THE EMBO JOURNAL

Manuscript EMBO-2016-95895

Fundamental Cell Cycle Kinases Collaborate to Ensure Timely Destruction of the Synaptonemal Complex During Meiosis

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Review timeline:

Submission date:	17 October 2016
Editorial Decision:	17 November 2016
Revision received:	20 April 2017
Editorial Decision:	18 May 2017
Revision received:	31 May 2017
Accepted:	02 June 2017

Editor: Ieva Gailite

Transaction Report:

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

1st Editorial Decision 17 November 2016

Thank you for submitting your manuscript for consideration by The EMBO Journal. It has now been seen by three referees whose comments are shown below.

As you can see from the comments, all three referees express interest in the presented analysis of DDK role in synaptonemal complex degradation and the resulting activation of DNA repair pathways. However, they also raise significant concerns with the analysis that would have to be addressed in order to consider publication here. I would like to invite you to submit your revised manuscript while addressing the comments of all three referees, and focusing in particular on the following points:

1. Referee 1 raises significant concerns regarding Dbf4/Cdc5 interaction before prophase I exit, which are crucial to address in the revised version.
2. Do biochemical analysis of in vivo interaction between Dbf4/Cdc5 (Referee 1).
3. Test the physiological relevance of Dbf4 phosphorylation by Cdc5 as requested by Referee 2.
4. Address the discrepancy between the regulation of synaptonemal complex degradation by Ccd5/Dbf4 in different genetic backgrounds as pointed out by Referee 1.
5. Include the data on the overexpression screen and Dbf4 mutant identification.
6. The data presentation in the manuscript should be adjusted to make it more accessible to the general audience of The EMBO Journal. We do not enforce strict manuscript length limitations, and in this case due to the complexity of experiments a clear presentation of the rationale and scientific background of the experiments is crucial.

Please feel free to contact me if have any further questions regarding the revision. Thank you for the opportunity to consider your work for publication. I look forward to your revision.

REFEREE REPORTS

Referee #1:

This manuscript focuses on the role of the Dbf4-dependent kinase (DDK) in prophase exit in budding yeast. Dbf4 was isolated in a screen for mutants that bypass the prophase I block of a repair-deficient mutant. Follow up analysis led to two interesting and novel findings. First, this work establishes a role for DDK downstream of Cdc5/Polo kinase in synaptonemal complex (SC) assembly. Second, data is also presented showing that destruction of the SC allows reactivation of DNA repair pathways mediated by Rad51. The manuscript is well written and the presented data is abundant and clear. Although the manuscript does not reveal the underlying mechanism by which DDK triggers SC disassembly, this is likely to involve a complicated interplay between multiple factors and is beyond the scope of the current study. The main concern is that the authors argue that an interaction between Dbf4 and Cdc5 is important for prophase I exit, but the presented data and previous observations do not provide convincing evidence to make this conclusion. Crucially, as previously reported by the Zachariae lab, and demonstrated in this manuscript, Cdc5 and Dbf4 only co-exist after prophase I exit, therefore it is difficult to reconcile the model presented with the available data.

Points to be addressed.
1. Details of the screen in which Dbf4 was identified should be presented. While it is not necessary to present all "hits" in this screen, it would be useful to know the background that led to the identification of Dbf4.

2. Similarly, no details as to the origin of the Dbf4 mutants are given in this study and it is not clear why the authors focused on these residues. This is central to the data presented and should be described in full.

3. The major conceptual problem mentioned above refers to Figure 1. In (A) the authors demonstrate that dbf4-E86K and dbf4-E86V are able to induce Ndt80 transcription in a dmc1Δ mutant, indicating prophase I exit. However, dbf4-E83E cannot promote prophase I exit. Figure 1E and 1F demonstrate that these mutants differ in their ability to interact with Cdc5 in vitro: dbf4-E86K and dbf4-E86V enhance the interaction, while dbf4-E83E abolishes it. Based on this data, the authors argue that the Cdc5-Dbf4 interaction is important for prophase I exit. The problem, as reported in Matos et al., and shown also in Figure 1B is that Cdc5 is not present prior to prophase I exit. So the ability to promote prophase I exit cannot be explained by the strength of interaction with Cdc5. Perhaps the E86K, E86V and E83E mutations affect some other aspect of Dbf4 function or interactions.
To start to address this point, the authors should determine whether Dbf4 can induce Ndt80 production, Zip1/Red1 destruction in cells with a meiotic depletion of Cdc5.
Note that data presented in Figure 4 indicate that Dbf4 is required downstream of Cdc5 induction for SC disassembly (at least in the ndt80 background), however again, this need not be direct.

4. Also, related to Figure 1. As the authors show later in the paper, Cdc5 and Dbf4 appear to collaborate to promote SC disassembly (Figure 4A). However, in Figure 1B (dbf4-E86K, E86V) both Dbf4 and Cdc5 are present but Red1 and Zip1 are both stable. How do the authors explain the discrepancy between the dmc1D mutant and the ndt80 block?

5. The interaction between Dbf4 and Cdc5 and the changes in this affinity in the Dbf4 point mutants in vitro are put forward as an explanation for many of the observations presented. However, evidence is not presented to show changes in complex formation in vivo. Co-immunopreciptation experiments of Dbf4-Cdc5 should be performed in different conditions/stages. This is particularly

important for the mutants (E86K, E86V, R83E). Also, is there an increased interaction observed upon bypass of either dmc1 or ndt80 arrest?

6. In Figure 2A. The induction of prophase I exit by Cdc5 under the control of the Dbf4 promoter occurs very late (indeed, Ndt80 production appears to precede it) and the effect is very minimal. This is possibly because Cdc5 is unstable in prophase I. Nevertheless, the difference between the DBF4 wild type control and R83E mutant is extremely small in this assay and this cannot be taken as a convincing argument that the interaction between Dbf4 and Cdc5 is important for enhanced progression.

7. dbf4-E86K can bypass the dmc1D block in the absence of Cdc5 induction. Can it also bypass the ndt80D block without Cdc5 induction? Non-induced controls should be shown in Figure 4 to address this point.

8. Role of CDK. Evidence is presented in Figure 6 that Cdc5 cannot phosphorylate Dbf4 without CDK. This leads to the hypothesis that CDK may mediate the interaction between Dbf4 and Cdc5. This should be tested in vivo by co-immunopreciptation.

9. FACS analysis should be presented for the experiments shown in Figures 6F and EV3.

Referee #2:

The transition from late meiotic prophase to metaphase I is marked by the resolution of recombination intermediates and by the disassembly of the synaptonemal complex (SC), a proteinaceus structure that is assembled between homologous chromosomes during early prophase. Understanding the mechanisms that regulate this key transition of the meiotic program is an important question for the fields of meiosis and chromosome biology. Previous studies demonstrated that Cdc5 (Polo-like kinase) is required both for SC disassembly and crossover formation, but how Cdc5 promotes these events remains largely unknown.

The manuscript by Argunhan et al. shows that an interaction between Cdc5 and DDK component Dbf4 promotes meiotic progression by inducing repair of recombination intermediates and destruction of SC components, in particular Red1. These conclusions are reached by using mutant versions of Dbf4 that show different strengths of interaction with Cdc5, combined with a detailed analysis of DSB repair and Red1 degradation in these mutants, as well by a cytological analysis of SC disassembly. In addition, these experiments show that following SC disassembly, DSB repair depends on the activity of the Rad51 recombinase, which is suppressed at earlier stages to promote Dmc1-mediated DSB repair, suggesting a switch in the mode of DSB repair during late prophase that is regulated by Dbf4-Cdc5. Overall, the data shown in these experiments supports the conclusion of the authors that an interaction between Dbf4 ad Cdc5 promotes SC disassembly and DSB repair at late prophase. The second part of the manuscript investigates how phosphorylation of Dbf4 affects SC disassembly. The authors show that Dbf4 is phosphorylated by Cdc5 and Cdk1 and that removal of Dbf4 compromises destruction of SC components, confirming the functional relevance of Dbf4 in SC disassembly. Finally, the authors attempt to demonstrate that phosphorylation of Dbf4 is directly responsible to induce SC disassembly. They successfully identify 4 residues that are phosphorylated in Dbf4, but the functional analysis of phospho-dead mutant is complicated by the apparent instability of the mutant protein. Clearly demonstrating the functional relevance of Dbf4 phosphorylation in SC disassembly would be an important addition to the manuscript. Can the authors overexpress dbf4-4A to achieve normal levels of the mutant protein and then determine if SC disassembly is affected? Or investigate if phosho-mimetic mutations in the residues mutated in dbf4-4A promote SC disassembly?

