PBAF chromatin remodeler complexes that mediate meiotic transitions in mouse

Rodrigo O. de Castro, Luciana Previato de Almeida, Agustin Carbajal, Irma Gryniuk and Roberto J. Pezza
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Original submission

First decision letter

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MS TITLE: PBAF chromatin remodeler complexes that mediate meiotic transitions in mouse

AUTHORS: Rodrigo DeCastro, Luciana Previato, Agustin Carbajal, Irma Gryniuk, and Roberto J Pezza

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

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision.

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

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

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Reviewer 1

Advance summary and potential significance to field

The paper by De Castro et al., entitled "PBAF chromatin remodeler complexes that mediate meiotic transitions in mouse" is an interesting study on the male gamete-specific function of ARID2 and BRG1. The authors mainly focus on the analyses of ARID2-deficient testes by generating three different Cre-diver mouse lines, Ddx4-Cre, Stra8-Cre and SPO11-Cre, to dissect out the stage-specific function of one of the SWI/SNF chromatin remodeling complexes in mice. The authors also generated conditional BRG1-deficient mice to compare the phenotype with that of ARID2. The authors firstly found that the expression of ARID2 begins in the diplotene spermatocytes and is highest in the round spermatids. The authors secondly found that the deficiency of ARID2 causes abnormal cell division in primary and secondary spermatocytes, which is associated with aberrations in chromosome orientation, chromosome congression failure and gross spindle aberration.
The deficiency ends up with severe apoptosis and defects in the second meiotic cell division. The ARID2 deficiency also causes overexpression of the Aurora B kinase. Finally the authors showed not only the possibility that BRG1 and ARID2 are required at different meiotic phases but also the evidence that ARID2/PBRM1 form a complex independent of BRG1.

Comments for the author

Major points I. Quality
-Experiments and data Overall, the quality of histological and western blot analyses is excellent. I have several suggestions to improve this work.
1. Gene deletion efficiency by Stra8-Cre and Spo11-Cre is different from one gene to another. Please evaluate the gene deletion efficiency of Arid2 by antibody staining for ARID2. If the gene deletion by Stra8-Cre is not efficient, the authors may delete these data.
2. The total protein level of Aurora B kinase is significantly increased in Arid 2 Δ/Δ mice which is quite interesting. Is this due to an increase of transcription or a decrease in the degradation of Aurora B kinase?
3. Given that Hec1/NDC80 is one of the key substrates for Aurora B kinase in meiosis and the phosphorylation status of Hec1/NDC80 links to stabilization of kinetochore-microtubule attachments (DeLuca et al. 2011), analyses of Hec1/NDC80 may help to understand the molecular mechanisms causing the phenotype shown in this manuscript.
Please check protein levels and phosphorylation levels of Hec1/NDC80 in the knockout mice.
4. For proper comparison, please identify the stages/steps where BRG1- and ARID2-deficient mice showed abnormal phenotypes. PNA lectin staining may help to characterize the stages/steps and the phenotypes.
-Completeness:
If the authors can respond to the reviewer's suggestions, this work will be complete.

II. Impact:
-Novelty: There is no report about the ARID2 deficiency in mice in the germ cell lineage.
Also, this work showed a stage-specific combinatory usage of SWI/SNF complex subunits during meiotic cell differentiation.
-Broad interest: The new findings of this in vivo study unveil the molecular behaviours of the members of SWI/SNF complexes in a stage-specific manner at meiotic phases. The reviewer assumes that this is an important result not only for the chromatin remodeling field but also for meiotic cell division and germ cell development studies.

Minor points
1. The enlargement should indicate the area wherein the pictures were enlarged.
2. Figure3C. Please show the number of mice the authors analyzed.
3. The scale bars in Fig6D, Figure S1 and Figure S2 are missing.
4. The round brackets before and after PBAF in the abstract should be deleted.
5. "R" of Aurora B is missing in Figure 4C (wild type).

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Reviewer 2

Advance summary and potential significance to field

In this study, the authors intend to demonstrate the functions and contributions of the chromatin remodeler complex “PBAF”. In general, chromatin remodelers such as PBAF and BAF are involved in DNA metabolism activities, gene transcription and cell development. To gain more information about the function of PBAF in gametogenesis, they decided to study two components of the complex, BRG1 and ARID2, which have been reported to be involved in germ cell development.
By immunostaining of the tests, they found out that BRG1 and ARID2 are expressed in the different germ cell developmental stage. BRG1 expression appears in the pachytene stage in spermatocytes, whereas ARID at the end of meiosis in spermatids. Conditional knocked-out mice are created using cre-loxp system, for stage specific BRG1 or ARID2 deletions: undifferentiated spermatogonia differentiating spermatogonia, and spermatocyte.
The results showed there was testis atrophy in ARID and BRG1 KO mice. Deleting ARID2 also caused azoospermia in adult testis. H&E and immune staining suggest there were gigantic polynuclear cells, lagging chromosomes, and spindle formation problems, revealing the failure of the metaphase-to-anaphase stage transition in meiosis. For this reason, Aurora Kinase B expression was measured. The western blot result showed the expression of it increased in ARID2 KO mice. Moreover, they double confirmed the fact by immunostaining that BRG1 has influences on chromosome pairing in the early meiosis stage. Last, using Immunoprecipitation, they verified that ARID2 can form an independent complex from BRG1.
To sum up, they demonstrate the respective contributions of the two PBAF components, BRG1 and ARID2, including their different expression times and functional influences during spermatogenesis. The implications of BRG1 and ARID2 being important for meiosis have been described earlier. The current manuscript dissected more detailed phenotypes in the loss of function mutant mice.

Comments for the author

The implications of BRG1 and ARID2 being important for meiosis have been described earlier. The current manuscript dissected more detailed functional significance.
Some specific comments can be addressed to polish the current manuscript for publication.
1. Several conditional KO mice mutating BRG1 and ARID2 were created for analyze the function of these factors at different developmental stage. However the rationale behind each experimental design and the reasons for selecting specific conditional mutant to address each scientific questions for Figures 1, 2 and 5, should be further clarified.
For Figure 2B, would it be better to show data from Spo11-ARID2 mutant as well? In case the authors found the data from other conditional mutants not behaving as expected, I would hope to see some discussion about these potential discrepancy.
2. If I have not mistaken, the authors mainly use explained the importance of ARID2 in meiotic cells mainly basing on ddx4-ARID2 mutant. However, DDX4-trigger conditional KO could create ARID2 mutant as early as embryonic stage. In addition the results from Stra8- ARID2 mutant should be the best design among the three conditional KO strain, but they mentioned that the data was weird.
3. In Fig. 4, the WB and immunostaining results of Aurora kinase B are not consistent. Would the authors please discuss into more details?
4. At Page 5, The authors stated that Stra8- ARID2 mutant mice showed a milder phenotype. However, the testes weight seems to imply otherwise?
5. Figure 4C. Which ARID2 mutant strain they chose?
And the expression level seems to be higher in wildtype?
And the overlap percentage between SYCP3 and Aurora B is higher in mutant?
6. It seems that further experiments to reveal chromatin structure and gene expression have not been addressed. Would the authors discuss about these perspectives?
7. Not all of the conditional mutant mice shown are directly supporting the title as indicated in some of the comments above. They authors would need to clarify or fine-tune the wording of the title a bit.

