CDC25B is required for the metaphase I-metaphase II transition in mouse oocytes

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We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

We are aware that you may be experiencing disruption to the normal running of your lab that makes 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 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. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The present manuscript aims at addressing the role of Cdc25B for metaphase-to-anaphase I transition in mouse oocyte meiosis. Studies of its role for progression through meiosis I were hindered for up to now by the fact that Cdc25B is required for entry into meiosis I (GVBD), through requirements of this phosphatase to activate Cdk1 by counteracting inhibitory phosphorylation mediated by Wee1/Myt1.
Without Cdc25B, oocytes are not able to enter meiosis I, and the authors show that activating Cdk1 by other means (expressing Cdc25A, or inhibiting Wee1/Myt1) allows GVBD in oocytes devoid of Cdc25B to address its role beyond GVBD. They find that oocytes are not able to progress into meiosis II without Cdc25B, and even though Cdc25A ectopic expression can rescue GVBD, it cannot rescue anaphase I onset.

It is however not clear what the role of Cdc25B could be for exit from meiosis I whether it targets Cdk1 (unlikely, because Cdk1 has to be inactivated for exit from meiosis I), the role of Cdc25B localization (essential for GVBD), etc. No hypothesis of the role of Cdc25B for anaphase I is proposed, no explanation of the phenotype is provided.

Comments for the author

Main points:
1) The authors state that oocytes without Cdc25B that are rescued to enter meiosis I arrest in metaphase I. No proper cell cycle analysis is provided, it is only shown that oocytes do not extrude polar bodies and have bipolar spindles without enough resolution to judge whether the chromosomes are indeed aligned. Do they arrest with high MPF activity? Is the spindle checkpoint activated? Are chromosomes aligned? Is progression through prometaphase comparable to control oocytes (time of spindle formation, kinetics of MPF accumulation, inactivation of the SAC, attachment of microtubules, ...)? With the current data the authors cannot conclude that oocytes are arrested in metaphase I and their data below actually indicate that it is not the case.
2) The conclusion that APC activity is reduced cannot be drawn by looking at a single substrate that is exogenously expressed. Maybe Cdc25B targets securin and not the APC? What about Cyclin B1, endogenous proteins,...? (Western blots of endogenous securin and cyclin B1 can be done in mouse oocytes, this has been published multiple times)
3) The statement that securin GFP degradation was reduced in oocytes arrested in MI does not make sense. If they are arrested, they shouldn't degrade securin otherwise it is not a MI arrest. (page 6, 1st paragraph)
4) an internal loading control for the kinase assays should be provided, to show how much unphosphorylated GST-LAMS22 was added to quantitate the phosphorylation of GST-LAMS22 relative to protein added to the reaction. (This control is also used in the publication cited by the authors for using GST-Laminin as a substrate). Immunodetection of a phosphosite is much less sensitive than incorporation of radioactivity that is usually used as a read-out for kinase assays in mouse oocytes. The authors have to show all the controls to demonstrate they have establish this assay in mouse oocytes where there is probably around 1000 times less protein than used for kinase assays in somatic cells, and show that they get the same profiles for Cdk1 activity such as previously published by multiple labs using radioactive ATP and Histone H1 as a substrate.

5) No controls for the specificity or efficiency of the Wee1/Myt1 inhibitor in oocytes at the indicated concentrations are provided.

Minor issues:
Legends are missing for graphs in supplementary figures, time of polar body extrusion should be indicated in Figure S3.

Contradiction with previous data published by the same group: Cdc25A overexpression arrests oocytes in metaphase I (Dev. Biol. 2008), whereas here they state that oocytes reach MII efficiently. At least it has to be determined whether differences in protein levels are the reason for this discrepancy. Additionally are there differences in Cdk1 activity with different levels of Cdc25A expression?

Reviewer 2

Advance summary and potential significance to field

This study reports a function for CDC25B for the MI to MII transition in mouse oocytes. Cdc25b knockout oocytes normally arrest in prophase as CDC25B is essential for CDK1 activation. The authors overcome the prophase arrest in Cdc25b-/- oocytes by several different strategies, including pharmacological inhibition of WEE1/MYT1 and expression of constitutively active CDK1. They subsequently observe that a large fraction of oocytes arrest in MI. Based on their data, they propose a function for CDC25B at the MI-MII transition.
The experiments are well executed and presented with quantifications throughout. However, as the authors need to trigger the Cdc25b-/- oocytes artificially to undergo GVBD (requiring to modulate Cdk1 activity in the first place) the interpretation of the results is difficult, given that Cdk1 regulation is also required to trigger anaphase. The main conclusion is hence not well supported. Moreover, the function of CDC25B in promoting progression into anaphase remains unclear. The mechanistic advance of the study is hence limited.

Comments for the author

1. Is there a way to acutely inhibit or remove CDC25B after the MI spindle has assembled? This would of course be the most convincing way to support the authors' main conclusion.
2. Is it possible to induce GVBD in milrinone arrested oocytes using one of the methods that the authors use to trigger GVBD in Cdc25b-/- oocytes? If so they could test in wildtype oocytes if these progress successfully into anaphase when GVBD is artificially triggered. If they also arrest in MI this would argue against a specific function of CDC25B.
3. Further insights into the mechanism by which CDC25B might promote anaphase onset would greatly strengthen and expand the scope of the study. However, to obtain meaningful results one would first have to find a better way of inactivating CDC25B after GVBD.
4. The authors argue that spindle assembly and chromosome behavior are not affected. It would be helpful if they support this point by quantifications.
5. The authors propose that the arrest is not due to SAC activation. This could be tested by inactivating the SAC, for instance by using reversine.
6. It would be helpful if the authors include all original blots for the Cdk1 kinase assay.

Reviewer 3

Advance summary and potential significance to field

Nicely describes a role for CDC25B in MI to MII transition in female meiosis in mammals.

Comments for the author

Comments to Editor: Ferencova et al

I think this manuscript is well written and presents a nice story that will be of interest to the readers of the journal. The experiments seem thorough and include the appropriate controls. I don't feel that there are any major flaws that would prevent it from being published. There are some minor things that could be fixed, some of which are simply changes to the writing.

First revision

Author response to reviewers' comments

Dear Professor Glover and Reviewers,

Enclosed please find the revision of our manuscript entitled "CDC25B is required for the metaphase I-metaphase II transition in mouse oocytes" submitted for consideration as a publication in the Journal of Cell Science. We have made our best effort to address the concerns raised in the initial review process; these changes are highlighted in yellow in therevised manuscript. What follows is a point-by-point response to the Reviewers concerns.

As Professor Glover is aware, Petr Solc, the corresponding author, passed away. He contributed significantly to the first version of the manuscript and the revision. Our wish is that he remains a co-author, noting that in the Acknowledgements, we dedicate the manuscript in his honor. We hope that the journal will honor our request.