The manuscript makes a strong case for the involvement of Dbf4 phosphorylation by Cdc5 and Cdk1 in regulating SC disassembly, but as mentioned above, a more clear demonstration of the direct effect of Dbf4 phosphorylation in SC disassembly would be an important addition.

Specific points:

1- Figure legends could be written with more detail to help the reader understand the different panels, specially the legend for Figure 2.
2- Figure 4A: Explain better what the graphs at the bottom of the panel represent in the figure legend.
3- Figure 4D: There is no apparent difference in the % of nuclei with polycomplex between the R83E and E86K/V mutants, while the same mutants show a clear difference in % of nuclei with linear SC tracks (Figure 4B)?
4- Model in Figure 4D. The finding that an interaction between Cdc5 and Dbf4 also affects DSB repair is not represented in the model.

Referee #3:

Summary
The manuscript from Bilge Argunhan et al identified a role of DDK (Dbf4+Cdc7) kinase complex in the prophase I to metaphase I transition of S. cerevisiae, required for dismantling of the SC, arrest of DSB formation, and relief of Rad51 HR repression. They show that DDK serves as the hub for signalling from CDC5 and CDC28, showing that the strength of this former interaction is important for function. This part of the paper is perhaps its most compelling with detailed mapping of the interaction sites and clever tethering experiments to prove that the physical interaction between these proteins is important for downstream events. The examination of the Dbf4 phosphorylation states and the connection to cdc28 is also well executed and deserving of publication in EMBO. Overall the experiments are well executed and interpreted and of significant interest. The manuscript, as written, however will appeal only to a very specialized audience. The logic of the experiments, the use of specific controls, the explanation of the figures, and the interpretation of results are often so cursory that only an aficionado can adequately evaluate the merit of aspects of the study. If this brevity is necessary to meet the needs of the EMBO Journal, then it behoves the authors to consider publication in a journal that will allow for more detailed explication of the experiments and results. Several egregious examples include: p 8, end of para 1 where the figure shows rad17Δ but not once is it explained in the text why this is used; the significance of the dmc1 ama1 double mutant; it is not stated in the text that the genetic background for most of the experiments is dmc1Δ.

Major comments
Experimentally, the only major issue the authors should address prior to publication is the nature of the poor spore viability. The authors imply, but never state, that the RAD51 dependent pathway that is ultimately used results in massive aneuploidy, presumably due to lack of crossovers. But, the authors do not report recombination frequencies to confirm that this is altered in the mutants (is it?).

Materials and methods are sparsely written and would be difficult to replicate.
Figure Legends 1 and 2 are particularly cursory and need further detail.
P values should be added to Figure 5B to be able to evaluate the extent of difference between +/- CDC% since the error bars overlap.

Minor Comments
Page numbers should have been included to facilitate editing

1. Introduction, p.3 paragraph 2. It is stated that "Sister chromatids condense and form chromosome axes". Chromosome axes are more likely formed by both chromatin and proteins (cohesins, Red1, etc). I find the statement confusing for a general audience and would suggest the author to precise that sister chromatids are organized around a proteinaceous axis.

2. p. 4, 4 lines from bottom: stated that "Production of CDC5 in prophase..." should read "during early stages of phophase I" since it is induced after pachytene but still in prophase I.

3. No methods describing the overexpression screen or the screen that identified E86V are provided

4. It appears that the screen was done in the dmc1Δ and hop1Δ backgrounds, but dmc1Δ is used

predominately throughout the rest of the text. Why?

5. Paragraph 3, p.7 The effect of the PDBF4-CDC5-DBF4 in a wild type background has not been stated has tested.

6. Figure legend 2B is incomplete and does not describe the right side of the figure. Also the nomenclature in this graph in 2B is confusing.

7. Figure 2B There is no explication for why CDC5 alone (3rd bar) can suppress dmc1Δ

8. Paragraph 1, p.7 In the statement "The cell cycle progression caused by mutations in DBF4", I assume the author mean "The cell cycle progression of dmc1Δ caused by mutations in DBF4". This should be clarified.

9. "Enforced" should be "forced"

10. "notably the R83E..." please add, "which did not suppress on its own,"...

11. Paragraph 1, p.8, In "Broken chromosomes were no longer repaired if the RAD51 gene was deleted", I am not sure if the authors tested the single rad51 mutant. I think the authors meant "if the RAD51 gene was deleted in a dmc1 mutant background." this sentence should be clarified.

12. Paragraph 2, p.8 The authors stated repaired by Rad51 but rather they should be more tenuous and state " by a Rad51-dependent pathway"

13. Paragraph 2, p.9 The authors refer to previous observations in a dmc1Δ ama1Δ double mutant. I think a short explanation of the known function of Ama1 would clarify their statement to a general audience.

14. Paragraph 2, p.10. In the statement "This uncoupling of SC destruction and DSB repair highlights the requirement for Rad51 in repairing DSBs that persist following destruction of the SC." and the following sentence, the authors omit to precise that this experiment is done in a dmc1 mutant background. Again, I think this is an important limit to the experiments that should be precise.

15. p. 11 Need to refer to the figure about SC lines in reference to dbf4-R83E

16. p11, paragraph 2, 1st sentence: providing the values for how much reduced broken chromosomes were would be useful.

17. It seems they are suggesting CDC5 has a role in feedback regulation of DSBs. If this is what they mean, they should suggest it directly.

18. Middle of paragraph, the sentence with reference to Valentin is awkward and the semicolon after the reference should be removed.

19. p12, first full paragraph. Authors need either tone down their interpretation or be more explicit how they reach conclusion that SC removes RAD51 inhibition, since this could be independent.

20. p 14, Paragraph 2, "neither led" should be "led neither"

21. p.14, Paragraph 3. I find confusing that the authors say "Next, we extended our analysis to prophase I" while they were already talking about prophase I, right?

22. Discussion: Authors should speculate more about the alternative HR pathway... are they suggesting sister vs homolog repair is different? (since Rad51 was not thought to have a role in sister repair). If this is an alternative HR pathway, why would it lead to reduced spore viability?

23. Discussion. In speculating about role of CDC5 in DSB attenuation, the authors should at least acknowledge the possibility that the progression in metaphase itself could function indirectly to turn

off DSBs and that it is not a direct function of the DDK. Or should point to their results that suggest this is a direct function of the DDK.

24. Discussion: the authors seem to be suggesting that they have discovered a new mode of HR-mediated repair in meiosis, yet they do not prove that the repair is via HR, as alternative Rad51 dependent pathways are known. In addition to the yeast references on Rad51 repair and Rad51 paralogues, there is precedence from C. elegans that late meiotic repair occurs and is distinct from HR-mediated CO or early sister repair in a requirement for RAD-50 (Hayashi et al 2007).

25. Figure 2 legend. When writing CDC5-dbf4-E83E fusion, I think they meant CDC5-dbf4-R83E.

26. Methods. Fluorescence polarization assay: Labeled not labelled--- please use spelling consistently throughout text.

27. Anchor Away assay: FRB should be written out at least once

1st Revision - authors' response 20 April 2017

Our concise response to six major points raised by the editor

Comments from the editor are shown in green and our responses are shown in black.

1. Referee 1 raises significant concerns regarding Dbf4/Cdc5 interaction before prophase I exit, which are crucial to address in the revised version.

We have presented evidence to support the notion that Dbf4 and Cdc5 interact before pachytene exit (stated as "prophase I exit" above). First, a small amount of Cdc5 already exists before pachytene exit (Figs 1B and EV2A). Second, pachytene exit is inherently "leaky", i.e., cells gradually exit pachytene even in the presence of small amounts of unrepaired DSBs. Combined with the fact that DSBs are gradually repaired even in the absence of Dmc1, cells will reach a point where the level of Cdc5 is steadily increasing as cells transition towards pachytene exit. Thus, the interaction strength between Dbf4 and Cdc5 can act as a critical effector that influences the progression of pachytene exit. A more thorough explanation of this can be found in the response to referee #1 point 3.