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Reviewer 3

Advance summary and potential significance to field

In this study, the authors explored a functional role for that PBAF regulatory subunit ARID2 in spermatogenesis. How chromatin is remodeled, and the process underlies spermatogenesis remains unclear, the study addresses a gap in knowledge. By generating a mouse model of conditional Arid2 knockout in the male germline, the authors report a key role in regulating meiotic progression. Although the knockout clearly produces a spermatogenic defect many of the experiments seem to lack appropriate rigor and controls needed for drawing firm conclusions.

Comments for the author

In regard to setting up the story, important background and lead-in information about spermatogenesis and ARID2 seems to be lacking in the introduction section. In addition, given the existing body of knowledge about the role of other SWI/SNF components in regulating spermatogenesis, in particular meiosis, the advance in understanding made by the current study seems incremental. The authors might want to consider using the introduction section to better setup the importance of exploring the role of ARID2.
The authors should consider bolstering the rigor of data presented in Figure 1A. First, specificity of the primary antibodies for immunostaining in cross-sections of adult testes should be addressed with appropriate controls. At present, the authors do not seem to have included controls to account for non-specific staining of either the primary or secondary antibodies. Also, demonstration that the primary antibodies specifically recognize BRG1 and ARID2 has not been achieved. Based on information available on the supplier website for the BRG1 primary antibody, evidence of specificity within mouse tissues is lacking. For the AIRD2 antibody, the supplier is listed as Sigma in Table S2 but the product # is lacking, thus making it difficult for this reviewer to explore whether specificity has been demonstrated in previous peer-reviewed studies. Second, the schematic at the bottom of the immunofluorescent images that is intended to show expression levels in different spermatogenic cell types requires quantitative supporting data. At present, the graphic appears to just represent subjective visualizations made by the authors. In the absence of adding quantitation and rigor to the immunostaining analyses, I find it difficult to agree with the authors' conclusions about distinctive expression patterns for BRG1 and ARID2 in mouse spermatogenesis.
For Figure 1C, because the Ddx4-Cre transgene is fist expressed in fetal male germ cells, I suggest that the authors change the cell label from Sg (Undiff.) to prospermatogonia. Several studies have shown that undifferentiated spermatogonia do not arise in mouse testes until postnatal day 3.
For Figure 1D, claims in the results section of measuring a substantial decrease in PBRM1 expression should be supported with quantitative data that is derived from proper biological replication. At present, the statement seems to be made from a single observation for which a difference in expression level is made subjectively.
For data presented in Figure 2, although the testes of Arid2 germ cell condition KO mice are clearly smaller and spermatogenesis appears to be disrupted in cross-sections, the claim of sever reduction in spermatozoa is not supported by solid empirical evidence. I suggest that the authors quantify epididymal spermatozoa to validate the subjective observations made from cross-sections of testicular parenchyma. In addition, the authors should consider testing fertility of the conditional knockout mice.
The authors have made statements of a quantitative nature for images of spermatogenic defects presented for Figure 2 g-i/l-m, such as rounded spermatids and a few elongated spermatids represented the most advanced spermatogenic cells. Consideration should be given to either removing verbiage of a quantitative nature or conducting quantitation to determine the difference in spermatogenic cell types of Arid2 conditional knockout mice compared to controls.
For quantitative data in Figures 3C and 4B, what that data point dots represented is not clear. The figure legends indicate that they are data from n=3 different mice of each genotype but the graphs contain 20+ dots for each genotype. A better description of the data presentation is needed and depending on what the data presented in the graph are will be important for assessing appropriateness of the statistical analyses. For example, if the dots represent cells or recombination sites, then ensuring that there is equal distribution across the 3 different mice analyzed for each genotype will impact validity of the statical tests that were used.
The Western blot data presented in Figure 4A should include quantitation of normalized band intensities from an appropriate number of biological replicates and statistical assessment for

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significant differences. At present, I cannot agree with the authors claim of reduced Aurora B expression in Arid2 conditional knockout mice because the supporting data are a single Western blot image derived from a single sample of seemingly a single mouse.
Lastly, the age of Arid2 conditional knockout mice that were analyzed is not sufficiently described. Although the authors did state that data for testis weights presented in Figure 1E are from 2-month-old males, I could not find indications of the ages examined for any of the other data presented in the manuscript. If all data were generated from 2-month-old mice, I suggest that the authors confirm the phenotype from Arid2 conditional knockout in the germline with 4-month-old mice. There are several examples in the literature where conditional gene knockout in the male germline or testicular soma produces a phenotype early in adulthood that is ameliorated at a later age.

First revision

Author response to reviewers' comments

Reviewer 1 Comments for the Author:
Major points
I. Quality
-Experiments and data

1. Gene deletion efficiency by Stra8-Cre and Spo11-Cre is different from one gene to another. Please evaluate the gene deletion efficiency of Arid2 by antibody staining for ARID2. If the gene deletion by Stra8-Cre is not efficient, the authors may delete these data.

1. Evaluation of Arid2 deletion efficiency in Spo11-Cre-ARID2 by western blot antibody staining can be found in Fig. 1E and Fig. S2A. Evaluation of *Arid2* deletion efficiency Ddx4-Cre-Arid2 by antibody staining can be found in Fig. 1E and Fig. S2A. Immunofluorescence evaluation of BRG1 in Ddx4-Cre-BRG1 deletion and ARID2 deletion efficiency in Ddx4-Cre-ARID2 can be found in Fig. S1.

We agree with the suggestion to delete results obtained with Stra8-CRE. Results obtained from Spo11-Cre and Ddx4-Cre are sufficient to support our conclusions regarding ARID2 and BRG1 functions in mouse gametogenesis.

2. The total protein level of Aurora B kinase is significantly increased in Arid 2 Δ/Δ mice, which is quite interesting. Is this due to an increase of transcription or a decrease in the degradation of Aurora B kinase?

2. qPCR evaluation of Aurora B kinase expression suggest that transcription is reduced in Arid2 knockout mice compared to wild type control. We added qPCR results in Figure S5C.

3. Given that Hec1/NDC80 is one of the key substrates for Aurora B kinase in meiosis, and the phosphorylation status of Hec1/NDC80 links to stabilization of kinetochore-microtubule attachments (DeLuca et al. 2011), analyses of Hec1/NDC80 may help to understand the molecular mechanisms causing the phenotype shown in this manuscript.
Please check protein levels and phosphorylation levels of Hec1/NDC80 in the knockout mice.

3. While western blot analysis using total Hec1/NDC80 mouse testis show no differences between wild type and Spo11-Cre-Arid2 knockout mice (see figure and corresponding quantitation below, 3 independent replicates), no western blot signal was detected when antibodies against phosphorylated Hec1/NDC80 was used (not shown).

