 2**	**Figure 2B**	**Figure 2B**
Metablots of BAZ2A and H3K14ac	**Rev. 2**	**-------**	**Figs. EV1E,F**
Volcano plot RNAseq	**Rev. 2**	**Suppl. Fig. S2E**	**Fig. EV2E**
Expression of BAZ2A-regulated genes in PC3 cells+BAZ2A-BRDi	**Rev. 1**	**-------**	**Fig. EV4C**
BAZ2A and H3K14ac levels in PC3 cells+BAZ2A-BRDi	**Rev. 2**	**-------**	**Fig. EV4D**
BAZ2A and BAZ2B expression levels in PC3 and PC3-CSCs	**Rev. 2**	**-------**	**Fig. EV4F**
Pten levels in organoids	**Rev. 2**	**Suppl. Fig. S4E**	**Fig. EV4H**

Referee #1:
In the manuscript from Pena-Hernandez et al the authors show how BAZ2A, through the recognition of H3K14ac on specific inactive enhancer regions, is able to silence genes involved in development and differentiation that are also commonly downregulated in aggressive PCa. In the characterization of the mechanism of action of BAZ2A the authors suggest also that EP300 is required to acetylate H3K14ac that is then recognize by the BRD of BAZ2A.
In the second part of the paper the authors focus on defining a potential role of BAZ2A in the transition of PC3 cells to a more cancer-stem like state. Suggesting that the inhibition of BAZ2A could be of relevance from a clinical point of view in targeting specific subpopulation of the tumor bulk with cancer stem-like features.

Major:
The authors specifically use the term "transition into a cancer stem like state", is there a reason why they use the term transition rather than selection or enrichment? The use of the term transition suggests plasticity, the experiments reported tackle selection/enrichment not plasticity of cell transition. To address this the authors should design different and appropriate experimentations.
Author. We agree with the Reviewer and modified the text accordingly.

In the experiments reported in figure 4C and D it is not clear when was the inhibitor added. Was the inhibitor added at the beginning of the culture in low adhesion plate or once the tumor-spheres were formed? Depending on when the inhibitor was added one could be affecting tumor-spheres formation or proliferation/survival. Again, the experiment designed is confusing and opens more questions then resolving them.
Author. We are sorry to not have clearly described the tumorspheres assay in the presence of the inhibitors. The inhibitors were added at the beginning of the culture in low adhesion plate. We clarified this point in the text.

The discrepancy between the effect on PC3 proliferation using the siRNA for BAZ2A or chemical inhibitors specific for BAZ2A-BRD could be due to a different efficiency in inhibiting BAZ2A expression/activity, did the authors check the status of known target of BAZ2A after the treatment with the inhibitor in the specific conditions analyzed?

Author. We included in Supplementary Fig. S4 (now Fig. EV4) the analysis of several Baz2A-regulated genes showing upregulation upon treatment with BAZ2-ICR.

In figure 4F and G the authors show that BAZ2A inhibitors affect the morphology of prostate organoids in which Pten is downregulated, although the change in morphology is clear a viability/proliferation assay should be showed, indeed also for ki67 staining in figure 4H a quantification of the staining in terms of percentage of ki67 positive nuclei should be added.

Author. We are sorry for the confusion and to have not precisely described the results of this experiment. The number of organoids did not differ between control and BAZ2-BRDi and we included these data in the new Fig. EV4G. These results are consistent with our previous work (Pietrzak et al. 2020) showing that BAZ2A-KO impairs the initiation of PCa mediated by PTEN depletion without affecting viability. In other words, organoids with PTEN depletion and BAZ2A-KO were morphologically and transcriptionally very similar to control organoids even if PTEN was downregulated. This phenotype is the same we obtained by treating organoids with BAZ2Ai, indicating that BAZ2A role is mediated by the bromodomain.

The Ki67 IHC images are consistent with previous reports by others and our laboratories showing the increase of $Ki67_{pos}$ nuclei in PTEN-deleted organoids compared to control organoids. Further, they confirmed our previous results showing less $Ki67_{pos}$ nuclei in organoids with PTEN depletion and BAZ2A-KO compared to PTEN-depleted organoids, indicating the important role of BAZ2A bromodomain. This is not a key result of our study but a result that confirmed the alterations in morphology shown and quantified in Figs. 4F,G. Unfortunately, for technical reasons we cannot provide percentage of $Ki67_{pos}$ nuclei. In these IHC images it is very difficult to visualize $Ki67_{neg}$ nuclei. This limitation does not allow us to provide percentage of $Ki67_{pos}$ nuclei that should be calculated as $Ki67_{pos}$ vs total nuclei ($Ki67_{pos}$+$Ki67_{neg}$). Although we cannot provide these quantifications, we think that the images of Figure 4H showing multiple organoids with distinct Ki67 signal between the different groups can be sufficient to further support the effect of BAZ2A inhibitor on PTEN-deletion mediated initiation of PCa in organoids.