Sincerely yours,

David Drutovic

Reviewer 1 Advance Summary and Potential Significance to Field: The present manuscript aims at addressing the role of Cdc25B for metaphase-to-anaphase I transition in mouse oocytemeiosis. Studies of its role for progression through meiosis I were hindered for up to now by the fact that Cdc25B is required for entry into meiosis I (GVBD), through requirements of thisphosphatase to activate Cdk1 by counteracting inhibitory phosphorylation mediated by Wee1/Myt1. Without Cdc25B, oocytes are not able to enter meiosis I, and the authors show that activating Cdk1 by other means (expressing Cdc25A, or inhibiting Wee1/Myt1) allows GVBD in oocytes devoid of Cdc25B to address its role beyond GVBD. They find that oocytesare not able to progress into meiosis II without Cdc25B, and even though Cdc25A ectopic expression can rescue GVBD, it cannot rescue anaphase I onset. It is however not clear what the role of Cdc25B could be for exit from meiosis I, whether it targets Cdk1 (unlikely, because Cdk1 has to be inactivated for exit from meiosis I), the role of Cdc25B localization (essential for GVBD), etc. No hypothesis of the role of Cdc25B for anaphase I is proposed, noexplanation of the phenotype is provided.

Reviewer 1
Comments for the Author:
Main points:

1) The authors state that oocytes without Cdc25B that are rescued to enter meiosis I arrest in metaphase I. No proper cell cycle analysis is provided, it is only shown that oocytes do not extrude polar bodies and have bipolar spindles without enough resolution to judge whether the chromosomes are indeed aligned. Do they arrest with high MPF activity? Is the spindle checkpoint activated? Are chromosomes aligned? Is progression through prometaphase comparable to control oocytes (time of spindle formation, kinetics of MPF accumulation, inactivation of the SAC, attachment of microtubules, ...)? With the current data the authors cannot conclude that oocytes are arrested in metaphase I and their data below actually indicatethat it is not the case.

a) To provide a detailed comparison of chromosome behavior and spindle formation kinetics between Cdc25b KO oocytes forced to resume meiosis and WT oocytes, we conducted three-color high-resolution live-imaging of chromosomes, microtubules, and MTOCs using light- sheet microscopy; see page 6 of the revised manuscript. First, we assessed chromosome alignment and compared KO oocytes at MI versus MII stage. Second, we assessed the time ittook for spindles to form after GVBD and to become a bipolar spindle. Third, we scored our imaging dataset for the presence of defective MTOCs clustering. These data are found in a new figure (Figure 2D-F) with the following revised text:

To assess chromosome behavior and microtubule organizing centres (MTOCs) clustering in KO oocytes treated with PD at high resolution, we microinjected oocytes stained with SiR- tubulin with cRNAs encoding an MTOC marker, mEGFP–CDK5RAP2, and H2B– mCherry—to visualize

chromosomes—as previously described (Blengini et al., 2021) and followed meiotic maturation and spindle formation by time-lapse light-sheet microscopy (Fig.2D and Movie 1). These experiments indicated no significant difference in the percentage of oocytes with segregation errors between WT and KO oocytes (Fig. S3B). At MI, WT oocytescontained a bipolar spindle with fully clustered MTOCs and centrally aligned chromosomes on the metaphase plate (Fig. 2D). At the same time, in the majority of KO oocytes, chromosomes were also aligned at the spindle equator (Fig. 2D, E). In KO oocytes, fragmented MTOCs formed two dominant MTOCs clusters similar to WT oocytes (Fig. 2D, F). We also observed that the onset of spindle formation and spindle elongation (bipolarization) were not affected in KO oocytes (Fig. S3C, D). These results provide furtherevidence that KO oocytes arrest in MI with normal bipolar spindles.

b) To ascertain whether persistent SAC activity causes meiosis I arrest, we first quantified the amount of kinetochore-associated MAD2, an accepted measure of SAC activity, in KO oocytes forced to resume meiosis and WT oocytes. Second, we inhibited SAC in KO oocytes by pharmacological inhibition of MPS1 using reversine and analyzed the first polar body extrusion in KO oocytes. These data are found in a new figure (Figure 5) with the following revised text:

To further confirm the role of SAC in MI arrest, we examined the localization of MAD2, an essential component of SAC signaling in mouse oocytes, by immunofluorescent staining (Fig.5A). First, we detected the MAD2 level on kinetochores at prometaphase I (4 h after meiotic resumption) in WT oocytes. We showed that MAD2 was enriched at the kinetochores in WT oocytes in this time interval. As anticipated, the relative protein expression levels (fluorescence intensity) of MAD2 were markedly reduced at kinetochores in WT oocytes treated with PD at MI (8 h after meiotic resumption). Although there was oocyte-to-oocyte variability, no significant difference in MAD2 intensity was measured between WT a KO oocytes suggesting that the SAC activity in KO oocytes is not elevated (Fig. 5B).
We then ascertained whether KO oocytes could progress to MII when SAC signaling is removed by treating oocytes simultaneously with PD and reversine to inhibit the SAC kinase Mps1. Meiotic progression and securin degradation were monitored by light-sheet microscopy(Fig. 5C and Movie 5). As expected, reversine treatment accelerated progression to Ana I, with securin degradation beginning around 4 h after meiotic resumption in WT oocytes (Fig. 5D). In the presence of reversine, KO oocytes treated with PD exhibited rates of securin degradation similar to WT oocytes (Fig. 5E). Surprisingly, all KO oocytes enter Ana I in the presence of reversine. Some of these oocytes either failed to extrude a polar body and complete MII or had a cytokinesis failure and retracted the polar body into the cytoplasm (Fig. 5C, F). Taken together, the comparable reduction of MAD2 at kinetochores in KO oocytes and failure to complete MII when the SAC is inhibited suggests that the SAC is notthe sole mediator of MI arrest in KO oocytes.

2) The conclusion that APC activity is reduced cannot be drawn by looking at a single substrate that is exogenously expressed. Maybe Cdc25B targets securin and not the APC? What about Cyclin B1, endogenous proteins,...? (Western blots of endogenous securin andcyclin B1 can be done in mouse oocytes, this has been published multiple times).

We appreciate this suggestion to confirm reduced APC activity in KO oocytes. Recent work shows that Cyclin B1 is already partially destroyed in prometaphase I due to its destruction motif insensitive to SAC signaling (Levasseur et al., 2019). In that study, the authors used a fluorescent non-CDK1-binding cyclin B1 reporter, cyclin B-Y170-VFP, that generated an almost identical destruction profile to WT cyclin B1 but did not affect the proportion of MI- arrested oocytes. Accordingly, we conducted live-imaging experiments of KO oocytes forcedto resume meiosis and WT oocytes to measure cyclin B-Y170-VFP destruction and calculatedcorresponding APC/C activity. This information can be found in lines 196-203 of the revised manuscript.