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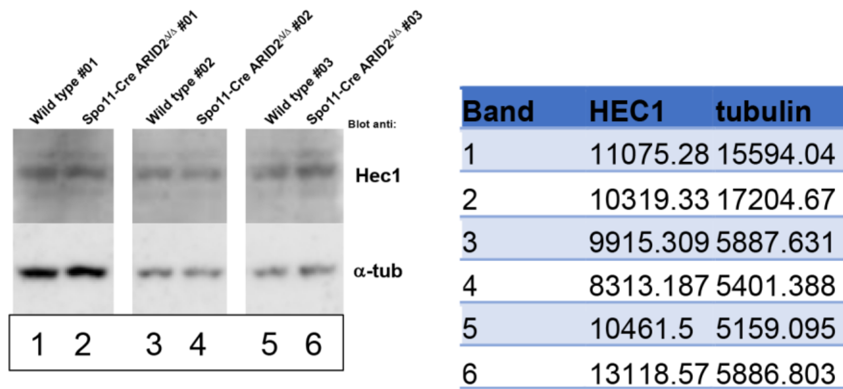
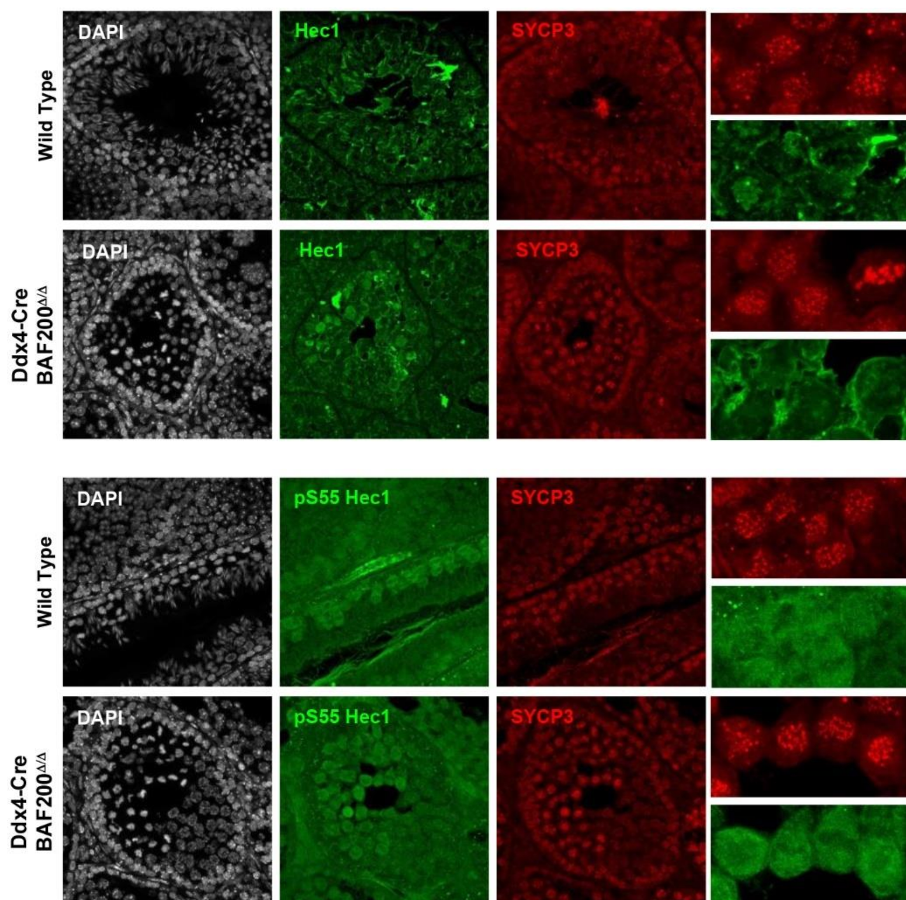


Band	HEC1	tubulin
1	11075.28	15594.04
2	10319.33	17204.67
3	9915.309	5887.631
4	8313.187	5401.388
5	10461.5	5159.095
6	13118.57	5886.803

Additionally, we have not been able to detect any specific immunosignal in paraffin embedded testis sections with antibodies against total or phosphorylated Hec1/NDC80 in either knockout or wild type mice. See figure below.



4. For proper comparison, please identify the stages/steps where BRG1- and ARID2-deficient mice showed abnormal phenotypes. PNA lectin staining may help to characterize the stages/steps and the phenotypes.

4. We have now added results corresponding to immunostaining with antibody specific for additional markers (PNA, SYCP3, and SYCP1) in paraffin embedded testis sections obtained from Spo11-Cre-ARID2, Ddx4-Cre-ARID2, and wild type control littermates (Fig. 3 and Fig. S4 and Fig. S7).

Minor points

1. The enlargement should indicate the area wherein the pictures were enlarged.

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1. The magnifications shown in figures 2 and figure 4 are independent. New figure 3, figure 5, figure S6A, and figure S7 show indicates the area that was enlarged.

2. Figure 3C. Please show the number of mice the authors analyzed.

In Figure 3 (now figure 4), we analyzed 3 mice of each phenotype. We indicate this in the figure legend.

3. The scale bars in Fig 6D, Figure S1 and Figure S2 are missing.

3. We added the scale bars to the corresponding figures.

4. The round brackets before and after PBAF in the abstract should be deleted.

4. Done.

5. "R" of Aurora B is missing in Figure 4C (wild type).

5. Done.

Reviewer 2 Comments for the Author:

1. Several conditional KO mice mutating BRG1 and ARID2 were created for analyze the function of these factors at different developmental stage. However, the rationale behind each experimental design and the reasons for selecting specific conditional mutant to address each scientific questions for Figures 1, 2 and 5, should be further clarified.

1.The main reason for selecting Ddx4-Cre and Spo11-Cre was the stage in which the Cre recombinase is expressed in these transgenic mice, allowing deletion of Arid2 or Brg1 at selected stages of prophase I.
1. See further clarification starting at page 5 last paragraph.

Ddx4-Cre (AKA Vasa) activity is directed to male and female germ cells starting at embryonic days 15-18.

Spo11-Cre recombinase expression is detected in spermatocytes that have initiated meiosis, as early as postnatal day 10 in spermatocytes.

For Figure 2B, would it be better to show data from Spo11-ARID2 mutant as well? In case the authors found the data from other conditional mutants not behaving as expected, I would hope to see some discussion about these potential discrepancy.

We added the required data to figure 2B.

2. If I have not mistaken, the authors mainly use explained the importance of ARID2 in meiotic cells mainly basing on ddx4-ARID2 mutant. However, DDX4-trigger conditional KO could create ARID2 mutant as early as embryonic stage. In addition, the results from Stra8- ARID2 mutant should be the best design among the three conditional KO strain, but they mentioned that the data was weird.

2. Yes, Ddx4-Cre activity at embryonic days 15-18. This has been shown to be adequate for generation of mutant germ cell lines (Generation of a germ cell-specific mouse transgenic Cre line, Vasa-Cre. Teresa Gallardo[1], Lane Shirley, George B John, Diego H Castrillon. PMID: 17551945. PMCID: PMC2597027).

As suggested by reviewer 1, we have eliminated results obtained with Stra8-cre. Examples in the bibliography show that use of Stra8-cre to ablate genes during gamete development resulted in the incomplete deletion of alleles, and further resulting in mosaic phenotypes (examples are doi:https://doi.org/10.1016/j.stem.2012.02.004 and doi: 10.1002/dvg.22389).

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3. In Fig. 4, the WB and immunostaining results of Aurora kinase B are not consistent. Would the authors please discuss into more details?

3. Evaluated by western blot and immunostaining in squashed seminiferous tubules we observed an increase of Aurora B protein levels in ARID2 knockout compared to wild type. Indeed, we observed similar tendency when we immunostained testis sections of Spo11/Ddx4-Cre-Arid2 (see figure 5C).

Regarding the images corresponding to immunostained squashed seminiferous tubules. Please note that we are showing a single section of a 3D image (in average 11 sections, 1um each), which could mislead the interpretation of the image. We replaced the images corresponding to wild type and knockout to better reflect the differences (Fig. S6B).

4. At Page 5, The authors stated that Stra8- ARID2 mutant mice showed a milder phenotype. However, the testes weight seems to imply otherwise?

4. We have eliminated this data from our manuscript.

5. Figure 4C. Which ARID2 mutant strain they chose?

5. Fig. 4C (Fig. S6B in the new version of our manuscript) show results obtained with Ddx4-ARID2. This information has been added to the corresponding figure legend.