2. Do biochemical analysis of in vivo interaction between Dbf4/Cdc5 (Referee 1).

This was addressed by performing in vivo co-immunoprecipitation experiments from meiotic cells (Fig 1D). The result clearly demonstrated that the interaction between full length Dbf4 and Cdc5 was distinctly affected in vivo by our three mutations in *DBF4*: *dbf4-R83E*, *dbf4-E86K* and *dbf4-E86V*. Compared to the interaction of Cdc5 with wild type Dbf4, we observed a poor interaction with Dbf4-R83E and an enhanced interaction with Dbf4-E86K/V. This result mirrored our prediction based on the genetic behaviour of these mutants as well as the biochemically measured K_d values using Dbf4 peptides and the C-terminal half of Cdc5. A more thorough explanation of this can be found in the response to referee #1 point 5.

3. Test the physiological relevance of Dbf4 phosphorylation by Cdc5 as requested by Referee 2.

The physiological relevance of Dbf4 phosphorylation was originally demonstrated by using the *dbf4*$^{S374A,\ T375A}$ and *dbf4-4A* mutants, where phosphorylation of Dbf4 was substantially reduced. In both mutants, SC protein destruction following Cdc5 induction was inefficient, although in the *dbf4-4A* strain, this interpretation was obscured because of the instability of the Dbf4-4A protein. We solved this problem by generating a strain that overproduces Dbf4-4A. In this strain, a clear reduction in Red1 destruction following Cdc5 induction was observed (Fig EV7C), although admittedly, the defect was milder than before, possibly due to the dosage effect of Dbf4-4A. Nevertheless, our results collectively argue the importance of Dbf4 phosphorylation in regulating SC protein destruction.

4. Address the discrepancy between the regulation of synaptonemal complex degradation by Ccd5/Dbf4 in different genetic backgrounds as pointed out by Referee 1.

In the data shown in Fig 1B, only a subset of the *dbf4-E86K/V* cell population (~20%) escape meiotic cell cycle arrest. It is only in this fraction of the cell population that Cdc5 induction and the subsequent destruction of Red1 and Zip1 takes place. Therefore, the majority of the population remains arrested within pachytene, with high levels of Red1 and Zip1, meaning that a reduction in the amount of Red1 and Zip1 is masked by the abundance of Red1 and Zip1 in the remaining ~80% of cells. In the case of Fig 4A, the Cdc5 induction system is more controllable and robust, thus Cdc5 induction is more efficiently achieved throughout the population, leading to population-wide destruction of Red1 and Zip1. Hence, a clear decline in the total levels of these proteins is detectable by immunoblotting.

5. Include the data on the overexpression screen and Dbf4 mutant identification.

A better description of the screen was included within the Results section (page 7, paragraphs 1 and 2). Specific details on how the screen was conducted were added to the Materials and Methods (page 34, paragraph 4 onwards).

6. The data presentation in the manuscript should be adjusted to make it more accessible to the general audience of The EMBO Journal. We do not enforce strict manuscript length limitations, and in this case due to the complexity of experiments a clear presentation of the rationale and scientific background of the experiments is crucial.

We completely agree that a clear explanation of the rationale behind the experiments is required to facilitate reader understanding. The brevity in our initial manuscript was due to concerns regarding length limitations, but these concerns have been allayed. We have rewritten several parts of our manuscript to include more detail to improve clarity and better explain the reasoning behind our experimental design. These include but are not limited to: a more detailed introduction and clearer definition of the substages of meiotic prophase I (page 4, paragraph 2); the origin of the *DBF4* mutations used (page 7, paragraphs 2 and 3); and an explanation of the control strains employed in the Cdc5-Dbf4 fusion experiments (page 9, paragraph 3 onwards).

Point-by-point response

Comments from reviewers' are shown in blue and our responses are shown in black.

Referee 1

We are encouraged by this referee's impression that our "manuscript is well written and the presented data is abundant and clear", and we would like to thank them for their constructive criticisms. Having addressed many of the points they raised experimentally, we certainly feel that our manuscript has improved. Furthermore, this referee highlighted a few instances where they found our explanation to be unclear or insufficient. We have added further explanations and descriptions to facilitate the understanding of both specialist and nonspecialist audiences. On the rare occasion where we disagreed with this referee's interpretation, we have provided a detailed description outlining our reasoning.

Referee #1:
This manuscript focuses on the role of the Dbf4-dependent kinase (DDK) in prophase exit in budding yeast. Dbf4 was isolated in a screen for mutants that bypass the prophase I block of a repair-deficient mutant. Follow up analysis led to two interesting and novel findings. First, this work establishes a role for DDK downstream of Cdc5/Polo kinase in synaptonemal complex (SC) assembly. Second, data is also presented showing that destruction of the SC allows reactivation of DNA repair pathways mediated by Rad51. The manuscript is well written and the presented data is abundant and clear. Although the manuscript does not reveal the underlying mechanism by which DDK triggers SC disassembly, this is likely to involve a complicated interplay between multiple factors and is beyond the scope of the current study.

The main concern is that the authors argue that an interaction between Dbf4 and Cdc5 is important for prophase I exit, but the presented data and previous observations do not provide convincing evidence to make this conclusion. Crucially, as previously reported by the Zachariae lab, and demonstrated in this manuscript, Cdc5 and Dbf4 only co- exist after prophase I exit, therefore it is difficult to reconcile the model presented with the available data.

Points to be addressed.

1. Details of the screen in which Dbf4 was identified should be presented. While it is not necessary to present all "hits" in this screen, it would be useful to know the background that led to the identification of Dbf4.

We have expanded on the background of the screen in the main text (page 7, paragraphs 1 and 2) and added further details to the Materials and Methods (page 34, paragraph 4 onwards).

2. Similarly, no details as to the origin of the Dbf4 mutants are given in this study and it is not clear why the authors focused on these residues. This is central to the data presented and should be described in full.

We have added an in-depth description of the origin of the *DBF4* mutations that were employed in this study and emphasised why particular mutations were chosen.

Briefly, we explained how the *dbf4-E86V* mutation was isolated (page 7, paragraph 2). Furthermore, we described the previous study that biochemically characterized the Dbf4-Cdc5 interaction and isolated the *dbf4-R83E* and *dbf4-E86K* mutations (page 7, paragraph 3). We also updated Fig 1A to reflect how these mutations affect the Dbf4-Cdc5 interaction, while clearly stating that the *dbf4-E86V* mutation was isolated in “this study”. Finally, we explained that the *dbf4-4A* mutant was generated by systematically mutating Ser/Thr residues that are highly conserved among six species of the *Saccharomyces* genus (page 22, paragraph 2).

Additional details can also be found in the Materials and Methods (page 34, paragraph 4 onwards).

3. The major conceptual problem mentioned above refers to Figure 1. In (A) the authors demonstrate that dbf4-E86K and dbf4-E86V are able to induce Ndt80 transcription in a dmc1Δ mutant, indicating prophase I exit. However, dbf4-E83E cannot promote prophase I exit. Figure 1E and 1F demonstrate that these mutants differ in their ability to interact with Cdc5 in vitro: dbf4-E86K and dbf4- E86V enhance the interaction, while dbf4-E83E abolishes it. Based on this data, the authors argue that the Cdc5-Dbf4 interaction is important for prophase I exit. The problem, as reported in Matos et al., and shown also in Figure 1B is that Cdc5 is not present prior to prophase I exit. So the ability to promote prophase I exit cannot be explained by the strength of interaction with Cdc5. Perhaps the E86K, E86V and E83E mutations affect some other aspect of Dbf4 function or interactions. To start to address this point, the authors should determine whether Dbf4 can induce Ndt80 production, Zip1/Red1 destruction in cells with a meiotic depletion of Cdc5. Note that data presented in Figure 4 indicate that Dbf4 is required downstream of Cdc5 induction for SC disassembly (at least in the ndt80 background), however again, this need not be direct.

This is an astute observation. However, there are three lines of evidence that argue against this notion.

First, as shown in Fig 1B, Cdc5 is in fact present before prophase I exit (i.e., before the robust induction of Cdc5 and Ndt80 at the end of pachytene), but the levels of protein are relatively low. This point is demonstrated more clearly when comparing between strains where *CDC5* is expressed from its own promoter or from the *CLB2* promoter, which is expressed in vegetative cells but heavily downregulated during meiosis (this allele is known as *cdc5-md*, for meiotic depletion). Clearly, basal levels of Cdc5 protein are detectable before pachytene exit when expression is driven from the native *CDC5* promoter but not from the *CLB2* promoter (Figs 6B and EV2A).