Moreover, after infection with shRNA PTEN cells have a more compact structure but still few translucent organoids are present, do these organoids have more PTEN compared to the compact ones? Is organoid density affected?

Author. The large amounts of organoids infected with shRNA-PTEN showed this compact structure and this is in agreement with previous work from ours and other lab (Karthaus *et al.*, 2014; Pietrzak *et al.*, 2020). It is possible that the few organoids with translucent phenotype could derive from cells that were less infected with shRNA-PTEN. However, considering that the majority of organoids showed this compact structure, we think that investigating the reason why few organoids are translucent is beyond the scope of this work that want to elucidate the function of BAZ2A-BRD.

In a previous work (Pietrzak et al PNAS 2020) the authors show that BAZ2A(TIP5) is critical for the initiation of PCa of luminal origin mediated by Pten-loss but dispensable once Pten-loss mediated transformation is established. In figure 4F cells were first depleted of Pten and then treated with the inhibitors, according to previous result one would expect no effect of the inhibitors instead the inhibitors seem to work, the authors should address this discrepancy.

Author. We are sorry for the confusion. As stated in the corresponding Figure legend, the organoids derived from mouse prostate cells were treated with DMSO, BAZ2-ICR (5μM), GSK2801 (5μM) or GSK126 (5μM) inhibitors followed by transduction with shRNA-Control or shRNA-*Pten*. We clarified this point in the result section. Thus, these results are consistent with Pietrzak et al showing that BAZ2A is required for the initiation of PCa driven by *Pten*-loss and highlighted an important role of BAZ2A-BRD in this process.

Finally, the authors should rethink the title, there is not sufficient evidence in their data that demonstrated transition of prostate cancer cells into a cancer stem-like state

Author. We modified the title

Minor:
(1) In Figure 4C in the schematic describing the experimental design I would change TIP5-BRDi with BAZ2A-BRDi.
Author. Thank you! We modified the text.

Referee #2:
The manuscript of Pena-Hernandez and colleagues examines the role of the epigenetic regulator BAZ2A in prostate cancer. They use a prostate cancer cell model and perform ChIP-seq and biochemical analyses to show that the BAZ2A bromodomain binds to H3K14ac-regions. Interestingly, BAZ2A-H3K14ac seems to mark a special class of repressive enhancers that silence developmental and differentiation genes. They use a tumorsphere assay and expression analyses to show that BAZ2A is necessary for cancer cells to transition to a stem cell like state. As the knock-downs of BAZ2A, pharmacological inhibition of the BAZ2A bromodomain with small molecules reduced the ability to form stem-cell like structures / tumorspheres offering a potential druggable strategy along this axis.
On the technical side, this manuscript is very well done. Apart from a couple of minor things (see below), I could not find any major controls missing. Experiments are replicated in sufficient amounts and well described. The bioinformatics is according to the state-of-the art and thoroughly explored, the raw data provided on GEO is complete. I envision the ChIP-seq profiles and RNA-seq datasets to be a valuable resource for the field and they may stimulate many follow up studies.
The biology of prostate cancer cells is not among my expertise, so I am unable to make an in-depth judgement of this part of the work. Though, I had no problems following the logic being a reader outside the field and this seemed well done, too.
In my view, the paper should be published and I would be happy to read a revised version where the following points are addressed:
Author. We thank the reviewer for the positive comments on our work.