To confirm reduced APC/C activity in MI-arrested KO oocytes, we monitored destruction of a non-CDK1-binding cyclin B1, another APC/C substrate, that is already partially destroyed in prometaphase I (Levasseur et al., 2019). Oocytes stained with SiR-tubulin were microinjected with *H2B-mCherry* and a non-CDK1-binding cyclin B1 reporter *cyclin B-Y170-Vfp* cRNAs, and degradation of the cyclin B1 reporter was monitored over time by live imaging (Fig. S5A-C and Movie 3). These experiments demonstrated that the rate of cyclin Bdegradation exhibits similar kinetics of destruction as securin in MI-arrested KO oocytes, thereby providing further evidence of reduced APC/C activity in KO oocytes.

3) The statement that securin GFP degradation was reduced in oocytes arrested in MI does not make sense. If they are arrested, they shouldn't degrade securin, otherwise it is not a MI arrest. (page 6, 1st paragraph)

We revised this sentence, which now reads:
In KO oocytes treated with PD, securin-EGFP was also degraded, but its rate of degradationwas reduced in oocytes failing to extrude the 1st polar body (Fig. 3C).

4) an internal loading control for the kinase assays should be provided, to show how much unphosphorylated GST-LAMS22 was added to quantitate the phosphorylation of GST LAMS22 relative to protein added to the reaction. (This control is also used in the publicationcited by the authors for using GST-Laminin as a substrate). Immunodetection of a phosphosite is much less sensitive than incorporation of radioactivity that is usually used as a read-out for kinase assays in mouse oocytes. The authors have to show all the controls to demonstrate they have establish this assay in mouse oocytes where there is probably around 1000 times less protein than used for kinase assays in somatic cells, and show that they get thesame profiles for Cdk1 activity such as previously published by multiple labs using radioactive ATP and Histone H1 as a substrate.

We apologize for the omission and have added the internal loading control and all originalblots for the kinase assays to Figure 3.

5) No controls for the specificity or efficiency of the Wee1/Myt1 inhibitor in oocytes at the indicated concentrations are provided.

In response to the Reviewer's concern and the suggestion from Reviewer 2, we tested MII progression in oocytes treated simultaneously with milrinone and PD0166285 (WEE1/MYT1inhibitor; PD). In a new Fig. S1, we show that 0.5 μM PD treatment can rescue milrinone- induced GV arrest of all WT oocytes, the majority of which progressed to MII. We reason that the ability of PD to rescue GV arrest without affecting MII progression confirms the specificity of the inhibitor and efficiency in a defined concentration. The description of these data can be found in lines 111-116:
To confirm that MI arrest in KO oocytes was due to the absence of CDC25B and not a non- specific effect of PD treatment, we monitored meiotic maturation of WT oocytes simultaneously treated with 2.5 μM milrinone (an inhibitor of meiotic maturation) and 0.5 μMPD. The results indicated that PD treatment could rescue milrinone-induced prophase I arrest of all WT oocytes and that ~ 90% of these oocytes progressed to MII, suggesting a specific role for CDC25B in the MI-MII transition (Fig. S1).

Minor issues:
1. Legends are missing for graphs in supplementary figures, time of polar body extrusionshould be indicated in Figure S3.

We apologize for the omission. We included the legends for graphs in supplementary figures. Regarding Figure S3, we are not sure what the Reviewer is referring to. Figure S3 (new Fig. 3D) is the CDK1 activity in vitro assay. We did not calculate the time of polar body extrusionfor this analysis but only defined the cell cycle stage in defined time intervals and collected cells to analyze CDK1 activity.

2. Contradiction with previous data published by the same group: Cdc25A overexpression arrests oocytes in metaphase I (Dev. Biol. 2008), whereas here they state that oocytes reach MII efficiently. At least it has to be determined whether differences in protein levels are the reason for this discrepancy. Additionally, are there differences in Cdk1 activity with differentlevels of Cdc25A expression?

The Solc et al. 2008 paper reported that the MI arrest observed following over-expression ofCDC25A depended on the amount of microinjected cRNA; 10% and 40% oocytes reached metaphase-II after microinjection of 500 and 100 ng/μl of Gfp-Cdc25a mRNA, respectively.We believe that differences in CDC25A protein levels are the basis for this apparent discrepancy.

Reviewer 2
Advance Summary and Potential Significance to Field:
This study reports a function for CDC25B for the MI to MII transition in mouse oocytes. Cdc25b knockout oocytes normally arrest in prophase as CDC25B is essential for CDK1 activation. The authors overcome the prophase arrest in Cdc25b-/- oocytes by several different strategies, including pharmacological inhibition of WEE1/MYT1 and expression of constitutively active CDK1. They subsequently observe that a large fraction of oocytes arrest in MI. Based on their data, they propose a function for CDC25B at the MI-MII transition. Theexperiments are well executed and presented with quantifications throughout. However, as theauthors need to trigger the Cdc25b-/- oocytes artificially to undergo GVBD (requiring to modulate Cdk1 activity in the first place) the interpretation of the results is difficult, given that Cdk1 regulation is also required to trigger anaphase. The main conclusion is hence not well supported. Moreover, the function of CDC25B in promoting progression into anaphase remains unclear. The mechanistic advance of the study is hence limited.

Reviewer 2
Comments for the Author:
1. Is there a way to acutely inhibit or remove CDC25B after the MI spindle has assembled?This would of course be the most convincing way to support the authors' main conclusion.

To the best of our knowledge, a specific CDC25B inhibitor is not available. To address the Reviewer's question, we used Trim-Away to promote CDC25B degradation after GVBD. This technique utilizes antibodies to target proteins for proteasome-mediated degradation via the ubiquitin ligase TRIM21. We first optimized this technique to obtain comparable GFP protein degradation in mouse oocytes using the protocol described by Clift et al., 2017. In brief, oocytes stained with SiR-DNA were co-injected with mRNA encoding for Cdc25-Gfp and Trim21-mCherry. Once the expressed CDC25B-GFP induced GVBD in KO oocytes, half of the oocytes were microinjected with anti-GFP antibody to induce CDC25B-GFP depletion.
Light-sheet microscopy confirmed expression of TRIM21-mCherry and efficient depletion ofCDC25B-GFP as fluorescence of GFP was reduced to the background level. In contrast to theKO+PD treated oocytes, we found that although CDC25B-GFP was degraded, only ~25% of these KO oocytes were arrested in MI. We suspect that the narrow time window required to deplete CDC25B after GVBD to prevent its function subsequent to GVBD is not sufficient, i.e., that while CDC25B is being degraded post-GVBD there nevertheless remain sufficient amounts of CDC25B to execute its post-GVBD function prior to its complete degradation.
Thus, we believe that PD treatment is the best approach for studying the role of CDC25B afterthe meiotic resumption.

2. Is it possible to induce GVBD in milrinone arrested oocytes using one of the methods thatthe authors use to trigger GVBD in Cdc25b-/- oocytes? If so they could test in wildtype oocytes if these progress successfully into anaphase when GVBD is artificially triggered. If they also arrest in MI this would argue against a specific function of CDC25B.