Second, pachytene exit proceeds progressively, as meiotic DSBs are gradually repaired. This is because the recombination checkpoint monitoring the status of homologous recombination during prophase I is inherently "leaky", i.e., cells gradually exit pachytene even in the presence of small

numbers of unrepaired DSBs. This reflects the dynamic nature of meiotic cell cycle progression. Meiotic DSB formation is continuously induced up until pachytene exit, thus it is unlikely that cells are within pachytene and do not have any DSBs.

Third, the cell cycle progression effect brought about by *dbf4-E86K* in the *dmc1* mutant requires Cdc5 (Fig EV2A). We tested this possibility directly by completely depleting Cdc5 in *dmc1 dbf4-E86K* meiotic cells through the use of the *CLB2* promoter. As expected, when Cdc5 levels were completely undetectable within pachytene, the *dbf4-E86K* mutation was unable to induce pachytene exit and meiotic completion in the *dmc1* mutant background, as judged by the robust induction of Ndt80 and formation of spores, respectively (Fig EV2A).

It is known that even in the absence of Dmc1, meiotic DSBs are gradually repaired by Rad51 (Tsubouchi & Roeder, Dev Cell, 2003). Thus, as the checkpoint response is gradually dampened, which probably occurs when the majority of DSBs have been repaired and DSB numbers are relatively low, Ndt80 (and Cdc5) levels are starting to increase. Consequently, the amount of DDK-Cdc5 complexes gradually increases, and as cells make the commitment to exit pachytene, these DDK-Cdc5 complexes collaborate to promote efficient destruction of the SC, which leads to rapid repair of any remaining DSBs through a Rad51-dependent mechanism. Thus, the interaction strength between Dbf4 and Cdc5 can act as a critical effector that influences the progression of pachytene exit.

A more detailed description covering these concepts has now been included throughout the text (e.g., page 7, paragraph 1; page 32, paragraph 2).

4. Also, related to Figure 1. As the authors show later in the paper, Cdc5 and Dbf4 appear to collaborate to promote SC disassembly (Figure 4A). However, in Figure 1B (dbf4-E86K, E86V) both Dbf4 and Cdc5 are present but Red1 and Zip1 are both stable. How do the authors explain the discrepancy between the dmc1D mutant and the ndt80 block?

In the data shown in Fig 1B, only a subset of the *dbf4-E86K/V* cell population (~20%) escape meiotic cell cycle arrest, and it is only in these cells that Cdc5 is induced and the subsequent destruction of Red1 and Zip1 takes place. Therefore, the majority of the population remains arrested within pachytene, with high levels of Red1 and Zip1, meaning that a reduction in the amount of Red1 and Zip1 is masked by the abundance of Red1 and Zip1 in the remaining ~80% of cells. In the case of Fig 4A, the Cdc5 induction system is more controllable and robust, thus Cdc5 induction is more efficiently achieved throughout the population, leading to population-wide destruction of Red1 and Zip1. Hence, a clear decline in the total levels of these proteins is detectable by immunoblotting.

An explanation of why the majority of Red1 and Zip1 persists in the strains where Cdc5 is induced was described as follows (page 14, paragraph 1):

"These results contrast with our findings in the *dmc1* background, where we did not observe a clear reduction in the levels of Red1 and Zip1 despite *dbf4-E86K/V* suppressing pachytene arrest (Fig 1B). This is because only a subset of the population exits pachytene in the *dmc1Δ dbf4-E86K/V* strains; the decline in SC protein levels in this fraction of the population (~20%) is masked by the persistent SC proteins in the population of cells that remain arrested in pachytene (~80%)."

5. The interaction between Dbf4 and Cdc5 and the changes in this affinity in the Dbf4 point mutants in vitro are put forward as an explanation for many of the observations presented. However, evidence is not presented to show changes in complex formation in vivo. Co-immunopreciptation experiments of Dbf4-Cdc5 should be performed in different conditions/stages. This is particularly important for the mutants (E86K, E86V, R83E). Also, is there an increased interaction observed upon bypass of either dmc1 or ndt80 arrest?

Many thanks for emphasizing this important point. Unfortunately, the examination of interaction strength under bypass conditions is not possible due to the following technical reason. In the *dmc1* background, only *dbf4-E86K/V* are able to bypass the cell cycle arrest and robustly induce Cdc5. Since *DBF4* and *dbf4-R83E* are not able to efficiently induce the production of Cdc5 in the *dmc1* background, there is a huge disparity in the intracellular levels of Cdc5 between these strains. Employing such a condition for co-IP would greatly hinder any attempt at a fair comparison of the

Dbf4-Cdc5 interaction strength between different *DBF4* mutants. The cell cycle arrest of the *ndt80* mutant is not bypassed by *dbf4* mutants (Fig EV5A), thus the interaction cannot be examined in that background.

To address this point as fairly as possible, we employed the *cdc20-md* mutant background, where cells undergo a natural exit from prophase I and subsequently arrest before commitment to anaphase I (Matos et al., 2008, Cell). Thus, the cellular environment for Dbf4-Cdc5 complex formation should be comparable to that seen at the prophase I-metaphase I boundary during the natural progression through meiosis. To avoid any possibility of a tag interfering with the Dbf4-Cdc5 interaction, we tagged Cdc7, which is the catalytic partner of Dbf4 and does not co-immunoprecipitate (co-IP) with Cdc5 in the absence of Dbf4 (Matos et al., 2008, Cell). Cdc7 was then used as an anchor to co-IP Dbf4-Cdc5 during the prophase I-metaphase I boundary (Fig 1D).

These co-IP experiments explicitly show that the *dbf4-E86K/V* mutations increase the association of Cdc5 with Dbf4 during meiosis, whereas the *dbf4-R83E* mutation weakens the interaction between Cdc5 and Dbf4 during meiosis (Fig 1D). Although the levels of Cdc5 in the *dbf4-R83E* strain were slightly lower, likely due to a mild delay in meiotic progression, the co-IP of Cdc5 with Dbf4 was reduced even at a later time point (6.5 hrs) when Cdc5 levels had increased.

Taken together, these in vivo co-IP results involving full-length proteins validate our in vitro data (Figs 1C and EV2B) and indicate that *dbf4-E86K/V* enhances and *dbf4-R83E* weakens the interaction between DDK and Cdc5.

These new findings have been summarized in the main text (page 9, paragraph 2)

6. In Figure 2A. The induction of prophase I exit by Cdc5 under the control of the Dbf4 promoter occurs very late (indeed, Ndt80 production appears to precede it) and the effect is very minimal. This is possibly because Cdc5 is unstable in prophase I. Nevertheless, the difference between the DBF4 wild type control and R83E mutant is extremely small in this assay and this cannot be taken as a convincing argument that the interaction between Dbf4 and Cdc5 is important for enhanced progression.

While it is true that the enhancement of cell cycle progression shown in Fig 2A is rather subtle, the observation is reproducible and the difference in cell cycle progression becomes more substantial at a later time point, as shown in Fig 2B. This is likely due to a combination of factors, such as the instability of Cdc5 within pachytene (Okaz et al., Cell, 2012), as noted by this referee, and also the fact that the *DBF4* promoter is rather weak (Murakami & Keeney, Cell, 2014). Furthermore, several findings all point towards the importance of the Dbf4-Cdc5 interaction in cell cycle progression (Figs 1C, 1D, EV2B). We believe the findings presented in Fig 2A, which are strongly supported by the related findings in Fig 2B, constitute an important piece of data that supports this notion.

7. dbf4-E86K can bypass the dmc1D block in the absence of Cdc5 induction. Can it also bypass the ndt80D block without Cdc5 induction? Non-induced controls should be shown in Figure 4 to address this point.

As indicated in point 3 above, bypass of cell cycle arrest in *dmc1* requires Cdc5, so it is incorrect to say that *dbf4-E86K* can bypass the *dmc1* block in the absence of Cdc5 induction. Although there are numerous ways to suppress the *dmc1* block (e.g., Rad51 overproduction, deletion of the Rad51 inhibitor *HED1*, deletion of lateral element kinase *MEK1*), it is not possible to suppress the *ndt80* block because Ndt80 is a master transcription factor that is essential for meiosis. In the absence of Ndt80, ~300 genes that are required for completion of meiosis (e.g., chromosome segregation, spore wall formation etc.) are not upregulated. Nonetheless, as shown in Fig EV5A, we have shown that suppression by *dbf4-E86K/V* requires Ndt80. We could not observe any increase in the levels of Cdc5 in these strains.