And the expression level seems to be higher in wildtype? And the overlap percentage between SYCP3 and Aurora B is higher in mutant?
Again, we note that the images shown represent a single section of a 3D image (in average 11 sections, 1 um each, squashed seminiferous tubules). We replaced the images corresponding to wild type and knockout to better reflect the differences (Fig. S6B).

6. It seems that further experiments to reveal chromatin structure and gene expression have not been addressed. Would the authors discuss about these perspectives?

6. We now discuss the possible specific effect of ARID2 (Swi/Snf) in chromatin and gene expression (starting line 10, page 15).

7. Not all of the conditional mutant mice shown are directly supporting the title, as indicated in some of the comments above. They authors would need to clarify or fine-tune the wording of the title a bit.

7. We believe the title is adequate as it reflects well the core message of the work.

Reviewer 3 Comments for the Author:
In regard to setting up the story, important background and lead-in information about spermatogenesis and ARID2 seems to be lacking in the introduction section.

Information regarding spermatogenesis has been added to the introduction (page 2, first paragraph under “Introduction” and page 3, starting at line 9).

In addition, given the existing body of knowledge about the role of other SWI/SNF components in regulating spermatogenesis, in particular meiosis, the advance in understanding made by the current study seems incremental. The authors might want to consider using the introduction section to better setup the importance of exploring the role of ARID2.

We changed the context of the introductory text. For example, see page 4, starting at line 1.

The authors should consider bolstering the rigor of data presented in Figure 1A. First, specificity of the primary antibodies for immunostaining in cross-sections of adult testes should be addressed with appropriate controls. At present, the authors do not seem to have included controls to account for non-specific staining of either the primary or secondary antibodies.

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Also, demonstration that the primary antibodies specifically recognize BRG1 and ARID2 has not been achieved. Based on information, available on the supplier website for the BRG1 primary antibody, evidence of specificity within mouse tissues is lacking. For the AIRD2 antibody, the supplier is listed as Sigma in Table S2 but the product # is lacking, thus making it difficult for this reviewer to explore whether specificity has been demonstrated in previous peer-reviewed studies.

Experimentation regarding specificity of antibodies against ARID2 and BRG1 for immunostained testis sections can now be found in supplementary figure S1.

Further validation of ARID2 and BRG1 antibodies can be found in western blots in figure 1E and 7B, and S2A.

The catalog number for ARID2 antibodies from Sigma has been added to table S2.

Second, the schematic at the bottom of the immunofluorescent images that is intended to show expression levels in different spermatogenic cell types requires quantitative supporting data. At present, the graphic appears to just represent subjective visualizations made by the authors. In the absence of adding quantitation and rigor to the immunostaining analyses, I find it difficult to agree with the authors' conclusions about distinctive expression patterns for BRG1 and ARID2 in mouse spermatogenesis.

Figure 1B show results obtained with immunofluorescence quantification corresponding to images in Figure 1A. The schematic at the bottom of the former Figure 1A has been eliminated.

For Figure 1C, because the Ddx4-Cre transgene is fist expressed in fetal male germ cells, I suggest that the authors change the cell label from Sg (Undiff.) to prospermatogonia. Several studies have shown that undifferentiated spermatogonia do not arise in mouse testes until postnatal day 3.

Done (example, see Fig 1D).

For Figure 1D, claims in the results section of measuring a substantial decrease in PBRM1 expression should be supported with quantitative data that is derived from proper biological replication. At present, the statement seems to be made from a single observation for which a difference in expression level is made subjectively.

Additional western blot showing differences in PBRM1 protein abundance in ARID2 knockout mice compared to wild type has been added to figure S2A. Quantification of western blots can be found in Fig. S2B and C.

For data presented in Figure 2, although the testes of Arid2 germ cell condition KO mice are clearly smaller and spermatogenesis appears to be disrupted in cross-sections, the claim of sever reduction in spermatozoa is not supported by solid empirical evidence. I suggest that the authors quantify epididymal spermatozoa to validate the subjective observations made from cross-sections of testicular parenchyma.

Representative images showing normal wild type and lack of spermatozoa content in ARID2 knockout caput and cauda sections of the epididymis are shown in Figure S3

In addition, the authors should consider testing fertility of the conditional knockout mice.

We believe the described phenotype is abundantly clear regarding deficiency in earlier stages of gamete production in the ARID2 knockout mice.

The authors have made statements of a quantitative nature for images of spermatogenic defects presented for Figure 2 g-i/l-m, such as rounded spermatids and a few elongated spermatids represented the most advanced spermatogenic cells. Consideration should be given to either removing verbiage of a quantitative nature or conducting quantitation to

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determine the difference in spermatogenic cell types of Arid2 conditional knockout mice compared to controls.

Regarding gamete development observations in Fig 2 g/i and l/m. We added “are apparently” to better reflect the qualitative appreciation of the phenotype at this stage of the manuscript. We have also included a quantitative characterization of primary spermatocyte and spermatids defects in the ARID2 knockout appearing in Fig. 3 and Fig. S4.

For quantitative data in Figures 3C and 4B, what that data point dots represented is not clear. The figure legends indicate that they are data from n=3 different mice of each genotype but the graphs contain 20+ dots for each genotype. A better description of the data presentation is needed and depending on what the data presented in the graph are will be important for assessing appropriateness of the statistical analyses. For example, if the dots represent cells or recombination sites, then ensuring that there is equal distribution across the 3 different mice analyzed for each genotype will impact validity of the statical tests that were used.

Figures now contain super plots showing data from each biological replicate in Fig. 1B, 3C, 4C, 5B, 5D. When possible, we have also clarified descriptive statistics in corresponding figure legend and in the text.

The Western blot data presented in Figure 4A should include quantitation of normalized band intensities from an appropriate number of biological replicates and statistical assessment for significant differences. At present, I cannot agree with the authors claim of reduced Aurora B expression in Arid2 conditional knockout mice because the supporting data are a single Western blot image derived from a single sample of seemingly a single mouse.

Quantitation corresponding to normalized western blot bands for Aurora B in ARID2 knockout and wild type mice shown in Fig 5A and Fig S5A can be found in Fig S B and D.

Lastly, the age of Arid2 conditional knockout mice that were analyzed is not sufficiently described. Although the authors did state that data for testis weights presented in Figure 1E are from 2-month-old males, I could not find indications of the ages examined for any of the other data presented in the manuscript. If all data were generated from 2-month-old mice, I suggest that the authors confirm the phenotype from Arid2 conditional knockout in the germline with 4-month-old mice. There are several examples in the literature where conditional gene knockout in the male germline or testicular soma produces a phenotype early in adulthood that is ameliorated at a later age.

When possible, in the text and figure legend we added the age of the mice used. We believe that data obtained from 2-month-old-mice is sufficient to support our claims of severely defective gametogenesis in ARID2 and BRG1 mice.

Second decision letter

MS ID#: DEVELOP/2021/199967

MS TITLE: PBAF chromatin remodeler complexes that mediate meiotic transitions in mouse

AUTHORS: Rodrigo DeCastro, Luciana Previato, Agustin Carbajal, Irma Gryniuk, and Roberto J Pezza

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

As you will see, the referees found the manuscript improved, but still have some significant criticisms and recommend further revision of your manuscript before we can consider publication.

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It is particularly important to fully address points raised by the reviewer 3. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision.