The Reviewer's point was most appreciated and see response #5 to Reviewer 1.

3. Further insights into the mechanism by which CDC25B might promote anaphase onset would greatly strengthen and expand the scope of the study. However, to obtain meaningfulresults one would first have to find a better way of inactivating CDC25B after GVBD.

Please see response #1.

4. The authors argue that spindle assembly and chromosome behavior are not affected. Itwould be helpful if they support this point by quantifications.

Please see response #1 to Reviewer 1.

5. The authors propose that the arrest is not due to SAC activation. This could be tested by inactivating the SAC, for instance by using reversine.

The Reviewer's point was most appreciated and see response #1b to Reviewer 1.

6. It would be helpful if the authors include all original blots for the Cdk1 kinase assay.

The loading control and all original blots for kinase assays are now shown (Fig. 3D).

Reviewer 3

Comments to authors
I think this manuscript is well written and presents a nice story about the role of the CDC25B phosphatase in female meiosis that will be of interest to the readers of the journal. The experiments seem thorough and include the appropriate controls. I don't feel that there are any major flaws that would prevent it from being published. There are some minor things that could be fixed, some of which are simply changes to the writing.

We are pleased that the Reviewer found our story interesting and address below theReviewer's comments.

I think HET is a more traditional abbreviation for heterozygote and would be a better abbreviation that HZ unless there is now some new convention. Also I wonder if AI is the usual abbreviation for Anaphase I or whether Ana I might be better. When I read AI I found ita bit difficult to follow.

These changes have been incorporated throughout the revised manuscript.

Line 82: CDC25B is essential for resumption of meiosis (Lincoln et al., 2002).Might be better if it read:
CDC25B is essential for resumption of meiosis in females (Lincoln et al., 2002). Or CDC25Bis essential for resumption of female meiosis in mammals (Lincoln et al., 2002).

The text now reads:
CDC25B is essential for resumption of meiosis in females (Lincoln et al., 2002).

Line 147: In contrast, ~ 90% of WT oocytes microinjected with the same amount of Cdk1afmRNA matured MII. Should read mRNA matured to MII.

The text now reads:
In contrast, ~ 90% of WT oocytes microinjected with the same amount of Cdk1af mRNAmatured to MII.

Line 154: Because of the significant delay in resumption of meiosis in KO oocytes microinjected with Cdk1af cRNA, we determined whether oocytes that resumed meiosis earlier reached MII, but oocytes resuming later did not. Results of these experiments indicatedthat the time of resumption of meiosis in KO oocytes arrested in MI was similar to KO oocytes that matured to MII (Fig. S2E). Very long sentence and confusing. Please edit and break up

We have edited this paragraph. It now reads:
Because there was a significant delay in resumption of meiosis in KO oocytes microinjectedwith Cdk1af cRNA, we determined whether oocytes that resumed meiosis arrested in MI. Results of these experiments did not show any significant difference in timing of resumptionof meiosis in KO oocytes at the MI versus MII stage.

Line 161: ....bypassed the requirement of CDC25B for resumption of meiosis and thereby unmasked a requirement of CDC25B for the MI-MII transition. Line 243: .....described Cdc25b-/- mice (Lincoln et al., 2002) and breed heterozygous males . Should read: .....and bred heterozygous males

The text now reads:
To obtain Cdc25b -/- mice on an inbred C57BL/6 genetic background, we used the previously described Cdc25b-/- mice (Lincoln et al., 2002) and bred heterozygous males with wildtype C57BL/6 females (Charles River) for six consecutive generations.

Figure 1D – there appear to be some oocytes from KO animals that do not remain arrested but progress to MI and even some that progress to MII. Has that been noted before or is that a new finding? Similarly, there appear to be some HZ oocytes that remain arrested. Again, has that been seen before on other strain backgrounds?

Observing that a small fraction of KO oocytes could progress to MII is a new finding. The Lincoln et al. (2002) paper reported that all analyzed Cdc25b-/- oocytes arrested in the GVstage and did not mature to MII. The authors did not analyze meiotic maturation in vitro inCdc25b +/- oocytes.

I note also that, according to the methods, the CDC25B strain was backcrossed onto the C57Bl/6 strain for 6 generation. What was the rationale for only 6 generations? Most backcrossing is carried out for 9 generations in order to ensure that the mutant allele is isolated onto the new background without much of the original genetic background beingpresent.

As we indicated in the cover letter, Petr Solc passed during the revision. As the principal investigator, Petr was responsible for the backcrossing of mice for this project. Unfortunately,we don't know what the rationale for why the mice were backcrossed for 6 generations.

Line 95: that did not, ~1/3 of the HZ oocytes did not emit a polar body should be written out by convention, IMHO. That is: "that did not, approximately a third of the HZ oocytes did not emit a polar body"

The text now reads:
In contrast to WT oocytes that successfully matured to MII, as evidenced by emission of a polar body (PB), and as expected KO oocytes that did not, approximately a third of the HETooocytes did not emit a polar body and were presumably arrested at MI (Fig. 1C, D).

Line 104: Because WEE1/MYT1 are responsible for the inhibitory phosphorylations of CDK1during prophase I arrest (Han et al., 2005; Oh et al., 2010)—CDC25B removes these phosphorylations during resumption of meiosis (Lincoln et al., 2002)— This is not actually shown in Lincoln et al. Is that correct? Maybe it would be better if it read: CDC25B presumably removes.....

The text now reads:
Because WEE1/MYT1 are responsible for the inhibitory phosphorylations of CDK1 duringprophase I arrest (Han et al., 2005; Oh et al., 2010)—CDC25B presumably removes these phosphorylations during resumption of meiosis (Lincoln et al., 2002)—PD treatment of KO oocytes resulted in resumption of meiosis as expected but 61% of these treated oocytesarrested in MI (Fig. 2A).

Line 109: In the second approach, we expressed CDC25A by microinjecting a Egfp-Cdc25a cRNA. CDC25A cooperates with CDC25B during resumption of meiosis to activate CDK1, but after resumption of meiosis, CDC25A degradation is required for the MI-MII transition (Solc et al., 2008). Injecting ~10 pl of 5 ng/µL of Egfp-Cdc25a cRNA did not compromise maturation to metaphase II in WT oocytes, but injecting ~10 pl of 10 ng/µL induced meiosis Iarrest (Fig. S1A), a finding consistent with the requirement of CDC25A destruction for the MI-MII transition (Solc et al., 2008). I wonder if there is an additional interpretation of this result, namely that CDC25A produced in the cytoplasm from injected cRNA might be able tosubstitute for CDC25B in the cytoplasm, whereas in the normal situation the two proteins are in different locations as described by the authors previously?

See response to Reviewer 1, minor issues, response b.