8. Role of CDK. Evidence is presented in Figure 6 that Cdc5 cannot phosphorylate Dbf4 without CDK. This leads to the hypothesis that CDK may mediate the interaction between Dbf4 and Cdc5. This should be tested in vivo by co-immunopreciptation.

This is an interesting point that certainly warrants further investigation. To test the possibility that CDK-dependent phosphorylation of Dbf4 affects the Dbf4-Cdc5 interaction, we employed the *dbf4-4A* mutant, in which four Ser/Thr residues are mutated to Ala. The mutant protein shows a reduction in the level of phosphorylation similar to what was seen when Cdk1 was inactivated and Cdc5 was depleted. Since our data indicated that the Dbf4-4A protein had reduced stability (Fig 7A), we employed a strain where *dbf4-4A* was overproduced during meiosis and Cdc5 was induced during prophase I. We then directly IP'd Dbf4 and Dbf4-4A with an anti-Dbf4 antibody and determined the amount of Cdc5 that co-IP'd in each case (Fig EV7D).

In both wild type *DBF4* and *dbf4-4A* backgrounds, Cdc5 co-immunoprecipitated with Dbf4, although we noticed that the efficiency of Dbf4 IP was substantially reduced in the *dbf4-4A* background for unknown reasons: solubility of Dbf4-4A was comparable to that of wild type Dbf4. Thus, at least this level of reduction in Dbf4 phosphorylation does not seem to drastically affect the interaction between Dbf4 and Cdc5, although it is possible that the interaction is maintained through residual levels of phosphorylation not visible by western blotting, possibly contributed by Cdk1 and other kinases.

These results and possibilities were included in the main text (page 23 paragraph 2).

9. FACS analysis should be presented for the experiments shown in Figures 6F and EV3.

We presume the concern here is that premeiotic DNA replication might be affected by Cdc5 induction and/or DDK depletion from the nucleus by the anchor away system.
In the anchor away system (Fig 6F), full establishment of the SC was cytologically confirmed at the time point when either Cdc7 or Dbf4 started being depleted. This means the cell cycle stage is way past the completion of premeiotic DNA replication. In EV3 (currently corresponding to EV5F), Cdc5 was induced at 3.5 hrs after entry into sporulation medium. This is the time when most/all premeiotic DNA synthesis has been completed (Valentin et al., JBC, 2006; Murakami & Keeney, Cell, 2014), although it is possible that some replication activity was still ongoing. Thus, we acknowledge the possibility that induction of Cdc5 at 3.5 hrs could overlap temporally with the final stages of DNA replication, which, if hindered, could also explain the reduction in meiotic DSB formation. However, we reproducibly observed a similar but less pronounced reduction in DSB formation when Cdc5 was induced at 6 hrs, where the SC is fully established in wild type and thus, premeiotic DNA synthesis is completed, arguing that Cdc5 can suppress DSB formation independently of any potential negative effect on DNA replication.

These possibilities were included in the main text (page 17, paragraph 2).

Referee 2

We appreciate the enthusiasm and positivity of this referee and agree whole-heartedly with their assertion that understanding how cells traverse the prophase I-metaphase I boundary is an "important question for the fields of meiosis and chromosome biology". Moreover, we are grateful for their feedback on our manuscript and hope that the audience of this study will benefit from the changes we have made. Below, we have detailed our response to the major point and minor points raised by this referee.

Referee #2:
The transition from late meiotic prophase to metaphase I is marked by the resolution of recombination intermediates and by the disassembly of the synaptonemal complex (SC), a proteinaceus structure that is assembled between homologous chromosomes during early prophase. Understanding the mechanisms that regulate this key transition of the meiotic program is an important question for the fields of meiosis and chromosome biology. Previous studies demonstrated that Cdc5 (Polo-like kinase) is required both for SC disassembly and crossover formation, but how Cdc5 promotes these events remains largely unknown.

The manuscript by Argunhan et al. shows that an interaction between Cdc5 and DDK component Dbf4 promotes meiotic progression by inducing repair of recombination intermediates and destruction of SC components, in particular Red1. These conclusions are reached by using mutant

versions of Dbf4 that show different strengths of interaction with Cdc5, combined with a detailed analysis of DSB repair and Red1 degradation in these mutants, as well by a cytological analysis of SC disassembly. In addition, these experiments show that following SC disassembly, DSB repair depends on the activity of the Rad51 recombinase, which is suppressed at earlier stages to promote Dmc1-mediated DSB repair, suggesting a switch in the mode of DSB repair during late prophase that is regulated by Dbf4-Cdc5. Overall, the data shown in these experiments supports the conclusion of the authors that an interaction between Dbf4 ad Cdc5 promotes SC disassembly and DSB repair at late prophase. The second part of the manuscript investigates how phosphorylation of Dbf4 affects SC disassembly. The authors show that Dbf4 is phosphorylated by Cdc5 and Cdk1 and that removal of Dbf4 compromises destruction of SC components, confirming the functional relevance of Dbf4 in SC disassembly. Finally, the authors attempt to demonstrate that phosphorylation of Dbf4 is directly responsible to induce SC disassembly. They successfully identify 4 residues that are phosphorylated in Dbf4, but the functional analysis of phospho-dead mutant is complicated by the apparent instability of the mutant protein. Clearly demonstrating the functional relevance of Dbf4 phosphorylation in SC disassembly would be an important addition to the manuscript. Can the authors overexpress dbf4-4A to achieve normal levels of the mutant protein and then determine if SC disassembly is affected? Or investigate if phosho-mimetic mutations in the residues mutated in dbf4-4A promote SC disassembly?

The manuscript makes a strong case for the involvement of Dbf4 phosphorylation by Cdc5 and Cdk1 in regulating SC disassembly, but as mentioned above, a more clear demonstration of the direct effect of Dbf4 phosphorylation in SC disassembly would be an important addition.

This is a great suggestion. The physiological relevance of Dbf4 phosphorylation was originally demonstrated by using the *dbf4-4A*, *dbf4*$^{S374A, T375A}$, and *dbf4*^{S375A} mutants, where phosphorylation was reduced. In all three mutants, SC protein destruction following Cdc5 induction was inefficient; the severity of this SC destruction defect closely correlated with the extent to which phosphorylation was reduced (Figs 7A and 7B). However, in the *dbf4-4A* strain, this interpretation was obscured because of the instability of Dbf4-4A protein. To circumvent this problem, we generated a strain overexpressing Dbf4-4A and induced Cdc5 in pachytene-arrested cells. In this experimental system, the amount of Dbf4-4A was similar to, if not more than, Dbf4. Importantly, we still saw a delay in the Red1 destruction kinetics, although the delay was milder than initially observed, possibly due to over-dosage of Dbf4-4A (compare Dbf4 and Dbf4-4A at 6hrs in Fig EV7C). Taken together with the data in Fig 7B, where Dbf4 phosphorylation was reduced without an accompanying protein stability defect, these results collectively argue the importance of Dbf4 phosphorylation in regulating SC protein destruction.

These findings were described in the main text (page 23, paragraph 1).

Specific points:

1- Figure legends could be written with more detail to help the reader understand the different panels, specially the legend for Figure 2.

We have added more details to all of the figure legends, particularly for Fig 2.

2- Figure 4A: Explain better what the graphs at the bottom of the panel represent in the figure legend.

As mentioned above, all of the figure legends now contain more detail and we hope that the added information will facilitate reader understanding.

3- Figure 4D: There is no apparent difference in the % of nuclei with polycomplex between the R83E and E86K/V mutants, while the same mutants show a clear difference in % of nuclei with linear SC tracks (Figure 4B)?

Firstly, we apologize profusely as the original graph that was submitted as part of Fig 4C was mislabeled. This has been corrected and we hope that will clear up any future confusion. With that said, it is indeed true that the difference in Zip1 destabilization between *dbf4-R83E* and *dbf4-E86K/V* is more pronounced for chromosomally associated Zip1 than extrachromosomal Zip1

(polycomplexes). This is possibly because Zip1 requires rigid chromosomal axes to be associated with chromosomes, but the foundation of the axes is provided by Red1, which itself is promptly destabilized upon induction of Cdc5.

This possibility was included in the main text (page 14, paragraph 2).

4- Model in Figure 4D. The finding that an interaction between Cdc5 and Dbf4 also affects DSB repair is not represented in the model.