Fig. 1 - The ChIP-seq has been generated with a plasmid-based approach, where HA-BAZ2A and mutant are expressed from. From Fig. S1D it looks like these proteins are highly overexpressed compared to endogenous BAZ2A. Since the authors provide a mutant and many other validations, this is fine in my view, but could the authors comment on that in the manuscript? Particularly, since Baz2a is overexpressed in cancer - how do these levels (Baz2a-Plasmid // Baz2a-Cancer // Baz2a-non-Cancer) relate?
Author. The plasmid-based approach was the only possibility to analyse the role of BAZ2A-BRD in PCa cells. We do not think that the overexpression of BAZ2A might have affected the results. For example, in the case of the ChIP, we validated some BAZ2A targets using a PC3 cell line expressing F/H-tagged BAZ2A. In the functional assay shown in Figure 3E, the number of spheres obtained from PC3 control (siRNA-Control) and cells expressing ectopic BAZ2A is very similar. BAZ2A levels are high in metastatic PCa, however, we cannot make direct comparisons between expression levels from plasmids and tumor vs. normal tissue.

Fig 1. F/G Could the authors create a metaplot instead of a boxplot? This would be more informative, since, it would show whether the pattern / local enrichment at these bound regions changes too.
Author. The metaplots can be found in Figures EV1E,F. The data are consistent with the corresponding heatmaps and quantifications shown in Figures 1A,F,G.

Fig. 1A shows n=96351 peaks. This is probably the total number of called peaks? However, in Fig. 2 and associated text, 527 genes with Baz2a at the promoter are mentioned. If this is 11% (Fig. 2B), that would relate to approximately 5000 peaks? Could the authors clarify these numbers and relationship between genes and peaks (maybe include a n= in Fig. 2B/C).
Author. We are sorry to not have sufficiently described the details of this analysis. Promoter annotation was done for BAZ2A peaks within -/+ 5kb from TSS. We have now included this information in the corresponding Figure 2B. The value 11.34% represents the fraction of BAZ2A peaks at promoters. Since some promoters contain more than one BAZ2A peak, the 11’480 peaks should not be interpreted as number of genes with BAZ2A-bound promoters, which are effectively 7’490. To analyse genes with BAZ2A-bound promoters we applied high stringency parameters: we shortened the window at the promoter to +/- 2kb from

TSS and selected these genes for BAZ2a peaks with high enrichment to input (≥3FC). We might have lost some candidate genes but at least we increased specificity. We included this information in the revised version.

p.5 / L.24 - This should probably read Phosphorylation of H3S10.
Author. Thank you for having highlighted this mistake. We replaced H3S19P with H3S10P.

Fig. S2E - the y-axis (-log10(p-value)) seems to display very high values. Can the authors verify these extremely high values are correct? Also, was the p-value or the padj-value plotted (the corrected, padj/FDR would be accurate in my view).
Author. We replaced Figure S2 with the y-axis corresponding to (-log10(Padj-value)). The significantly affected genes remained the same. It is true that the *P* and *Padj* values are high. We think that this is mainly due to the fact that these data originate from duplicates. Unfortunately, the sequencing of one si-control and one siBAZ2A sample had very low quality and we had to discard them. However, as also discussed below, the data we obtained were very similar to transcriptomic data obtained from microarray sequencing that we published in Gu et al. Nat Genetics (2015).

p. 7 / L.7 - P<0.05 or Padj/FDR<0.05?
Author. We replaced P values with Padj values since we modified Figure S2 (now Fig. EV2). The differentially expressed genes and their number remained the same.

Fig. 2C - some numbers seem randomly floating at the plot (e.g. a 0 at the legend/scale). Or would this be a 10? And a 30?
Author. We corrected the labelling of Figure 2C.

p.7/L9 - Since this is an RNA-seq experiment, that informs on steady-state expression, rather than transcription per se, I would rephrase this sentence to:
Among the 527 genes with a BAZ2A peak at their promoter region, only a minority was affected upon BAZ2A depletion (30 up- and 25 downregulated).
Author. We replaced the text with the suggested sentence.

Fig. 3C: Why are both datapoints for the PC3 at 1? Was there no variation in the PC3 condition among the replicates or were these datapoints mathematically set to 1?
Author. The values represent the measurements of two independent experiments that were performed and measured at different times and by different researchers. In this case, we set as 1 the control since the readout is the fold change in expression between PC3_CSCs and PC3 cells in each experiment. We would also like to clarify that these measurements are validation of the RNAseq shown in Figure 2A.

Fig 3: How do BAZ2A and BAZ2B expression levels change in the tumorsphere RNA-seq data? Could this be provided as a Supp. panel?
Author. BAZ2A and BAZ2B expression between PC3 and tumorspheres is not affected. Indeed, they are not listed as differentially expressed genes in tumorspheres compared to PC3 cells (Suppl. Table S4). To better clarify this point, we included a RNAseq track profile in Fig. EV4F.