Line 156: Results of these experiments indicated that the time of resumption of meiosis in KO oocytes arrested in MI was similar to KO oocytes that matured to MII (Fig. S2E). Might be good to have a conclusion statement here for readers to follow along.

We have added a conclusion statement to the paragraph to clarify this. These details are found starting at line 178:
We conclude that acceleration of meiotic resumption does not affect MII progression in KOoocytes.

Fig 3A – would it be possible to show Securin-GFP images at the same timepoint, e.g. the last timepoint shown for WT plus PD v KO plus PD is very different? Might be good to see the KO image

at the timepoint shown for WT and perhaps reinforce the point the authors are trying to make?

In response to the Reviewer's suggestion, we changed the timepoint 16:40 for the KO+PDgroup to timepoint 12:50, similar to WT and WT+PD groups.

Line 176: APC/C activation (Fig. 3A, B), normal bipolar spindle formation and chromosome alignment (Fig. 2B) in KO oocytes treated with PD suggest that MI arrest is not due to persistent SAC activity, Please define SAC at first use and also put it into context for the reader with regard to the aforementioned APC/C, normal bipolar spindle formation and chromosome alignment.

The text now reads:
APC/C activation (Fig. 3A, B), normal bipolar spindle formation and chromosome alignment(Fig. 2B) in PD-treated KO oocytes suggest that MI arrest is not due to persistent spindle assembly checkpoint (SAC) activity, although MI-arrested oocytes did not reach maximal APC/C activity (Fig. 4C). SAC monitors attachment of microtubules to kinetochores, and itsactivation caused by loss of kinetochore-microtubule (K-MT) attachments prevents APC/C activation and delays anaphase entry. In addition, inappropriate K-MT attachment could result in chromosome misalignment (Musacchioa and Salmon, 2007).

Line 180: When PLK1 is acutely inhibited at the end of MI (Solc et al., 2015), oocytes remain arrested in MI, and APC/C activation exhibits similar pattern as KO oocytes treated with PD Exhibits similar pattern of what? Not clear the way the sentence is written Line 180: When PLK1 is acutely inhibited at the end of MI (Solc et al., 2015), oocytes remainarrested in MI, and APC/C activation exhibits similar pattern as KO oocytes treated with PD. Might also read better as:
"When PLK1 is acutely inhibited at the end of MI (Solc et al., 2015), oocytes remain arrestedin MI, and APC/C activation exhibits similar pattern as CDC25B KO oocytes treated with PD.
Since there might be confusion between whether KO in this context refers to PLK1 orCDC25B.

The text now reads:
When PLK1 is acutely inhibited at the end of MI (Solc et al., 2015), oocytes remain arrestedin MI, and degradation of securin exhibits a similar pattern as CDC25B KO oocytes treated with PD.

Line 176: APC/C activation (Fig. 3A, B), normal bipolar spindle formation and chromosome alignment (Fig. 2B) in KO oocytes treated with PD suggest that MI arrest is not due to persistent SAC activity, although MI arrested oocytes did not reach the full APC/C activity (Fig. 4C). I find this statement a little problematic since the authors acknowledge that APC/Clevels do not reach full activity. Doesn't that mean that SAC could be in place still? Also isn'tit within the authors toolset to knockdown PLK1 while at the same time expressing CDC25Bto follow up on their speculation about the role of PLK1 in regulating CDC25B and their theory that the MI arrest in KO oocytes is not due to SAC activity?

Although the Reviewer proposes some interesting experiments, we believe that determiningPLK1-CDC25B interaction is beyond the scope of this study.

Second decision letter

MS ID#: JOCES/2020/252924

MS TITLE: CDC25B is required for the metaphase I-metaphase II transition in mouse oocytes

AUTHORS: Ivana Ferencova, Michaela Vaskovicova, David Drutovic, Lucie Knoblochova, Libor Macurek, Richard M Schultz, and Petr Solc
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers gave favourable reports but raised some critical points that will require amendments to your manuscript. I hope that you will be able to carry these out because I would like to be able to accept your paper, depending on further comments from reviewers.

We are aware that you may be experiencing disruption to the normal running of your lab that makes 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 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. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The present manuscript aims at addressing the role of Cdc25B for metaphase-to-anaphase I transition in mouse oocyte meiosis. Studies of its role for progression through meiosis I were hindered for up to now by the fact that Cdc25B is required for entry into meiosis I (GVBD), through requirements of this phosphatase to activate Cdk1 by counteracting inhibitory phosphorylation mediated by Wee1/Myt1.
Without Cdc25B, oocytes are not able to enter meiosis I, and the authors show that activating Cdk1 by other means (expressing Cdc25A, or inhibiting Wee1/Myt1) allows GVBD in oocytes devoid of Cdc25B to address its role beyond GVBD. They find that oocytes are not able to progress into meiosis II without Cdc25B and remain arrested in metaphase I, independently of the spindle assembly checkpoint.
Heterozygote Cdc25B+/- oocytes are impaired in metaphase to anaphase transition of meiosis I because of only partial APC/C activation.

Comments for the author

The manuscript has greatly improved, and the authors have responded to most of my comments in a satisfying manner. It can be published ONCE the following concerns have been addressed.

Main points:
1) Figure 2D: pannel KO+PD MII, 10:40. The authors cannot call this an MII oocyte because it is in anaphase I. The authors claim that KO oocytes are arrested in MI however they show an anaphase I oocyte which they label as M II. I guess they mean that most oocytes arrest in meiosis I with aligned chromosomes "KO+PD MI"), but some can enter anaphase I and then remain arrested in a kind of anaphase I like state without being able to exit meiosis I and enter meiosis II. This is not clear at all from the labeling and the images shown in Figure 2D. Can the authors show later time points of ALL stages (at BD +12h) to illustrate this better, and revise their labeling (how about KO+PD AI) and/or conclusion in saying that some KO oocytes are able to go through the metaphase-to-anaphase transition of meiosis I but are unable to exit meiosis I?
2) It should be indicated how many oocytes have been used for generating the graphs that are shown. This is the standard in the field.