This concept of our study has been included in the model as Rad51-dependent HR to reflect the nature of the DSB repair, as seen in Fig 7C.

Referee 3

We are delighted by the very positive response of this referee and are happy that they think "the experiments are well executed and interpreted and of significant interest". We would like to thank this referee in particular for their encouraging words and rigorous evaluation of our manuscript. Finally, we are very grateful for the suggestions this referee has provided to make our manuscript more appealing to the general audience of EMBO J.

Referee #3:
Summary
The manuscript from Bilge Argunhan et al identified a role of DDK (Dbf4+Cdc7) kinase complex in the prophase I to metaphase I transition of S. cerevisiae, required for dismantling of the SC, arrest of DSB formation, and relief of Rad51 HR repression. They show that DDK serves as the hub for signalling from CDC5 and CDC28, showing that the strength of this former interaction is important for function. This part of the paper is perhaps its most compelling with detailed mapping of the interaction sites and clever tethering experiments to prove that the physical interaction between these proteins is important for downstream events. The examination of the Dbf4 phosphorylation states and the connection to cdc28 is also well executed and deserving of publication in EMBO. Overall the experiments are well executed and interpreted and of significant interest. The manuscript, as written, however will appeal only to a very specialized audience. The logic of the experiments, the use of specific controls, the explanation of the figures, and the interpretation of results are often so cursory that only an aficionado can adequately evaluate the merit of aspects of the study. If this brevity is necessary to meet the needs of the EMBO Journal, then it behoves the authors to consider publication in a journal that will allow for more detailed explication of the experiments and results. Several egregious examples include: p 8, end of para 1 where the figure shows rad17Δ but not once is it explained in the text why this is used; the significance of the dmc1 ama1 double mutant; it is not stated in the text that the genetic background for most of the experiments is dmc1Δ.

Major comments

Experimentally, the only major issue the authors should address prior to publication is the nature of the poor spore viability. The authors imply, but never state, that the RAD51 dependent pathway that is ultimately used results in massive aneuploidy, presumably due to lack of crossovers. But, the authors do not report recombination frequencies to confirm that this is altered in the mutants (is it?).

We thank this referee for their intelligent suggestion. Elucidation of the DSB repair pathway would certainly add to the mechanistic insight offered by our study. To test whether the DSB repair seen in *dmc1 E86V* and *dmc1 E86Vx2* strains results in interhomolog crossovers, we employed a physical assay utilizing the *HIS4-LEU2* hotspot. At a time point in meiosis where DSBs are partially/fully repaired in the two *dbf4-E86V* strains, interhomolog crossing over was found to be reduced ~2.5-fold compared to wild type cells with fully repaired DSBs (Figs EV4B-D). These results provide a reasonable explanation for the poor spore viability observed in both of the *dmc1 E86V* strains (Fig EV4A). We have now included an explanation of these results in the main text (page 12 paragraph 1).

Materials and methods are sparsely written and would be difficult to replicate. Figure Legends 1 and 2 are particularly cursory and need further detail.

We have added substantial details to the Materials and Methods as well as all figure legends, especially for Figs 1 and 2.

P values should be added to Figure 5B to be able to evaluate the extent of difference between +/- CDC% since the error bars overlap.

At the time when Cdc5 was induced, we noticed variability between duplicate cultures in the amount of DSBs that had formed (Fig 5B, ~90% broken chromosomes; Fig EV5D, ~70% broken chromosomes). When DSB levels were far below their peak, as in Fig EV5D, the induction of Cdc5 had a more obvious inhibitory effect on further DSB formation; this is seen more clearly when comparing the difference between ±Cdc5 in Figs 5B and EV5D. This variation between the two experiments was too big to reject the null hypothesis for the difference between ±Cdc5 conditions. Nevertheless, in an effort to better evaluate the effect of Cdc5 on DSB formation, Cdc5 was induced at a much earlier time point (3.5 hrs). Under this condition, further DSB formation, and the lack of it, would be more obvious. We conducted this experiment in triplicate cultures and found that the difference in DSB formation with or without Cdc5 induction was statistically significant (Fig EV5F). In order to portray the reproducibility of the former condition (6 hr induction), individual results of the duplicate experiments (rather than the average result) were included (Figs 5B and EV5D). Statistical analysis was included for the experiments where Cdc5 was induced at the 3.5 hr time point (Fig EV5F).

Our interpretation of the results obtained with the 6 hr induction condition, as well as our reasoning for employing the 3.5 hr induction condition, have now been clearly stated in the main text (page 16, paragraph 2 onwards).

Minor Comments
Page numbers should have been included to facilitate editing

1. Introduction, p.3 paragraph 2. It is stated that "Sister chromatids condense and form chromosome axes". Chromosome axes are more likely formed by both chromatin and proteins (cohesins, Red1, etc). I find the statement confusing for a general audience and would suggest the author to precise that sister chromatids are organized around a proteinaceous axis.

This suggestion has been implemented.

2. p. 4, 4 lines from bottom: stated that "Production of CDC5 in prophase..." should read "during early stages of phophase I" since it is induced after pachytene but still in prophase I.

This suggestion has been implemented.

3. No methods describing the overexpression screen or the screen that identified E86V are provided

In-depth descriptions of the screening procedures have been added to the Materials and Methods (page 34, paragraph 4 onwards).

4. It appears that the screen was done in the dmc1Δ and hop1Δ backgrounds, but dmc1Δ is used predominately throughout the rest of the text. Why?

We infer that this referee means *dmc1* and *hop2* backgrounds (not *hop1*). The two different backgrounds were employed because of the phenotypic differences between the BR1919 and SK1 strain backgrounds. The *dmc1* mutant, which has been extensively characterized for studying prophase I arrest caused by meiotic recombination defects in SK1 strains, does not induce efficient prophase I arrest in the BR1919 background, while *hop2* causes complete pachytene arrest even in BR1919 strains. A note of this has been made in the Materials and Methods to help readers (page 34, paragraph 2).

5. Paragraph 3, p.7 The effect of the PDBF4-CDC5-DBF4 in a wild type background has not been stated has tested.

We determined the spore viability of a wild type strain with and without this transgene integrated at an ectopic locus to be 98% and 99%, respectively (80 spores examined). This result suggests that expression of the Cdc5-Dbf4 fusion protein does not have any drastic affect on an otherwise wild type meiosis. This spore viability data has now been mentioned in the relevant section (page 9, paragraph 3).

6. Figure legend 2B is incomplete and does not describe the right side of the figure. Also the nomenclature in this graph in 2B is confusing.

What this reviewer has identified as being the right side of Fig 2B is actually part of Fig 2A. The two different data have now been rearranged so that it is more obvious that they belong to separate parts of the figure. The nomenclature in Fig 2B is now color-coded; this will hopefully make it easier for readers to interpret the data.

7. Figure 2B There is no explication for why CDC5 alone (3rd bar) can suppress dmc1Δ

Similar to how Dbf4 overproduction can suppress pachytene arrest, Cdc5 overproduction has been shown to suppress pachytene arrest (Acosta et al., 2011, Mol. Biol. Cell.). Here, we showed that this suppression can be achieved by expressing Cdc5 from the *DBF4* promoter, but only in a genetic background where Cdc5 can interact with Dbf4 (i.e., not in *dbf4-R83E*). An explanation of this was added to the text, along with the reasoning behind why several of the strains in Fig 2 were employed (page 9, paragraph 3 onwards). We hope this genetic data will now be easier to interpret, especially for non-specialist readers.

8. Paragraph 1, p.7 In the statement "The cell cycle progression caused by mutations in DBF4", I assume the author mean "The cell cycle progression of dmc1Δ caused by mutations in DBF4". This should be clarified.

This suggestion has been implemented.

9. "Enforced" should be "forced"

This suggestion has been implemented.

10. "notably the R83E..." please add, "which did not suppress on its own,"...

This suggestion has been implemented.

11. Paragraph 1, p.8, In "Broken chromosomes were no longer repaired if the RAD51 gene was deleted", I am not sure if the authors tested the single rad51 mutant. I think the authors meant "if the RAD51 gene was deleted in a dmc1 mutant background." this sentence should be clarified.

This suggestion has been implemented.

12. Paragraph 2, p.8 The authors stated repaired by Rad51 but rather they should be more tenuous and state " by a Rad51-dependent pathway"

This suggestion has been implemented.