Related to Fig. 4: Do bulk H3K14ac levels and BAZ2A levels change upon treatment with the BRD inhibitors in PC3 cells?
Author. We included a western blot in Figure EV4 showing that H3K14ac levels and BAZ2A levels are not affected in PC3 cells upon treatment with BAZ2-ICR inhibitor.

Fig. S4E - no replicates for the shRNA control? The legend I think also misses the information on replicates (Values are from n=? experiments).
Author. We are sorry for the confusion. The data were from two experiments. We corrected the Figure (now Fig. EV4H) and the corresponding legend.

Almost all of the imaging panels are missing scale bars.
Author. The images were taken with a camera attached to a light microscope that does not show a bar. We would like to clarify that the results from these images do not concern the size of tumorspheres and organoids but mainly only their number and morphology.

The manuscript is well written. Though, I had sometimes a hard time finding my way with all the abbreviations that were used (I am aware of the issue with the maximum word count). Could the authors improve on that aspect (for example BRD could be written as bromodomain, so that it is not confused with bromodomain proteins such as BRD4)?
Author. We replaced BRD with bromodomain all over the text.

The authors only provide 2 replicates of their RNA-seq. I think this is fine, because a similar dataset has been previously published (which is acknowledged and cited by the authors, Gu et al., 2015). Since there are only 2 Reps, could the authors though provide some information on how well their dataset overlaps with the Gu et al. paper?
Author. Considering the different techniques and bioinformatic pipelines used for the 2 datasets (microarray in Gu et al.; RNAseq in this study), the data overlap quite well. The RNAseq could identify > 60% of genes detected as differentially expressed in the published microarray analysis of PC3 cells upon BAZ2A-KD. Further, upregulation and downregulation states of BAZ2A regulated genes among the two dataset is practically identical (>99%) with only 4 genes showing differential expression states.

In the GEO upload, I noticed that the authors also generated ChIP-seq profiles from spheres. Why was this data not included in the manuscript?
Author. We thank the reviewer to have informed us about this. We are in contact with GEO to delete the ChIPseq profiles of the tumorspheres since these data were not used in this manuscript.

Referee #3:

The manuscript by Pena-Hernandez et al "BAZ2A association with H3K14ac is required for the transition of prostate cancer cells into a cancer stem-like state" reports on a key role of the bromodomain containing protein BAZ2A in regulating cell state transition of prostate cancer cells into a stem-like state. The authors show that BAZ2A associates with inactive enhancers that are characterized by H3K14ac marks.
Bromodomain dependent recruitment to these enhancers results in repression of genes that are frequently silences in prostate cancer. Mutation, knockdown as well as treatment with BAZ2A-BRD inhibitors BAZ2-ICR or GSK2801 affected the growth of prostate cancer cell lines in 3D and impaired a Pten-loss mediated transformed phenotype.
This is a very interesting and comprehensive report with new data on the role of the BAZ2A bromodomain and consequences of its pharmacological inhibition. The study has been designed well and data are appropriately discussed and documented. I have therefore only minor suggestions and recommend publication with high priority.

Author. We thank the reviewer for the positive comments on our work.

My only concern is that studies are "only" cell lines based and study have been carried out often with ectopically expressed protein. Prostate cancer is certainly not an easy model. However, recent reports suggest that patient derived organoid cultures can be established that may allow studies in the future on tumor tissue and in the context of the prostate microenvironment. This should not be a requirement for the current study

Author. The establishment of organoids from metastatic PCa of specimens remains still a challenge and only few organoid lines could be established. Further, to our knowledge, the establishment of long-term cultures of PCa organoids from primary PCa is not possible due to the overgrowth of non-cancer cells. It is true that our study is mainly based on the analysis of PCa cell lines, however, this still represents a good system to generate new concepts and hypotheses that can subsequentially be examined using more complex systems such as organoids. This is the strategy that we also used in this study where we analysed the effect of BAZ2A inhibitors using organoids that model the initiation of PCa driven by *PTEN* deletion.

The authors should however comment on potential roles of BAZ2B as inhibitors target both bromodomains and these proteins may have overlapping functions.