3) The manuscript is written in a quite confusing manner, making it often hard to understand the logic of experiments (for example p5 line 10: “both WT oocytes and WT oocytes treated with PD”. Replace with something like “WT and PD-treated oocytes”)

Minor points:
In Figure 3D, the error bar for the wt+PD condition at 8 hours is missing.
P5, line 132: they describe the effect of Wee1/Myt1 inhibition as “more” rapid”.
It is not statically significant in the fig… there is no point to mention this in my opinion.
P8, line 221: “Cdk1 activity was lower in metaphase I (18h) in comparison to WT oocytes”. This experiment was done with PD, which inhibits Myt1/Wee1. Why is Cdk1 lower ??? Does it means that PD has an unspecific effect? An increase in Cdk1 activity would be expected. Unfortunately, there is no western blot showing pY15-Cdk1 to support the data.
P9, Line 252: “As anticipated” > Why ??? and line 255: “a” should be replaced by or “between WT a KO oocytes”

Reviewer 2

Advance summary and potential significance to field

In their revisions, the authors addressed all points that I raised. This is even more impressive given the difficult circumstances under which the revisions were carried out. My thoughts are with the authors in these difficult times.
I now support publication in JCS. However, before publication I recommend the following small changes:

Comments for the author

1. The authors successfully used Trim-Away to deplete GFP-Cdc25B in Cdc25B-/- oocytes after GVBD. They observed that the oocytes progressed into anaphase relatively efficiently. However, they decided against including these data in their manuscript. They argue that the short time upon NEBD while Cdc25B is present might be sufficient for it to execute its stimulatory effect on anaphase.
I would recommend that the authors include these interesting data in their manuscript, as they indicate that Cdc25B promotes progression into anaphase by dephosphorylating targets other than Cdk1 during an early stage of meiotic maturation. In my view, it is even possible that this happens even already prior to GVBD. This is an additional interesting piece of information that could be discussed by the authors.
From what the authors write in their response, the GFP-Cdc25B depletion seems to be fairly complete. However, if there was substantial residual GFP-Cdc25B they could of course also discuss that this could contribute to the weaker MI arrest.
2. The authors had argued that the MI arrest is not due to SAC activation. However, they observed progression into anaphase upon SAC inactivation by reversine, although progression through the first meiotic division was not normal. The observation that Mad2 levels at kinetochore were similar in WT and KO oocytes at 4 hours after meiotic resumption is not so surprising, as Mad2 levels only drop more substantially closer to anaphase onset. If the authors wanted to compare the levels they would have to fix oocytes very close to time of anaphase onset in control oocytes. Given the current data, I would phrase the interpretation of these results more carefully, and discuss that they cannot exclude that Mad2 levels might show differences closer to anaphase. They could write that CDC25B-/- oocytes might activate the SAC, but that progression into anaphase is not normal and that CDC25B therefore likely has functions beyond those required for SAC inactivation.

Reviewer 3

Advance summary and potential significance to field

The findings show that Cdc25B has a role not only in resumption of female meiosis but also in the transition from metaphase I to metaphase II.

Comments for the author

Please accept my apologies for the delay and my sincere condolences on the death of your friend and co-author. I still think this is a good piece of work and that it tells an interesting story that will be of interest to the readers of the journal. I still have one question however. In my previous review I noted that the authors had concluded that:
“APC/C activation (Fig. 3A, B), normal bipolar spindle formation and chromosome alignment (Fig. 2B) in KO oocytes treated with PD suggest that MI arrest is not due to persistent (spindle assembly checkpoint) SAC activity, although MI arrested oocytes did not reach the full APC/C activity (Fig. 4C).”

I noted in my review that I found this statement a little problematic since the authors acknowledge that APC/C levels do not reach full activity in KO oocytes.
Doesn't that mean that the SAC could be in place still? This question was similar to one raised by the other two reviewers I think. The authors have responded to this critique by carrying out experiments using reversine, to inhibit the SAC kinase Mps1 and to first examine the change in expression of MAD2.

The images in Figure 5A of MAD2 staining in WT and KO oocytes treated with PD are difficult to see. I realize that is part of the point is that MAD2 levels are reduced under these conditions relative to the WT prometaphase but right now the other two panels look blank. Would it be possible to enhance each of those three MAD2 staining images to an equivalent amount (and explain in the figure legend) so as to be able to at least see the staining in the WT and KO panels? I realize also that it might also mean that the WT prometaphase image might become a bit overexposed, but right now the other two panels look blank. I think the rationale for the image enhancement could be explained in the text and figure legend.

The authors then go on to say:
“To further confirm the role of SAC in MI arrest, we examined the localization of MAD2, an essential component of SAC signaling in mouse oocytes, by immunofluorescent staining (Fig. 5A). First, we detected the MAD2 level on kinetochores at prometaphase I (4 h after meiotic resumption) in WT oocytes. We showed that MAD2 was enriched at the kinetochores in WT oocytes in this time interval. As anticipated, the relative protein expression levels (fluorescence intensity) of MAD2 were markedly reduced at kinetochores in WT oocytes treated with PD at MI (8 h after meiotic resumption). Although there was oocyte-to-oocyte variability, no significant difference in MAD2 intensity was measured between WT a KO oocytes suggesting that the SAC activity in KO oocytes is not elevated 256 (Fig. 5B).”

I am no statistician but the plots of WT plus PD and KO plus PD (Figure 5B) look slightly different to me. I wonder if there are any other statistical tests that could be used to analyze that data to determine if there are in fact subtle differences between the WT and KO in that regard.

Then the authors state:
“We then ascertained whether KO oocytes could progress to MII when SAC signaling is removed by treating oocytes simultaneously with PD and reversine to inhibit the SAC kinase Mps1. Meiotic progression and securin degradation were monitored by light-sheet microscopy (Fig. 5C and Movie 5). As expected, reversine treatment accelerated progression to Ana I, with securin degradation beginning around 4 h after meiotic resumption in WT oocytes (Fig. 5D). In the presence of reversine, KO oocytes treated with PD exhibited rates of securin degradation similar to WT oocytes (Fig. 5E). Surprisingly, all KO oocytes enter Ana I in the presence of reversine.
Why is that surprising? If in fact there is some residual SAC activity in KO oocytes would not this be the expected result?

They then go on to say:
“Some of these oocytes either failed to extrude a polar body and complete MII or had a cytokinesis failure and retracted the polar body into the cytoplasm (Fig.
5C, F). Taken together, the comparable reduction of MAD2 at kinetochores in KO oocytes and failure to complete MII when the SAC is inhibited suggests that the SAC is not the sole mediator of MI arrest in KO oocytes.”

Indeed they may be correct in suggesting that the SAC is not the sole mediator of MI arrest in KO oocytes but they seem a bit reluctant to admit that it has any role at all. Could it not be that Cdc25B has some interaction with the SAC or some component of it that might be required for MI entry? Also I am curious if the authors simply treated KO oocytes with reversine and then added PD or not. It might be interesting to see what happens.
In any case I think perhaps my question about the SAC might simply be addressed by re-writing of the manuscript rather than new experiments.

Second revision

Author response to reviewers' comments

Reviewer 1 Advance Summary and Potential Significance to Field: The present manuscript aims at addressing the role of Cdc25B for metaphase-to-anaphase I transition in mouse oocytemeiosis. Studies of its role for progression through meiosis I were hindered for up to now by the fact that Cdc25B is required for entry into meiosis I (GVBD), through requirements of thisphosphatase to activate Cdk1 by counteracting inhibitory phosphorylation mediated by Wee1/Myt1. Without Cdc25B, oocytes are not able to enter meiosis I, and the authors show that activating Cdk1 by other means (expressing Cdc25A, or inhibiting Wee1/Myt1) allows GVBD in oocytes devoid of Cdc25B to address its role beyond GVBD. They find that oocytesare not able to progress into meiosis II without Cdc25B and remain arrested in metaphase I, independently of the spindle assembly checkpoint. Heterozygote Cdc25B+/- oocytes are impaired in metaphase to anaphase transition of meiosis I because of only partial APC/C activation.