13. Paragraph 2, p.9 The authors refer to previous observations in a dmc1Δ ama1Δ double mutant. I think a short explanation of the known function of Ama1 would clarify their statement to a general audience.

A short explanation of the roles of Ama1 in meiosis has been included to clarify our comments about the *dmc1 ama1* double mutant (page 13, paragraph 1). We hope that the general audience of EMBO J will find this explanation useful.

14. Paragraph 2, p.10. In the statement "This uncoupling of SC destruction and DSB repair highlights the requirement for Rad51 in repairing DSBs that persist following destruction of the SC." and the following sentence, the authors omit to precise that this experiment is done in a dmc1 mutant background. Again, I think this is an important limit to the experiments that should be precise.

We have now added a clear statement that these experiments were conducted in a *dmc1* mutant (page 16, paragraph 1).

15. p. 11 Need to refer to the figure about SC lines in reference to dbf4-R83E

The corresponding figure was cited as suggested. In addition, we numbered each column in Fig 4D and included references to the exact data to further facilitate reader understanding (page 15, paragraph 2).

16. p11, paragraph 2, 1st sentence: providing the values for how much reduced broken chromosomes were would be useful.

The actual numbers have been included in the main text to emphasize the differences (page 16, paragraph 2).

17. It seems they are suggesting CDC5 has a role in feedback regulation of DSBs. If this is what they mean, they should suggest it directly.

We have suggested this possibility directly, while also mentioning the limitations of this experiment (as suggested by Reviewer #1 point 9; page 17, paragraphs 2 and 3).

18. Middle of paragraph, the sentence with reference to Valentin is awkward and the semicolon after the reference should be removed.

This section of the text has been rewritten in the revised manuscript to improve clarity.

19. p12, first full paragraph. Authors need either tone down their interpretation or be more explicit how they reach conclusion that SC removes RAD51 inhibition, since this could be independent.

We have toned-down our conclusion as suggested. That particular part now reads: "Taken together, we conclude that the upregulation of Cdc5 upon pachytene exit is sufficient to drive SC destruction, which coincides with unshackling of the mitotic recombinase Rad51" (page,17 paragraph 3)

20. p 14, Paragraph 2, "neither led" should be "led neither"

This suggestion has been implemented.

21. p.14, Paragraph 3. I find confusing that the authors say "Next, we extended our analysis to prophase I" while they were already talking about prophase I, right?

In the previous paragraph, we were describing our results in the *cdc20-md* background (metaphase I-arrested cells; Fig 6A). We then employed the *ndt80* background (pachytene-arrested cells i.e., prophase I; Figs 6B and 6C). This part of the text has now been changed to “Next, we examined pachytene-arrested cells by introducing the *ndt80Δ* mutation”. We hope this statement will avoid any further confusion (page 19, paragraph 3).

22. Discussion: Authors should speculate more about the alternative HR pathway... are they suggesting sister vs homolog repair is different? (since Rad51 was not thought to have a role in sister repair). If this is an alternative HR pathway, why would it lead to reduced spore viability?

We interpret this comment as meaning that this referee would like us to include more discussion on the second mode of HR that we have proposed, which is referred to as the “alternative HR pathway” in their comment. During meiosis, it is thought that Rad51 plays a relatively minor role in directly promoting crossover formation between homologs, which is preferentially performed by Dmc1.

Indeed, several mechanisms exist to limit the direct contribution of Rad51 to HR, including the existence of SC proteins such as Red1. Furthermore, recent publications have suggested that the meiotic role of Rad51 is solely to assist Dmc1. Our findings suggest that as cells make the exit from pachytene, when SC proteins are targeted for destruction by DDK-Cdc5-CDK, Rad51 inhibition is attenuated and persisting DSBs are repaired. We have now provided evidence in the revised manuscript (as suggested in the major point of this referee) that this Rad51-dependent repair does not lead to efficient crossover formation (Figs EV4B-D), suggesting that it resembles a mitotic mode of HR. Presumably, this reduction in crossovers represents a reduction in the number of physical interhomolog linkages, which are required for correct chromosome segregation, thus leading to aneuploidy and loss of spore viability (Fig EV4A). We have expanded our discussion to better describe our interpretation of the relevant data (page 30, paragraph 3 onwards).

23. Discussion. In speculating about role of CDC5 in DSB attenuation, the authors should at least acknowledge the possibility that the progression in metaphase itself could function indirectly to turn off DSBs and that it is not a direct function of the DDK. Or should point to their results that suggest this is a direct function of the DDK.

In the experiments that were conducted to test whether Cdc5 has a role in attenuating DSB formation (Figs EV5E and EV5F), Cdc5 production was induced through the *GAL* promoter during early prophase I in the *ndt80* mutant background. This means cells cannot progress beyond pachytene, and from the ~300 genes that comprise the Ndt80 regulon, only Cdc5 is upregulated. Thus, we can confidently say that the attenuation in DSB formation observed in our experimental system is solely due to the untimely production of Cdc5 before the prophase I-metaphase I transition. However, we acknowledge the possibility that, in wild type cells, other Ndt80-dependent/-independent factors may contribute to this Cdc5-driven downregulation of DSB formation during the prophase I-metaphase I boundary. This possibility has now been stated in the text (page 29, paragraph 1). Regarding the potential involvement of DDK, it was recently shown that DDK collaborates with Cdc5 and CDK to regulate the resolution of recombination intermediates during mitosis (Princz et al., 2017, EMBO J), supporting the notion that these three fundamental cell cycle kinases likely regulate multiple events during both mitosis and meiosis. Thus, we feel that our speculation that attenuation of DSB formation and regulation of meiotic recombination intermediate resolution by Cdc5 may involve DDK is a reasonable one that does not require modification.

24. Discussion: the authors seem to be suggesting that they have discovered a new mode of HR-mediated repair in meiosis, yet they do not prove that the repair is via HR, as alternative Rad51 dependent pathways are known. In addition to the yeast references on Rad51 repair and Rad51 paralogues, there is precedence from C. elegans that late meiotic repair occurs and is distinct from HR-mediated CO or early sister repair in a requirement for RAD-50 (Hayashi et al 2007).

In regards to the repair pathway, we have presented evidence that low levels of Rad51-dependent crossovers form between homologs (Fig EV4C and EV4D), strongly suggesting that the mechanism responsible for repairing DSBs involves HR. We regret not having cited the *C. elegans* paper in the previous version of the manuscript. The study has been cited with more in-depth discussion in our revised manuscript (page 31, paragraph 3).

25. Figure 2 legend. When writing CDC5-dbf4-E83E fusion, I think they meant CDC5-dbf4-R83E.

This mistake has been corrected.

26. Methods. Fluorescence polarization assay: Labeled not labelled--- please use spelling consistently throughout text.

The manuscript has been checked to ensure consistent spellings throughout.

27. Anchor Away assay: FRB should be written out at least once

This suggestion has been implemented and a more detailed explanation of the experimental system has been included (page 21, paragraph 1).

2nd Editorial Decision 18 May 2017

Thank you for submitting a revised version of your manuscript. The manuscript has now been seen by all original referees, who find that their main concerns have been addressed. There remain only a few minor issues that have to be dealt with before formal acceptance of the manuscript.

1. Please incorporate the minor textual changes suggested by the referees #1 and #3.
2. Statistics: in several figures (Figures 1B, 2A, 3A, 3B, 4B, 4C, 5B, 5D, EV4D) it is stated that data is represented as a mean +/-SEM from two experiments. In duplicate experiments use of error bars and statistical tests can be misleading and can suggest a false level of significance. Please remove the error bars and statistical analysis information accordingly.
3. Please add titles for the following sections: Conflict of interest, Figure legends and EV Figure legends.
4. We normally publish up to five EV figures, therefore I would suggest to move two EV figures that are less central to the manuscript flow to the Appendix.
5. Please submit Appendix as a pdf file.
6. We generally encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. We would need one file per figure (which can be a composite of source data from several panels) in jpg, gif or PDF format, uploaded as "Source data files". The gels should be labelled with the appropriate figure/panel number, and should have molecular weight markers; further annotation would clearly be useful but is not essential. These files will be published online with the article as supplementary "Source Data". Please let me know if you have any questions about this policy.

Finally, papers published in The EMBO Journal include a 'Synopsis' to further enhance discoverability. Synopses are displayed on the html version of the paper and are freely accessible to all readers. The synopsis includes a short introductory paragraph - written by the handling editor - as well as 2-5 one-sentence bullet points that summarise the paper and are provided by the authors. Please send us your suggestions for bullet points and a synopsis image. This image should provide a rapid overview of the question addressed in the study, but still needs to be kept fairly modest, since the image size cannot exceed 550x400 pixels.