Author. The function of BAZ2B is not well studied and there is no evidence that BAZ2B might regulate PCa. This is also evident by our analysis showed in Figure EV4E indicating that BAZ2B-KD does not affect PC3-CSCs. Finally, we think it is unluckily that BAZ2A and BAZ2B have overlapping functions since BAZ2A acts mainly as repressor whereas BAZ2B functions has so far only related to gene activation (i.e. Arumugam et al., 2020, Cell Reports). Thus, we would prefer to not include further comments on BAZ2B since so far there is no indication that BAZ2B might play a role in PCa.

1st Revision - Editorial Decision 26th Jul 2021

Dear Prof. Santoro,

Thank you for the submission of your revised manuscript to our editorial offices. I 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, the referees now support the publication of your study. However, referees #1 and #2 have remaining points and suggestions to improve the manuscript I ask you to address in a final revised version of the manuscript. Please also provide a point-by-point-response addressing the remaining concerns of the referees.

Moreover, I have these editorial requests:

- I would suggest a more active and direct title. How about:
BAZ2A-mediated repression via H3K14ac-marked enhancers promotes prostate cancer stemness [or 'stem cells'].

- We plan to publish your manuscript in the Report format. For a Scientific Report we require that results and discussion sections are combined in a single chapter called "Results & Discussion". Please do this for your manuscript. For more details please refer to our guide to authors:
http://www.embopress.org/page/journal/14693178/authorguide#researcharticleguide

- Please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (also of the EV figures), and that statistical testing has been done where applicable. Please avoid the phrase 'independent experiment', but clearly state if these were biological or technical replicates. If statistical testing was done but there is no significant difference, please also mark this in the diagrams (n.s.).

- Please add scale bars of similar style and thickness to all the microscopic images, using clearly visible black or white bars (depending on the background). Please place these in the lower right corner of the images. Please do not write on or near the bars in the image but define the size in the respective figure legend.

- Per journal policy, we do not allow 'data not shown', which is stated in the manuscript (page 20). All data referred to in the paper should be displayed in the main or Expanded View figures, or an Appendix. Thus, please add these data (or change the text accordingly, if these data are not important). See:
http://embor.embopress.org/authorguide#unpublisheddata

- EV Tables 1, 2, 3 (named 'Supplementary Table 3') and 4 are datasets and too large to be shown as tables. Please name and upload these as datasets using the nomenclature Dataset EVx and add their legends on the first tab of the excel file. Then please change their callouts in the manuscript file and remove the EV table legends from the manuscript text file.

- Table EV5 contains 3 separate tables. I would suggest uploading these as main tables to the methods sections. Please add these as Table 1, 2 and 3 to the main manuscript text with a legend and change the callouts in the manuscript text.

- Thanks for submitting the Western blot source data. Please submit this as one PDF file per figure.

- Please remove the words 'Accession numbers' form the data availability section.

- Please confirm compliance to the Arrive guidelines in box 10 of the author checklist. Please also fill in every box (in case use n.a.).

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

In addition, I would need from you:
- a short, two-sentence summary of the manuscript (not more than 35 words).
- two to four 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.

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.

Yours sincerely,

Achim Breiling
Editor
EMBO Reports

Referee #1:

The authors have addressed most of the point raised.

The authors stated in the rebuttal: "The number of organoids did not differ between control and BAZ2-BRDi and we included these data in the new Fig. EV4G." A minor point is that the figure is EV4I. Moreover they state: "These results are consistent with our previous work (Pietrzak et al. 2020) showing that BAZ2A-KO impairs the initiation of PCa mediated by PTEN depletion without affecting viability". How is viability not affected if, as they state further, "The Ki67 IHC images are consistent with previous reports by others and our laboratories showing the increase of Ki67pos nuclei in PTEN-deleted organoids compared to control organoids."? Given that I do believe that the shift in morphology is a consequence of the treatment, the point remains that this data should be complemented by quantification of the number of proliferating cells. This can be achieved by measuring Ki67 staining on IHC and relate this to the total amount of nuclei. In alternative, given the technical difficulties the authors could perform a viability assay performing the Cell Titer 3D Glo.

To my understanding the authors want to demonstrate that the effects of BAZ2A are dependent on the activity of the bromodomain. The activity of the inhibitors tested in not clear. The doubt that I have comes from the fact that the downstream targets are not significantly affected (Fig EV4C) by the presence of the inhibitor. Might the effect that the authors see on the organoids not be mediated by the bromodomain itself but an off target? Are the differences between the BAZ2A targets statistically significant? What about in the mouse organoids, is there an effect on these targets?