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

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

Reviewer 1

Advance summary and potential significance to field

In this revision, the authors are presenting a revised manuscript that is improved and addressing key suggestions from the reviewers, but there are some missing points to improve this work. Also, please make clear where the authors have rewritten the manuscript from the previous one.

Comments for the author

1. The effect of conditional gene deletion by Cre varies from tubule to tubule and from individual to individual in mice. These cannot be evaluated by Western blotting. In order to assess the effect, it is necessary to stain the testes of Spo11-ARID2Δ/Δ with an anti-ARID antibody, like Figure S1. The predicted result is that the ARID2 protein will be remained in spermatogonia but should disappear from the spermatocyte. At that time, it is necessary to evaluate how many seminiferous tubules still have ARID2 in the spermatocytes and the spermatids. IHC representative photos and the results of the statical evaluation are needed. Please also provide higher magnification images of the figures at multiple stages.
1’. In Sertoli cells, the ARID2 signal should remain in Ddx4-ARID2Δ/Δ mice (Figure S1). So why did the ARID2signals in the Sertoli cells almost disappear? Please provide higher magnification images of the figures at multiple stages.
2. Given that Aurora B is already shown to be ubiquitinated for degradation, the reviewer had hoped for the authors to check whether Aurora B kinase in Spo11-ARID2Δ/Δ reveals less ubiquitinated or not. This could explain the increase of Aurora B kinase. Please check it.
The fact that transcription and translation/degradation are changing in exactly the opposite direction is surprising and interesting.
3. The authors can include the discussion about Ndc80/Hec1-independent pathway.

Reviewer 2

Advance summary and potential significance to field

The authors tackle a very important question about the epigenetic modulatory machinery during the meiotic transition. The authors applied conditional knockout approach to illuminate the germ cell developmental stage dependent composition and function for members of SWI/SNF chromatin remodelers at critical stages of male germ cell development.

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Comments for the author

I understand that the authors took some major effort for this revision. For the QA Session, I was hoping to see a bit more depth in the responser to various reviewers' comments or questions. The authors disagree with some of the reviewers' comments, which is fine. But I would appreciate to have more rationale for the disagreement.
1.As we already mentioned last time, the conditional knockout mice used in this paper maybe not be the ideal choice. DDX4 starts to express in the embryonic stage; the expression timing of SPO11 and ARID2 are too close to delete ARID2 expression properly (The efficiency seems to be compromised in Figure S2).
Suggestion: If distinguish the roles of ARID2 in meiosis I and after meiosis I is the critical question, two conditional knockout systems, one before meiosis I and another just after meiosis I could be applied. This point can be discussed in the discussion session. Although the authors indicated that Stra8 driven conditional mutant may have mosaicism and removed that part of dara, one recent publication indicated otherwise..
2. Can we clarify whether the results from relative fluorescence intensity between control and mutant mice were from the sage stage? If not, it can be a concern..
3. We do not think the specificity of the ARID2 is good enough to calculate fluorescence intensity in seminiferous tubules. (Figure 1B: It seems that the expression level in spermatogonia is higher than L and Z spermatocytes.) (It is not consistent with other published results, RNA-seq or protein) (Maybe caused by background or non-specific signals)
4.In addition, for the research on cell division, the location of proteins could be more important than quantity. (Especially when the authors did not separate different developmental stages of cells for western blot, and neither did the staining data.)
(For AURKB and H3S10P)
Moreover, I was wondering if tubulin is an appropriate internal control for western blot in this case? (If arid2 deficiency influences microtubule assembly)
Others:
5. The authors really need to not clarify the age of mice they used in every data.
(Timing is quite important for developmental biology). Based on the response letter to Reviewer 3's comments, the authors may use 2 month old mice for all experiment. It's still necessary to put this info in materials and methods and/or Figure legends 6. Figure 2D: why spo11cko MII are ND 7. Figure S5: The protein level of Aurora B kinase (AURKB) is higher in mutant mice (But RNA level is lower?) (The result is different with another paper)
(because they use different internal control?)
The labeling is still not clear:
1. Figure 4c spo11-cko?
2. What is Figure S5 refer to? (Legend not clear)
3. Figure S6: what's the sample in S6A (different with legend), and the labeling in S6B also non-clear
Minor: There are still many small points need to polish. I did not list all.
Page7 line 2: maybe should be Fig. 2A panel g, i, l, m
Page 10 line 18: should we call signal decrease in wild type?

Reviewer 3

Advance summary and potential significance to field

The authors have acceptably addressed many, but not all, of the concerns raised in the initial review. There are still some outstanding issues that should be considered to improve the rigor and validity of the findings.

Comments for the author

A hold over concern from the first version of the manuscript is that conditional knockout mice were not examined beyond two months of age. Again, I suggest that the authors show that the phenotype of disrupted spermatogenesis is confirmed at 3-4 months of age. In mice, spermatogenesis has not reached a full fledged steady-

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state until 3-4 months of age. Thus, by limiting analyses to only 2 months of age conclusions about the role of Arid2 in regulating spermatogenesis are not fully supported. Also, the authors have still not proven that the conditional knockout mice are sterile. They have assumed this based on smaller testis size and subjective observations of testicular cross-sections. In the interest of thorough scientific investigation, I still recommend that they simply pair conditional knockout males with wild-type females to test whether a pregnancy can occur.

The authors should revise the Figure S3 legend. Spermatocytes should not be p resent in cross-sections of epidydimal tubules. Rather, the authors likely mean t o note the absence of spermatozoa.

Second revision

Author response to reviewers' comments

Reviewer #1:

1. The effect of conditional gene deletion by Cre varies from tubule to tubule and from individual to individual in mice. These cannot be evaluated by Western blotting. In order to assess the effect, it is necessary to stain the testes of Spo11-ARID2Δ/Δ with an anti-ARID antibody, like Figure S1. The predicted result is that the ARID2 protein will be remained in spermatogonia but should disappear from the spermatocyte. At that time, it is necessary to evaluate how many seminiferous tubules still have ARID2 in the spermatocytes and the spermatids. IHC representative photos and the results of the statical evaluation are needed. Please also provide higher magnification images of the figures at multiple stages.

Although it is formally possible that the use of Cre for gene deletion may increase variability to some level, we still think western blots are a reasonably good tool to account for global changes. This is the intention of the western blots we show in our work. Note that the phenotype we observed in Spo11-Cre and Ddx4-Cre Arid2 knockout mice are consistent with a recent publication PMID: 34772938 (published when the first version of our manuscript was under review).

Per this new reviewer's request, we assessed ARID2 expression in Spo11-Arid2 knockout and wild type littermates and show magnified images, including spermatogonia, spermatocytes, spermatids, and Sertoli cells. Please, see new supplementary figure 2A and B.

1'. In Sertoli cells, the ARID2 signal should remain in Ddx4-Arid2Δ/Δ mice (Figure S1). So why did the ARID2 signals in the Sertoli cells almost disappear? Please provide higher magnification images of the figures at multiple stages.

Higher magnification of Sertoli are now show in supplementary figure 1. In our images, we see no difference in ARID2 immunosignal in Sertoli cells when comparing wild type and knockout testis.

2. Given that Aurora B is already shown to be ubiquitinated for degradation, the reviewer had hoped for the authors to check whether Aurora B kinase in Spo11-ARID2Δ/Δ reveals less ubiquitinated or not. This could explain the increase of Aurora B kinase. Please check it. The fact that transcription and translation/degradation are changing in exactly the opposite direction is surprising and interesting.