Reviewer 1 Comments for the Author:
The manuscript has greatly improved, and the authors have responded to most of my commentsin a satisfying manner. It can be published ONCE the following concerns have been addressed.

Main points:
1) Figure 2D: pannel KO+PD MII, 10:40. The authors cannot call this an MII oocyte, becauseit is in anaphase I. The authors claim that KO oocytes are arrested in MI, however they show an anaphase I oocyte which they label as M II. I guess they mean that most oocytes arrest in meiosis I with aligned chromosomes "KO+PD MI"), but some can enter anaphase I and then remain arrested in a kind of anaphase I like state without being able to exit meiosis I and entermeiosis II. This is not clear at all from the labeling and the images shown in Figure 2D. Can theauthors show later time points of ALL stages (at BD +12h) to illustrate this better, and revise their labeling (how about KO+PD AI) and/or conclusion in saying that some KO oocytes are able to go through the metaphase-to-anaphase transition of meiosis I, but are unable to exit meiosis I?

We show that significantly more HET oocytes (Fig. 1D), as well as KO oocytes, (Fig. 2A) arrestat metaphase I (labeled as MI in manuscript) when CDK1 activity is experimentally increased.HET or KO oocytes that do not arrest at metaphase I enter anaphase I and extrude the first polarbody (MII in manuscript). See lines 148-150. In Fig. 2D, we point out that there is no differencein spindle formation and chromosome alignment between MI and MII in PD-treated KO oocytes. We added one additional time point in Fig. 2D to show that a fraction of PD-treated KO oocytes extrude the first polar body.

2) It should be indicated how many oocytes have been used for generating the graphs that are shown. This is the standard in the field.

We apologize for the omission and have added the number of oocytes for all groups in the figure legends.

3) The manuscript is written in a quite confusing manner, making it often hard to understand the logic of experiments (for example p5 line 10: "both WT oocytes and WT oocytes treated with PD". Replace with something like "WT and PD-treated oocytes")

We thank the reviewer for this suggestion. We replace the phrase "treated with PD" with "PD-treated" throughout the manuscript.

Minor points:
In Figure 3D, the error bar for the wt+PD condition at 8 hours is missing.

As indicated in Fig. 3D legend, CDK1 activity in MI WT oocytes treated with PD was arbitrarily set to 1. The standard deviation is equal to zero.

P5, line 132: they describe the effect of Wee1/Myt1 inhibition as "more" rapid". It is not statically significant in the fig… there is no point to mention this in my opinion.

We thank the reviewer for this suggestion. We removed this sentence and added information describing the effect of WEE1/MYT1 inhibition and *Egfp-Cdc25a* cRNA microinjection on GVBD compared to WT oocytes. The text now reads:
"Whereas inhibition of WEE1/MYT1 did not have a significant effect on the timing of GVBD,the process of GVBD initiated by *Egfp-Cdc25a* cRNA microinjection was significantly slowerin comparison to WT oocytes ".

P8, line 221: "Cdk1 activity was lower in metaphase I (18h) in comparison to WT oocytes". This experiment was done with PD, which inhibits Myt1/Wee1. Why is Cdk1 lower ??? Does it means that PD has an unspecific effect? An increase in Cdk1 activity would be expected. Unfortunately, there is no western blot showing pY15-Cdk1 to support the data.

We do not know why CDK1 activity is lower in PD-treated WT oocytes. We agree with the reviewer that the immunoblot of pY15-CDK1 could support the data. We could conduct such experiments but note that it will require optimizing immunoblotting conditions using WT oocytes because we have not conducted such experiments in the past and will likely require collecting hundreds of KO oocytes. If conducting this experiment is absolutely required for publication, we will attempt to conduct it, noting that it will likely result in a delay of several months before we can submit the re-revised manuscript.

P9, Line 252: "As anticipated"> Why ??? and line 255: "a" should be replaced by or "betweenWT a KO oocytes"

We removed the phrase "as anticipated" and replace the phrase "between WT a KO oocytes" with "between PD-treated WT and PD-treated KO oocytes".

Reviewer 2 Advance Summary and Potential Significance to Field:
In their revisions, the authors addressed all points that I raised. This is even more impressive given the difficult circumstances under which the revisions were carried out. My thoughts are with the authors in these difficult times. I now support publication in JCS. However, before publication I recommend the following small changes:

Reviewer 2 Comments for the Author:
1. The authors successfully used Trim-Away to deplete GFP-Cdc25B in Cdc25B-/- oocytes afterGVBD. They observed that the oocytes progressed into anaphase relatively efficiently. However, they decided against including these data in their manuscript. They argue that the short time upon NEBD while Cdc25B is present might be sufficient for it to execute its stimulatory effect on anaphase. I would recommend that the authors include these interesting data in their manuscript, as they indicate that Cdc25B promotes progression into anaphase by dephosphorylating targets other than Cdk1 during an early stage of meiotic maturation. In my view, it is even possible that this happens even already prior to GVBD. This is an additional interesting piece of information that could be discussed by the authors. From what the authors write in their response, the GFP-Cdc25B depletion seems to be fairly complete. However, if there was substantial residual GFP-Cdc25B they could of course also discuss that this could contribute to the weaker MI arrest.

In a supplemental Fig. S2E, F we show the effect of EGFP-CDC25B depletion in KO oocytes after GVBD on MII progression. We find no significant difference in the percentage of oocytesarrested in MI. Therefore, we prefer to include these data in the Supplementary material section.The

description of these data can be found in lines 128-136.

“In a third approach, we used Trim-Away to promote EGFP-CDC25B degradation aftergerminal vesicle breakdown (GVBD) in KO oocytes (details in Methods section). Light-sheet microscopy confirmed expression of mCherry-TRIM21 and efficient depletion of EGFP- CDC25B as fluorescence of EGFP was reduced to the background level (Fig. S2E and Movie 1). In contrast to the PD-treated KO oocytes (Fig. 2A), we found that although EGFP-CDC25Bwas degraded, only around 25% of these KO oocytes were arrested in MI (Fig. S2F). We suspectthat the narrow time window required to deplete CDC25B after GVBD to prevent its function subsequent to GVBD is not sufficient, i.e., that while CDC25B is being degraded post-GVBD,there nevertheless remain sufficient amounts of CDC25B to execute its post-GVBD function prior to its complete degradation.”

In the re-revised version, we include details concerning the Trim-Away method in the methods section.