Please let me know if you have any further questions regarding this or any previous points. You can use the link below to upload the revised version.

Thank you again for giving us the chance to consider your manuscript for The EMBO Journal. I am looking forward to seeing the final version.

REFEREE REPORTS

Referee #1:

The authors have addressed my concerns with the addition of new experiments and more detailed arguments in the text. The manuscript is suitable for publication, following one minor change:

- The first sentence of the discussion should be modified because, while this work presents the importance of an interplay between 3 kinases, the "mechanism" still needs to be uncovered. Therefore "we uncovered the mechanism whereby three major cell cycle kinases....coordinate to dismantle the SC" is an over-statement. Similarly the last sentence of this paragraph should be toned down.

Referee #2:

The authors have addressed the main issues that I raised on the previous version, therefore I support

publication of the manuscript.

Referee #3:

The revised manuscript from Argunhan on the interactions between DDK, Polo, and CDK1 in regulating pachytene progression and SC disassembly is highly improved and appears to adequately address both my concerns and those of all of the other reviewers. The writing is significantly improved with sufficient detail in the methods and figure legends. The paper is now accessible to a broader audience and warrants publication in EMBO Journal.

Minor issues to address:

Page 5 This sentence does not make sense: "However, unlike Cdc5, whose production is downregulated before pachytene exit (Okaz et al., 2012), DDK is believed to function primarily before pachytene exit."

p. 12 "This low spore viability combined with the requirement for Rad51" this is directly refer to Figure 3A

p17 "These results suggest that Cdc5 acts during the prophase I-metaphase I transition to shut-off meiotic DSB formation." It would be worth including the recent Colaiacovo paper in the discussion of this topic (p. 29??) since this that papers shows that a PLK-dependent phosphorylation events of the SC feeds back to restrict DSBs in C. elegans. Two other worm papers, Machovina et al 2016 also showed a requirement for plk-2 in crossover feedback to regulate DSB competency and SC stabilization that should be noted.

p. 27 'The demonstration that the catalytic activity of Cdc5, DDK and Cdc28 is" should be "catalytic activities....are..."
"Congruently, it has been reported" I think congruently is used incorrectly and should read "Consistently"

2nd Revision - authors' response 31 May 2017

Response to the editor.

1. Please incorporate the minor textual changes suggested by the referees #1 and #3.

Referees' suggestions have been incorporated as detailed in the response letter.

2. Statistics: in several figures (Figures 1B, 2A, 3A, 3B, 4B, 4C, 5B, 5D, EV4D) it is stated that data is represented as a mean +/-SEM from two experiments. In duplicate experiments use of error bars and statistical tests can be misleading and can suggest a false level of significance. Please remove the error bars and statistical analysis information accordingly.

Error bars and statistical analysis have been removed in the enlisted figures. Descriptions regarding them have been removed from the main text.

3. Please add titles for the following sections: Conflict of interest, Figure legends and EV Figure legends.

Titles for the above sections have been added.

4. We normally publish up to five EV figures, therefore I would suggest to move two EV figures that are less central to the manuscript flow to the Appendix.

Figs EV3 to EV7 have been renamed as Figs EV1 to EV5, and Figs EV1 and EV2 are now Appendix Figs S1 and S2.

5. Please submit Appendix as a pdf file.

The Appendix file was prepared.

6. We generally encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. We would need one file per figure (which can be a composite of source data from several panels) in jpg, gif or PDF format, uploaded as "Source data files". The gels should be labeled with the appropriate figure/panel number, and should have molecular weight markers; further annotation would clearly be useful but is not essential. These files will be published online with the article as supplementary "Source Data". Please let me know if you have any questions about this policy.

Source data to key results have been prepared for publication. Source data for other results have been included as much as possible.

Finally, papers published in The EMBO Journal include a 'Synopsis' to further enhance discoverability. Synopses are displayed on the html version of the paper and are freely accessible to all readers. The synopsis includes a short introductory paragraph - written by the handling editor - as well as 2-5 one-sentence bullet points that summarise the paper and are provided by the authors. Please send us your suggestions for bullet points and a synopsis image. This image should provide a rapid overview of the question addressed in the study, but still needs to be kept fairly modest, since the image size cannot exceed 550x400 pixels.

A synopsis image and a file with 5 one-sentence bullet points have been prepared.

Response to referees' comments.

Referee #1:

The authors have addressed my concerns with the addition of new experiments and more detailed arguments in the text. The manuscript is suitable for publication, following one minor change:

- The first sentence of the discussion should be modified because, while this work presents the importance of an interplay between 3 kinases, the "mechanism" still needs to be uncovered. Therefore "we uncovered the mechanism whereby three major cell cycle kinases....coordinate to dismantle the SC" is an over-statement. Similarly the last sentence of this paragraph should be toned down.

The relevant text has been modified and the conclusions have been toned-down. It now reads as follows:

P.25, line 2 ~
"In this work, we demonstrated that three major cell cycle kinases, DDK, Polo, and CDK1, coordinate to dismantle the SC, a meiosis-specific chromosomal structure, at the prophase I-metaphase I transition"

P.25, line 12 ~
"Our findings shed light on how SC destruction is temporally coordinated with the cell cycle and point towards the existence of a change in the mode of HR to promote faithful chromosome segregation and reinforce gamete viability."

Referee #2:

The authors have addressed the main issues that I raised on the previous version, therefore I support publication of the manuscript.

Thanks a lot.

Referee #3:

The revised manuscript from Argunhan on the interactions between DDK, Polo, and CDK1 in regulating pachytene progression and SC disassembly is highly improved and appears to adequately address both my concerns and those of all of the other reviewers. The writing is significantly improved with sufficient detail in the methods and figure legends. The paper is now accessible to a broader audience and warrants publication in EMBO Journal.

Minor issues to address:

Page 5 This sentence does not make sense: "However, unlike Cdc5, whose production is downregulated before pachytene exit (Okaz et al., 2012), DDK is believed to function primarily before pachytene exit."

p.5, line 18 ~
That particular sentence was changed. It now reads:
“However, unlike Cdc5 which functions after pachytene exit (Okaz et al., 2012), DDK is believed to function primarily before pachytene exit.”

p. 12 "This low spore viability combined with the requirement for Rad51" this is directly refer to Figure 3A

Referral to Figure 3A was added (p.12 line 6)

p17 "These results suggest that Cdc5 acts during the prophase I-metaphase I transition to shut-off meiotic DSB formation." It would be worth including the recent Colaiacovo paper in the discussion of this topic (p. 29??) since this that papers shows that a PLK-dependent phosphorylation events of the SC feeds back to restrict DSBs in C. elegans. Two other worm papers, Machovina et al 2016 also showed a requirement for plk-2 in crossover feedback to regulate DSB competency and SC stabilization that should be noted.

The first publication (Colaiacovo) was cited in the discussion, in the context of possible involvement of a polo kinase in DSB regulation (p.29, line 3~).
The second publication (Machovina) was also cited in the discussion, in the context of involvement of a polo kinase in SC destruction (p.26, line 1 ~).

p. 27 'The demonstration that the catalytic activity of Cdc5, DDK and Cdc28 is" should be "catalytic activities....are..."
"Congruently, it has been reported" I think congruently is used incorrectly and should read "Consistently"

These suggestions were implemented accordingly.

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PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Hideo Tsubouchi
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2016-95895

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	We followed common practices of the field, and feasable and practical numbers were chosen for analysis.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	N/A
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	N/A
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	N/A
For animal studies, include a statement about randomization even if no randomization was used.	N/A
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	no
4.b. For animal studies, include a statement about blinding even if no blinding was done	N/A
5. For every figure, are statistical tests justified as appropriate?	Statistical methods employed were specified in the figure legends.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Chi square test was used in Fig 4D , which is a common test for comparing two propotions.
Is there an estimate of variation within each group of data?	N/A
Is the variance similar between the groups that are being statistically compared?	N/A

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Catalog numbers and sources with citations were specified for used antibodies, which can be found in the materials and methods section in the main text and the Appendix.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	N/A

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

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	All the strain details were described in the materials and methods section in the main and appendix documents.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	N/A
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	N/A

E- Human Subjects

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

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	N/A
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	N/A
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	N/A