Minor points:
All images are missing scale bars which should be all included even if they are not quantifying size.
Also the magnification image of the organoids in figure 4F is of poor quality (out of focus).
The legend of Fig EV4 does not correspond to the figure.
In the text (line 2 page 10) "Similarly, treatment of PC3 cells with siRNA BAZ2A reduced the number of CSC (Fig3E)". The figure shows a reduction of tumor spheres, the authors should changed CSC in the text to tumor spheres.
On Line 7 page 10 the authors claim that "Collectively, these results indicate that a functional BAZ2A-bromodomain is required for PC3-CSCs and supported a role of BAZ2A in driving aggressive and poorly differentiated PCas." This sentences should be toned down given that the data they show is in vitro.

Referee #2:

The manuscript has been greatly improved after revision and all my concerns have been addressed in the RTR and/or the manuscript. I recommend acceptance.
(P.S. I spotted two minor details that should be fixed before publication:
p. 36 / Legend should read I (instead of G)
EV4H Figure / y-axis should probably read "Rel. mRNA levels" (as all other qPCR panels)

Referee #3:

My first review suggested only minor changes to the paper. The authors have addresses all issues I raised and my suggestion trying to establish organoids was in my point of view not a requirement for the acceptance of the paper. I see therefore no more issues that would need to be addressed and congratulate the authors to this interesting study.

Referee #1:

The authors have addressed most of the point raised.

The authors stated in the rebuttal: "The number of organoids did not differ between control and BAZ2-BRDi and we included these data in the new Fig. EV4G." A minor point is that the figure is EV4I.

Author: We corrected the legend of the corresponding Figure (now Fig. 6E). In the text, the Figure was correctly cited.

Moreover they state: "These results are consistent with our previous work (Pietrzak et al. 2020) showing that BAZ2A-KO impairs the initiation of PCa mediated by PTEN depletion without affecting viability". How is viability not affected if, as they state further, "The Ki67 IHC images are consistent with previous reports by others and our laboratories showing the increase of Ki67pos nuclei in PTEN-deleted organoids compared to control organoids."?

Author: We would like to clarify that Ki67 is not a marker of cell viability. Instead, Ki67 is a marker of cell proliferation. As demonstrated by our previous work (Pietrzak et al. PNAS, 2020), BAZ2A-KO does not affect cell viability but impairs the transformation mediated by *PTEN*-loss. In this work, we observed the same phenotypes (morphology and number of $Ki67^+$-cells) using the two BAZ2A inhibitors.

Given that I do believe that the shift in morphology is a consequence of the treatment, the point remains that this data should be complemented by quantification of the number of proliferating cells. This can be achieved by measuring Ki67 staining on IHC and relate this to the total amount of nuclei. In alternative, given the technical difficulties the authors could perform a viability assay performing the Cell Titer 3D Glo.

Author: To overcome the technical difficulties described in the previous response to measure the percentage of $Ki67^+$-cells in IHC, we now provide quantifications corresponding to the number of $Ki67^+$-cells in each organoid. These data are displayed in the **new Fig. 6D** and they are consistent with the representative IHC images of **Fig. 6C** showing that the treatment with BAZ2A inhibitors significantly decreases Ki67 signal in Pten-KD organoids.

To my understanding the authors want to demonstrate that the effects of BAZ2A are dependent on the activity of the bromodomain. The activity of the inhibitors tested in not clear. The doubt that I have comes from the fact that the downstream targets are not significantly affected (Fig EV4C) by the presence of the inhibitor. Might the effect that the authors see on the organoids not be mediated by the bromodomain itself but an off target? Are the differences between the BAZ2A targets statistically significant?

Author: We used two different inhibitors targeting BAZ2A-BRD and demonstrated that they impair BAZ2B-BRD association with H3K14ac. We showed that both inhibitors caused similar changes in both tumorspheres and organoids and are consistent with previously published data showing that BAZ2A-KO impair *Pten*-loss mediate transformation. Further, the results in tumorspheres could be reproduced using BAZ2A BRD-mutant with impaired ability to associate with H3K14ac. Finally, we showed that these effects

are specific to BAZ2A and not to BAZ2B. Thus, we think that the possibility that both inhibitors do not target BAZ2A-BRD or have the same off-targets in tumorspheres and organoids is a very unlucky event.
The data of Fig. EV4C (now EV4B) were requested by this Reviewer to show that treatment of PC3 cells with BAZ2Ai upregulates the expression of BAZ2A-target genes in PC3 cells (not organoids). The data are from two independent experiments and, consequently, we cannot calculate statistical significance. However, the upregulation in PC3 cells upon treatment with BAZ2-ICR compound is clear in both experiments. To further clarify this point, we have now represented the values of each experiment with different colours.