To satisfy this new request, we attempted performing immunoprecipitations using antibodies against Aurora B in Arid2 knockout and wild type testes. The rationale was that products or IP would be evaluated in western blots with antibodies specific for ubiquitin. Unfortunately, the available antibodies were not proficient in immunoprecipitating Aurora B from mouse testis extracts (BD Biosciences, 611082 and BioSource, MBS8242227). We note that this may not come as a surprise because, to the present moment, we are not aware of any antibody with the ability to immunoprecipitate Aurora B from mouse testis extract. So far, works immunoprecipitating Aurora B in other models has been done with tagged Aurora B.

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3. The authors can include the discussion about Ndc80/Hec1-independent pathway.

Our results on Hec1/NDC80 levels or phosphorylation status (see our previous response) were not conclusive.

Reviewer #2:

I understand that the authors took some major effort for this revision. For the QA. Session, I was hoping to see a bit more depth in the responser to various reviewers' comments or questions. The authors disagree with some of the reviewers' comments, which is fine. But I would appreciate to have more rationale for the disagreement.

1. As we already mentioned last time, the conditional knockout mice used in this paper maybe not be the ideal choice. DDX4 starts to express in the embryonic stage; the expression timing of SPO11 and ARID2 are too close to delete ARID2 expression properly (The efficiency seems to be compromised in Figure S2). Suggestion: If distinguish the roles of ARID2 in meiosis I and after meiosis I is the critical question, two conditional knockout systems, one before meiosis I and another just after meiosis I could be applied. This point can be discussed in the discussion session.
Although the authors indicated that Stra8 driven conditional mutant may have mosaicism and removed that part of dara, one recent publication indicated otherwise.

"As we already mentioned last time, the conditional knockout mice used in this paper maybe not be the ideal choice. DDX4 starts to express in the embryonic stage."

We agree that Ddx4-Cre is expressed in embryonic stages of testis development (e-15 - e-18, PMID: 17551945). However, we believe that this is actually an advantage, because allows to study the first triggered phenotype in any developmental stage from primordial germ stem cells throughout spermatogenesis. For example, deletion of Chd4 (the catalytic subunit of the NURD chromatin remodeling complex) using Ddx4-Cre (aka Vasa-Cre) resulted in arrest at early stages of spermatogonia development (PMID: 35568926 and PMID: 33961790), while deletion of Brg1 with Ddx4-Cre exhibited developmental defects at pachytene stage (meiosis I) (PMID: 22495890, PMID: 22318225, PMID: 3104342).

In our work, deletion of the Arid2 gene with Ddx4-Cre result in neither obvious premeiotic or an early meiotic phenotype, but a phenotype at meiosis exit.

"The expression timing of SPO11 and ARID2 are too close to delete ARID2 expression properly (The efficiency seems to be compromised in Figure S2)."

We do not think the efficiency of Arid2 deletion is compromised using Spo11-Cre, because: 1- Immunocytochemistry analysis (new experiments now incorporated in the last version of the manuscript) of ARID2 expression in wild type versus knockout testis shows that the level ARID2 depletion by Spo11-Cre and Ddx4-Cre are comparable (Fig. S1 and Fig. S2). 2- Regarding Fig. S2A (now S3A) expression levels. Western blot immunosignal show a reduction of ARID2 in Spo11-knockouts compared to wild type. Note that a remaining signal in the Spo11-ARID2 knockout likely originate from testis connective tissues, in which ARID2 is not deleted.

We also note that although Spo11-Cre delete ARID2 at later stages respect to Ddx4-Cre, the phenotype observed in Spo11-Arid2 knockout and Ddx4-Arid2 knockout are notably similar. We believe, all the above indicate that Spo11-Cre efficiently deletes later meiotic expressing genes such as ARID2 (ARID2 expression peaks at late pachytene – diplotene).

"Suggestion: If distinguish the roles of ARID2 in meiosis I and after meiosis I is the critical question, two conditional knockout systems, one before meiosis I and another just after meiosis I could be applied. This point can be discussed in the discussion session."

Distinguishing the specific roles of ARID2 in meiosis I and after meiosis I is not the focus of our work. However, we have now included in the discussion section (See page 14, starting lane 15).

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In our knowledge PRM1-cre (PMID: 3754219) is the only available Cre for events occurring post meiosis I. However, *Prm1* is expressed during the terminal, haploid stages of spermatogenesis, which will be too late for the mention purposes.

"Although the authors indicated that Stra8 driven conditional mutant may have mosaicism and removed that part of dara, one recent publication indicated otherwise."

Please note that removal of Stra8 data was suggested by one of the reviewers in the previous version. Regardless, if this reviewer refers to the publication PMID: 34772938 (published when the first version of our manuscript was under review), the authors of this publication indicate that deletion of Arid2 using Stra8-Cre results in "inefficient Cre-mediated deletion" (see Supplementary Figure 2 and text in Results of this cited manuscript).

NOTE: *Figure provided for reviewer has been removed. It showed part of Supplementary Fig. 2 from Menon et al. (2021) Mammalian SWI/SNF chromatin remodeler is essential for reductional meiosis in males. Nat Commun. 2021 Nov 12;12(1):6581. doi: 10.1038/ s41467-021-26828-1.*

2. Can we clarify whether the results from relative fluorescence intensity between control and mutant mice were from the sage stage? If not, it can be a concern.

Immunofluorescent intensity measurements for H3pS10 (diplotene cells, fig. 5B) and Aurora (metaphase-anaphase, fig, 5C and D) in wild type and Arid2 knockouts were obtained from cells at the same developmental stage. We clarify the stage of the cells used for these measurements in the figure legend.

3. We do not think the specificity of the ARID2 is good enough to calculate fluorescence intensity in seminiferous tubules. (Figure 1B: It seems that the expression level in spermatogonia is higher than L and Z spermatocytes.) (It is not consistent with other published results, RNA-seq or protein) (Maybe caused by background or non-specific signals)

Please consider the following. First, in our results, the median fluorescence intensity for ARID2 in Fig.1B (lower panel) show no statistically significant differences when comparing ARID2 immunosignal between spermatogonia and leptotene (P=0.98) or zygotene (P=0.23). Second, in general, RNA measurements (PMID: 34772938) cannot be directly compared with protein measurement (our work). Third, actually, ARID2 immunosignal data in PMID: 34772938 do agree with our results, in which ARID2 protein levels increased as prophase I progresses. However, the authors in PMID: 34772938 do not evaluate the level of ARID2 immunosignal in spermatogonia, thus this data cannot be compared to our studies in regard to the argument raised by this reviewer here.

4. In addition, for the research on cell division, the location of proteins could be more important than quantity. (Especially when the authors did not separate different developmental stages of cells for western blot, and neither did the staining data.) (For AURKB and H3S10P). Moreover, I was wondering if tubulin is an appropriate internal control for western blot in this case? (If arid2 deficiency influences microtubule assembly).

"In addition, for the research on cell division, the location of proteins could be more important than quantity. (Especially when the authors did not separate different developmental stages of cells for western blot, and neither did the staining data.) (For AURKB and H3S10P)."

Figure 5C-D shows immunostaining assessment of Aurora B protein levels in cells at metaphase-anaphase I, and Figure 5B shows results obtained in diplotene spermatocytes.

"Moreover, I was wondering if tubulin is an appropriate internal control for western blot in this case? (If arid2 deficiency influences microtubule assembly)."

Total tubulin assessed by western blot (which amounts in wild type and knockouts are not expected to change) used in our experiments is independent of any possible changes in microtubule assembly. Additionally, Topo II isomerase shows similar results as tubulin.