2. The authors had argued that the MI arrest is not due to SAC activation. However, they observed progression into anaphase upon SAC inactivation by reversine, although progressionthrough the first meiotic division was not normal. The observation that Mad2 levels atkinetochore were similar in WT and KO oocytes at 4 hours after meiotic resumption is not so surprising, as Mad2 levels only drop more substantially closer to anaphase onset. If the authorswanted to compare the levels they would have to fix oocytes very close to time of anaphase onset in control oocytes. Given the current data, I would phrase the interpretation of these results more carefully, and discuss that they cannot exclude that Mad2 levels might show differences closer to anaphase. They could write that CDC25B-/- oocytes might activate the SAC, but that progression into anaphase is not normal and that CDC25B therefore likely has functions beyond those required for SAC inactivation.

As indicated in lines 260-263, we detected MAD2 level on kinetochores in WT oocytes at prometaphase I (4 h after GVBD) and at MI in PD-treated WT and PD-treated KO oocytes (8 hours after GVBD). As the reviewer suggested, we fixed oocytes very close to the time of anaphase onset. We modified the sentence to highlight that PD-treated WT and KO oocytes were fixed at MI. The text now reads:

"Although there was oocyte-to-oocyte variability, no significant difference in MAD2 intensitywas measured at MI between PD-treated WT and PD-treated KO oocytes suggesting that the SAC activity in KO oocytes is not elevated (Fig. 5B)."

We modified the conclusion statement in the result section based on the reviewer's suggestions.The text now reads:
"Taken together, the comparable reduction of MAD2 at kinetochores in KO oocytes and failureto complete MII when the SAC is inhibited suggests that the CDC25B has an additional role inpromoting MII progression beyond SAC satisfaction ".

Reviewer 3 Advance Summary and Potential Significance to Field:
The findings show that Cdc25B has a role not only in resumption of female meiosis but also inthe transition from metaphase I to metaphase II.

Reviewer 3 Comments for the Author:
Please accept my apologies for the delay and my sincere condolences on the death of your friendand co-author. I still think this is a good piece of work and that it tells an interesting story that will be of interest to the readers of the journal. I still have one question however. In my previousreview I noted that the authors had concluded that:

"APC/C activation (Fig. 3A, B), normal bipolar spindle formation and chromosome alignment(Fig. 2B) in KO oocytes treated with PD suggest that MI arrest is not due to persistent (spindleassembly checkpoint) SAC activity, although MI arrested oocytes did not reach the full APC/Cactivity (Fig. 4C)."

I noted in my review that I found this statement a little problematic since the authors acknowledge that APC/C levels do not reach full activity in KO oocytes. Doesn't that mean thatthe SAC could be in place still? This question was similar to one raised by the other two reviewers I think. The authors

have responded to this critique by carrying out experiments usingreversine, to inhibit the SAC kinase Mps1 and to first examine the change in expression of MAD2.

The images in Figure 5A of MAD2 staining in WT and KO oocytes treated with PD are difficultto see. I realize that is part of the point is that MAD2 levels are reduced under these conditionsrelative to the WT prometaphase but right now the other two panels look blank. Would it be possible to enhance each of those three MAD2 staining images to an equivalent amount (and explain in the figure legend) so as to be able to at least see the staining in the WT and KO panels? I realize also that it might also mean that the WT prometaphase image might becomea bit overexposed, but right now the other two panels look blank. I think the rationale for the image enhancement could be explained in the text and figure legend.

We agree that it is difficult to view MAD2 in PD-treated WT and KO oocytes, in part becauseMAD2 levels are reduced. We have enhanced the MAD2 signal to highlight the MAD2 stainingbetter and modified the figure.

The authors then go on to say:

"To further confirm the role of SAC in MI arrest, we examined the localization of MAD2, an essential component of SAC signaling in mouse oocytes, by immunofluorescent staining (Fig.5A). First, we detected the MAD2 level on kinetochores at prometaphase I (4 h after meiotic resumption) in WT oocytes. We showed that MAD2 was enriched at the kinetochores in WT oocytes in this time interval. As anticipated, the relative protein expression levels (fluorescenceintensity) of MAD2 were markedly reduced at kinetochores in WT oocytes treated with PD at MI (8 h after meiotic resumption). Although there was oocyte-to-oocyte variability, no significant difference in MAD2 intensity was measured between WT a KO oocytes suggestingthat the SAC activity in KO oocytes is not elevated 256 (Fig. 5B)."

I am no statistician but the plots of WT plus PD and KO plus PD (Figure 5B) look slightly different to me. I wonder if there are any other statistical tests that could be used to analyze thatdata to determine if there are in fact subtle differences between the WT and KO in that regard.

As we indicate in the Method section, the Mann-Whitney test was used because the relative fluorescence intensity values of the MAD2 signal were not normally distributed. We are confident that we selected the appropriate statistical method for these data—there is no statistical difference between groups.

Then the authors state:

"We then ascertained whether KO oocytes could progress to MII when SAC signaling is removed by treating oocytes simultaneously with PD and reversine to inhibit the SAC kinase Mps1. Meiotic progression and securin degradation were monitored by light-sheet microscopy(Fig. 5C and Movie 5). As expected, reversine treatment accelerated progression to Ana I, withsecurin degradation beginning around 4 h after meiotic resumption in WT oocytes (Fig. 5D). Inthe presence of reversine, KO oocytes treated with PD exhibited rates of securin degradation similar to WT oocytes (Fig. 5E). Surprisingly, all KO oocytes enter Ana I in the presence of reversine.

Why is that surprising? If in fact there is some residual SAC activity in KO oocytes would notthis be the expected result?

We agree with the reviewer and removed the word "surprisingly".

They then go on to say:

"Some of these oocytes either failed to extrude a polar body and complete MII or had a cytokinesis failure and retracted the polar body into the cytoplasm (Fig. 5C, F). Taken together,the comparable reduction of MAD2 at kinetochores in KO oocytes and failure to complete MIIwhen the SAC is inhibited suggests that the SAC is not the sole mediator of MI arrest in KO oocytes."
Indeed they may be correct in suggesting that the SAC is not the sole mediator of MI arrest in KO oocytes but they seem a bit reluctant to admit that it has any role at all. Could it not be that

Cdc25B has some interaction with the SAC or some component of it that might be required forMI entry? Also I am curious if the authors simply treated KO oocytes with reversine and then added PD or not. It might be interesting to see what happens.

In any case I think perhaps my question about the SAC might simply be addressed by re-writingof the manuscript rather than new experiments.

Based on reviewers 2 and 3 suggestions, we modified the conclusion statement in the result section, which now reads:
„Taken together, the comparable reduction of MAD2 at kinetochores in KO oocytes and failureto complete MII when the SAC is inhibited suggests that the CDC25B has an additional role inpromoting MII progression beyond SAC satisfaction.“

Third decision letter

MS ID#: JOCES/2020/252924

MS TITLE: CDC25B is required for the metaphase I-metaphase II transition in mouse oocytes

AUTHORS: Ivana Ferencova, Michaela Vaskovicova, David Drutovic, Lucie Knoblochova, Libor Macurek, Richard M Schultz, and Petr Solc
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.