What about in the mouse organoids, is there an effect on these targets?

Author: BAZ2A-regulated genes were identified in human metastatic PC3 cells using the intersection of ChIPseq and RNAseq analyses. The prostate organoids used in this study are from mouse and represent a model of primary PCa. We do not possess BAZ2A-ChIPseq data to identify BAZ2A-target genes in organoids. Consequently, we cannot provide measurements for the expression of BAZ2A-target genes in organoids.

Minor points:

All images are missing scale bars which should be all included even if they are not quantifying size.

Author: This point was explained in the previous response to the Reviewer 2. The images were taken with a camera attached to a light microscope that does not show a bar. We would like to clarify that the results from these images do not concern the size of tumorspheres and organoids but mainly only their number and morphology (i.e. translucent or compact structure).

Also the magnification image of the organoids in figure 4F is of poor quality (out of focus).

Author: We modified the images to improve quality.

The legend of Fig EV4 does not correspond to the figure.

Author: We corrected the y-axis of Fig. EV4H (now EV5A)

In the text (line 2 page 10) "Similarly, treatment of PC3 cells with siRNA BAZ2A reduced the number of CSC (Fig3E)". The figure shows a reduction of tumor spheres, the authors should changed CSC in the text to tumor spheres.

Author: We modified the text accordingly.

On Line 7 page 10 the authors claim that "Collectively, these results indicate that a functional BAZ2A-bromodomain is required for PC3-CSCs and supported a role of BAZ2A in driving aggressive and poorly differentiated PCas." This sentences should be toned down given that the data they show is in vitro.

Author: We kindly disagree. The sentence does not only refer to the role of BAZ2A in tumorspheres (in vitro) but also support the data described in Fig. 2 ,3 EV2 ,and previous work (Gu et al., Nat Genetics 2015) that implicated BAZ2A in aggressive disease using molecular and clinical data from the patients

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Referee #2:

The manuscript has been greatly improved after revision and all my concerns have been addressed in the RTR and/or the manuscript. I recommend acceptance.

(P.S. I spotted two minor details that should be fixed before publication:

p. 36 / Legend should read I (instead of G)

EV4H Figure / y-axis should probably read "Rel. mRNA levels" (as all other qPCR panels)

Authors: We thank the Reviewer for the constructive comments. We corrected the legend and the y-axis of Figure EV4H (now EV5A).

Referee #3:

My first review suggested only minor changes to the paper. The authors have addresses all issues I raised and my suggestion trying to establish organoids was in my point of view not a requirement for the acceptance of the paper. I see therefore no more issues that would need to be addressed and congratulate the authors to this interesting study.

Authors: Thank you!

2nd Revision - Editorial Decision 2nd Aug 2021

Prof. Raffaella Santoro
University of Zurich
Molecular Mechanisms of Disease
Winterthurerstrasse 190
Zurich 8057
Switzerland

Dear Prof. Santoro,

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

At the end of this email I include important information about how to proceed. Please ensure that you take the time to read the information and complete and return the necessary forms to allow us to publish your manuscript as quickly as possible.

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Corresponding Author Name: Raffaella Santoro
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2021-53014

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)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	not relevant to this study
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	not relevant to this study
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No data were excluded from the analyses
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.	No
For animal studies, include a statement about randomization even if no randomization was used.	not relevant to this study
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.	No
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Is there an estimate of variation within each group of data?	No
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).	All the details of the antibodies were in cluded in the Table EV5
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	The cell lines were purchased from ATCC. Tests for mycoplasma contamination are routinely performed (one a month). All cell lines used in this study are mycoplasma free.

* 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.	Prostate organoids were derived from 6-8 weekd old wt C57BL/6 male mice.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	The experiments were performed according to the ethical regulation that was approved by Organentnahme bei Kanton Zurich Gesundheitdirektion Veterinäramt, ZH240/18.
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 to the ARRIVE guidlines

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	
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.	
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	
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.	
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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	Data availabity section was included
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).	The datasets produced in this study are available in the following database: RNA-Seq and ChIP-Seq data: Gene Expression Omnibus GSE131268 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE131268)
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).	
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.	

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