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Others:

5. The authors really need to not clarify the age of mice they used in every data. (Timing is quite important for developmental biology). Based on the response letter to Reviewer 3's comments, the authors may use 2 month old mice for all experiment. It's still necessary to put this info in materials and methods and/or

We have indicated mice age in main text, material and methods, and legends. We have now included mice age in several parts of the text, figures, and supplementary figures. We also clarified in material and methods that unless specified all analyzed mice were 2-months-old.

Figure legend

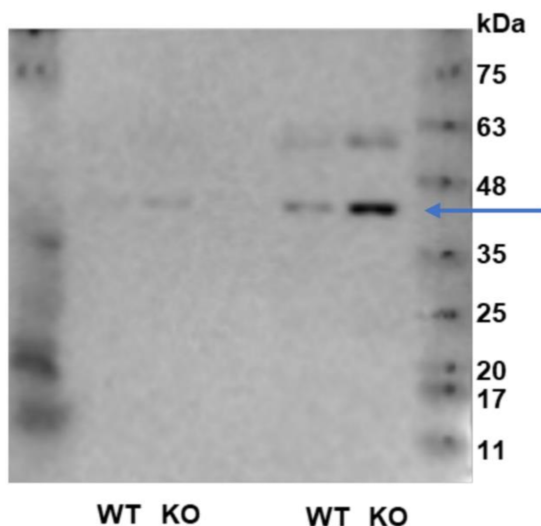
6. Figure 2D: why spo11cko MII are ND

Cells at meiosis I are reliably identified by SYCP3 staining at centromeres, thus our detailed analysis and quantification were focused on this stage.

7. Figure S5: The protein level of Aurora B kinase (AURKB) is higher in mutant mice (But RNA level is lower?) (The result is different with another paper) (because they use different internal control?)

7. Independent of the internal control used, our results on RNA measurements agree with those published in PMID: 34772938 (see our supplementary figure 7c) in that Aurora B mRNA levels are reduced in the knockout compared to wild type.

Regarding the levels of protein expression in wild type versus KO: We have not been able to distinguish a specific band for Aurora B in the corresponding figure of manuscript PMID: 34772938. In our manuscript, we show a number of independent experiments with clear and specific western blot immunosignal (with the predicted MW for Aurora B) that agrees with results obtained with complementary methods such as immunohistochemistry (see uncropped gel below, left panel).



The labeling is still not clear:

1. Figure 4c spo11-cko?

1. The figure 4C legend indicate that the knockout used was Ddx4-Arid2.

2. What is Figure S5 refer to? (Legend not clear)

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2. (now figure S7) it refers to changes in Aurora B protein and RNA levels observed in knockout Arid2 mice compared to wild type.

3. Figure S6: what's the sample in S6A (different with legend), and the labeling in S6B also non-clear

3. (now fig S8) We changed the figure legend to clarify.

Minor: There are still many small points need to polish. I did not list all. Page7 line 2: maybe should be Fig. 2A panel g, i, l, m Page 10 line 18: should we call signal decrease in wild type?

We changed the wording in text corresponding to Fig. 2A.

Regarding page 10, the manuscript states: "We observed a significantly increase in Aurora B signal in Arid2 knockout (Ddx4-Arid2$^{\Delta/\Delta}$, 142.5 ± 63.7, n=421 cells obtained from 3 mice, two tailed P<0.0001. Spo11-Arid2$^{\Delta/\Delta}$, 126.0 ± 57.9, n=493 obtained from 3 different mice, two tailed P<0.0001) compared to wild type controls (78.8 ± 15.5 relative fluorescence intensity, n=373 cells obtained from 2-month-old 3 different mice) (Fig. 5C and D and Fig. S8A)."

Reviewer #3:

A hold over concern from the first version of the manuscript is that conditional knockout mice were not examined beyond two months of age. Again, I suggest that the authors show that the phenotype of disrupted spermatogenesis is confirmed at 3-4 months of age. In mice, spermatogenesis has not reached a full-fledged steady-state until 3-4 months of age. Thus, by limiting analyses to only 2 months of age, conclusions about the role of Arid2 in regulating spermatogenesis are not fully supported.

We have now included analysis of 4-month-old mice in our studies.

H&E analysis of testis sections revealed that 4-month-old Spo11-Arid2 knockout mice show similar phenotype (e.g., arrested spermatogenesis and lagging chromosomes) to that observed in 2-month-old (Fig. S5B).

Also, the authors have still not proven that the conditional knockout mice are sterile. They have assumed this based on smaller testis size and subjective observations of testicular cross-sections. In the interest of thorough scientific investigation, I still recommend that they simply pair conditional knockout males with wild-type females to test whether a pregnancy can occur.

We performed male fertility tests using controls (Arid2$^{+/+}$ or Arid2$^{+/-}$) and Spo11-Arid2$^{-/-}$ knockouts males, which were mated with Arid2$^{+/+}$ females for a period of approximately 4 months. While control mating (male Arid2$^{+/+}$X female Arid2$^{+/+}$ or male Spo11-Arid2$^{+/-}$X female Arid2$^{+/+}$) produced siblings, Spo11-Arid2$^{-/-}$ knockout males mated with female Arid2$^{+/+}$ did not (see Fig. S5A and Table S1).

The authors should revise the Figure S3 legend. Spermatocytes should not be present in cross-sections of epidydimal tubules. Rather, the authors likely mean to note the absence of spermatozoa.

Changed "spermatocyte" for "spermatozoa".

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Third decision letter

MS ID#: DEVELOP/2021/199967

MS TITLE: PBAF chromatin remodeler complexes that mediate meiotic transitions in mouse

AUTHORS: Rodrigo DeCastro, Luciana Previato, Agustin Carbajal, Irma Gryniuk, and Roberto J Pezza

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

The overall evaluation is positive and we would like to publish a revised manuscript in Development, provided that the referees' comments can be satisfactorily addressed. Please editorially attend to comments raised by reviewers 1 and 2 in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so. If it would be helpful, you are welcome to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating your plans for addressing the referee’s comments, and we will look over this and provide further guidance.

Reviewer 1

Advance summary and potential significance to field

The revised version shows improvements. However, there are problems with the quality of some figures.

Comments for the author

I found that the description of ARID2 expression during spermatogenesis in this manuscript is still difficult to understand. The authors measured the fluorescence intensity of individual spermatogonia in Figure 1B, showing some of the spermatogonia moderately expressed ARD2. However, Menon et al., PMID:34772938, clearly say that the expression of ARID2 is estimated to be almost undetectable.
Do the authors here think that a subpopulation of spermatogonia expresses ARID2? Or, do the authors think that ARID2 expression in spermatogonia is barely detectable. If ARID2 is not expressed before the early meiotic stages, the arguments in this manuscript, showing ARID2 and BRG1 function differently during spermatogenesis, are more convincing and meaningful. In addition, the data shown in Figure 7, where Brg1 is knocked out with Spo11-Cre, becomes a more worthy outcome.
Therefore, the problems at this point are that the authors of this manuscript did not clearly mention whether spermatogonia expressed ARID2 or not. The authors need to be clarified whether moderate ARID2 expression in spermatogonial is correct or mistaken for other types of cells. What is of concern to proof on this point is that the expression levels of spermatogonia are measured even though they are probably not stained with spermatogonia markers.
In addition, in Figure S2, staining for ARID2 and SYCP3 is at unacceptable levels and must be reexamined. SYCP1 and SYCP3 staining in Figure S9 is also low quality. There is little difference between signal and noise. Thus, these results are not convincing. The authors should pay much attention to the quality of the data. Otherwise, I cannot support this work anymore.

Reviewer 2

Advance summary and potential significance to field

The detailed molecular and epigenetic machineries associated with different developmental stages (especially during the meiosis process) is a perfect example of endogenous physiological condition where dynamic genome assembly and intensive DNA double strand breaks takes place. The authors compared specific SWI/SNF chromatin remodeler components, different subunits of PBAF/BAF

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complexes, and suggested specific functions of these subunits being critical for different stages of meiosis stages in vivo. These data may provide insight into pathological circumstances for genome integrity problems in general and provide possible novel solutions.

Comments for the author

The authors may consider revising the wording of the Title. "PBAF chromatin remodeler complexes mediate meiotic transitions in mice" can be considered.
Although one partly overlapped, from some aspects better, work published while this current manuscript is under review, the detailed characterization of conditional mutant mouse lines independently generated in this paper is still worth documenting. The consensus and some discrepancies that discussed in the revised manuscript as well as in the QA section (which will be published as well) can serve as material for the readers to make final judgement.
The authors may not have responded to all comments head on, but I am OK with their response to my part.

Reviewer 3

Advance summary and potential significance to field

The authors have acceptably addressed my concerns. The data are now convincing or a role for PBAF chromatin remodeler complexes in mouse spermatogenesis.

Comments for the author

I have no further concerns with the manuscript.

Third revision

Author response to reviewers' comments

Reviewer 1 Comments for the Author:
I found that the description of ARID2 expression during spermatogenesis in this manuscript is still difficult to understand. The authors measured the fluorescence intensity of individual spermatogonia in Figure 1B, showing some of the spermatogonia moderately expressed ARD2. However, Menon et al., PMID:34772938, clearly say that the expression of ARID2 is estimated to be almost undetectable.

As we mentioned in our last round of responses, it is currently understood that RNA measurements (PMID: **34772938**) cannot be directly compared with protein measurement (our work), thus this type of comparison may be misleading. Note that both experiments (mRNA versus protein immunostaining measurements, see inserted images below) show the same curve pattern for ARID2 and BRG1 expression in the different germ cell population.

Importantly, ARID2 immunosignal data in PMID: **34772938 do agree with our results, in which** ARID2 protein levels increased as prophase I progresses. However, the authors in PMID: **34772938** do not evaluate the level of ARID2 immunosignal (protein levels) in spermatogonia.

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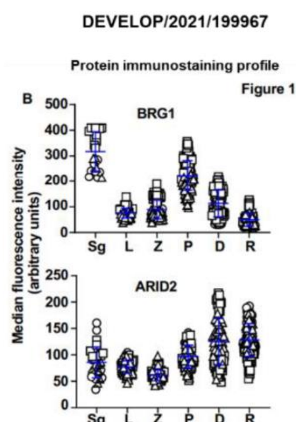

***NOTE**: Figure provided for reviewer has been removed. It showed part of Fig. 1 from Menon et al. (2021) Mammalian SWI/SNF chromatin remodeler is essential for reductional meiosis in males. Nat Commun. 2021 Nov 12;12(1):6581. doi: 10.1038/s41467-021-26828-1.*

Do the authors here think that a subpopulation of spermatogonia expresses ARID2? Or, do the authors think that ARID2 expression in spermatogonia is barely detectable. If ARID2 is not expressed before the early meiotic stages, the arguments in this manuscript, showing ARID2 and BRG1 function differently during spermatogenesis, are more convincing and meaningful. In addition, the data shown in Figure 7, where Brg1 is knocked out with Spo11-Cre, becomes a more worthy outcome.

Regardless the expression level of ARID2 in spermatogonia cells, the results we obtain using genetic disruption and phenotypic analysis clearly indicate possible different functions for BRG1 and ARID2 during spermatogenesis.

We also note that our recent work has demonstrated that deletion of ARID2 gene in spermatogonia has not major effects, indicating minimal contribution of ARID2 (and so its expression) during pre-meiotic events.

Therefore, the problems at this point are that the authors of this manuscript did not clearly mention whether spermatogonia expressed ARID2 or not. The authors need to be clarified whether moderate ARID2 expression in spermatogonial is correct or mistaken for other types of cells. What is of concern to proof on this point is that the expression levels of spermatogonia are measured even though they are probably not stained with spermatogonia markers.

In our experiments, spermatogonia cells are clearly identified in base of the following criteria: 1- relative cell positioning within the seminiferous tubule, 2- specific patters of DAPI staining (which we have previously show correspond to spermatogonia specific markers such as PLZF. PMID: 35568926), 3- specific spermatogonia cell morphology, and 4- absence of markers for other cell types such as pre-meiotic S-phase (e.g., yH2AX) and SYCP3 at chromosome axes in primary spermatocytes.

In addition, in Figure S2, staining for ARID2 and SYCP3 is at unacceptable levels and must be reexamined. SYCP1 and SYCP3 staining in Figure S9 is also low quality. There is little difference between signal and noise. Thus, these results are not convincing. The authors should pay much attention to the quality of the data. Otherwise, I cannot support this work anymore.

We do not agree with this reviewer's assessment of figure S2 and S9. Our argument for figure S2 is the following: 1- the nuclear signal of ARID2 in wild type testis is directly correlated with the stage of spermatocyte. This is, near no signal is observed in leptotene/zygotene cells and increased immunofluorescence is observed in nuclei of pachytene and diplotene cells. 2- More importantly, ARID2 immunostaining is absent in nuclei of Spo11-Arid2 knockout pachytene and diplotene cells, clearly indicating antibody specificity. Note than in the legend of figure S2, we comment regarding

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background problem (red foci) in both wild type and knockout seminiferous tubules, possibly caused by a different antibody lot used in these experiments.

Respect to Figure S9. Primary spermatocytes in this figure show a prominent nuclear staining with typical axis like staining for both SYCP1 and SYCP3. As expected, some background signal is expected given the thickness of the testis section used and also possible binding of antibodies to SYCP1/3 that are not participating in the synaptonemal complex. This do not invalidate the fact that the immunostaining indicate location of primary spermatocytes in the images presented.

Reviewer 2 Comments for the Author:
The authors may consider revising the wording of the Title. "PBAF chromatin remodeler complexes mediate meiotic transitions in mice" can be considered.

We believe our current title is adequate as it comprehensibly describe our findings.

Although one partly overlapped, from some aspects better, work published while this current manuscript is under review, the detailed characterization of conditional mutant mouse lines independently generated in this paper is still worth documenting. The consensus and some discrepancies that discussed in the revised manuscript as well as in the QA section (which will be published as well) can serve as material for the readers to make final judgement.

The authors may not have responded to all comments head on, but I am OK with their response to my part.

Thanks for the overall constructive comments.

Reviewer 3 Advance Summary and Potential Significance to Field:
The authors have acceptably addressed my concerns. The data are now convincing or a role for PBAF chromatin remodeler complexes in mouse spermatogenesis.

Reviewer 3 Comments for the Author:
I have no further concerns with the manuscript.

Fourth decision letter

MS ID#: DEVELOP/2021/199967

MS TITLE: PBAF chromatin remodeler complexes that mediate meiotic transitions in mouse

AUTHORS: Rodrigo DeCastro, Luciana Previato, Agustin Carbajal, Irma Gryniuk, and Roberto J Pezza
ARTICLE TYPE: Research Article

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

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