Stabilization of the metaphase spindle by Cdc14 is required for recombinational DNA repair

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1st Editorial Decision 04 January 2016

Thank you for submitting your manuscript on Cdc14 roles in DSB repair in budding yeast to The EMBO Journal. I apologize for the delay in getting back to you with a decision, owed to the end-of-the-year holiday break. We have now received comments from three expert referees, which I am copying below for your information. As you will see from these comments, all referees appreciate the overall interest of the topic and the potential importance of several of your present findings.

However, a majority of reviewers is not convinced that the various new observations come together to provide a sufficiently coherent and conclusive picture on the role of Cdc14 and SPBs in DSB repair, to already make the study a compelling candidate for EMBO Journal publication at this stage.

In this respect, the major specific concerns of referees 1 and 3 relate to key experiments not being sufficiently conclusive and not all data being highly convincing, but also to significant discrepancies of some of the present results and conclusions with a body of previously published work and our current knowledge of Cdc14 and SPB regulation. In addition, the main conceptual concern -how the current findings may be integrated with the current understanding on DSB relocalization and its functional importance- is also echoed in the comments and questions of the overall more positive reviewer 2. In light of these well-taken concerns, I am afraid we had to conclude that the study remains still too preliminary to presently warrant concrete further considerations for publication in The EMBO Journal. In any case, I would like to thank you for having had the opportunity to consider this work. I am sorry that I cannot be more positive in light of these evaluations at this point, but nevertheless hope that you will find our referees' detailed comments and suggestions helpful for improving and proceeding further with this study.
REFEREE REPORTS

Referee #1:

Stabilization of the metaphase spindle by Cdc14 is required for recombinational DNA repair

By Villoria et al.

This manuscript analyses the function of budding yeast Cdc14 in DNA repair.

Cdc14 is a conserved dual specificity phosphatase. The budding yeast CDC14 is an essential gene, is entrapped in the nucleolus during most of the cell cycle, is released from the nucleolus with anaphase onset by the actions of the FEAR and MEN pathways and then dephosphorylates substrates that previously have been phosphorylated by cyclin dependent kinase Cdk1. Substrate dephosphorylation by Cdc14 in anaphase/cytokinesis follows a defined program that ensures the correct order of events and finally also inactivation of Cdk1-Clb2 to reset the cell cycle to Start. Recent data indicated a role of yeast Cdc14 in DNA repair. Two papers in Mol Cell have shown that Cdc14 dephosphorylates the nuclease Yen1. This regulation is important for the activity of the nuclease and its cellular localization. Human cells encode hCDC14A and hCDC14B. The function of these hCDC14 paralogues is muss less understood but data suggest that they may have a role in DNA repair.

This paper by Villoria et al. now analyses the role of budding yeast Cdc14 in DNA repair. First, they show convincingly that conditional lethal mutants of CDC14 are super-sensitive towards DNA damaging drugs at a semi-permissive temperature. They next suggest that cdc14-1 cells have defects in DSB repair. Moreover, they show convincingly that a fraction of Cdc14 becomes released from the nucleolus via FEAR and dephosphorylates the SPB component Spc110. The authors suggest that the DSB somehow becomes recruited to the SPB via microtubule action. Finally, they show that phospho-inhibitor spc110 S36-91A cells have defects in DSB recruitment close to the SPB and in inter-chrom. repair.

The model of DNA damaged release of Cdc14 from the nucleolus is attractive and the data on the dephosphorylation of Spc110, a role of CDC14 in repair pathways and the spc110 S36-91A are convincing. These data suggest that the authors may have a story worth publishing in EMBO J.

However, the manuscript also has a number of problems: Lack of statistics; Southern blots were only performed twice; the spc110-220 experiment does not tell much; the DSB is not at SPBs it is close to it; the effect of the DSB on astral microtubules is unclear. Importantly, the critical spc110 S36-91A experiment (Fig. 6) lacks a complementation control (Southern blot only done twice).

The data presented here are also not in line with published data. Ling and Wang reported that the Chk1 pathway prevents FEAR pathway-dependent Cdc14 release in the presence of DNA damage. The paper by S. Gasser (Mol Cell) suggest that DSB bind in S and G2 phase via Mps3 to the nuclear envelope. Thus, the DSB signal is at the nuclear envelope and not 400 nm away from it as shown here. Mps3 is at the SPB and this Mps3 pool may bind the DSB to the SPB - this would be in line with this paper. However, the Gasser paper does not present evidence for SPB association of DSB. The N-terminus of Mps3 that binds DSB is not essential for SPB duplication - so it is easy to test whether Mps3 binds DSB to SPBs. Moreover, the Huisman et al paper does not describe an enhanced SPB velocity in spc110 S36-91A cells although they carefully analyzed spindle behavior.

What to suggest? The authors have to make their story more convincing by providing statistics, complementation of phenotypes, analyzing DSB with LacO arrays as done by Gasser (to compare), analyze the localization of DSB in ΔN-mps3 cells.

Specific points:
1. Fig. 1: the authors should explain the genomic situations of the mating type locus better (Fig. 1B). Comparing B with C do not allow me to judge the size of the DNA fragments in the Southern blot. Moreover, experiments in C and D were only done twice. The authors give SD from two experiments which does not make sense. The differences between Wt and cdc14-1 are relatively small. Sure, with two experiments statistical analysis is missing. I wonder whether the differences
are significant. The authors have to repeat the experiment at least another time in order to get a clear answer. Same for Fig. 1E.

2. Fig. 2A: The localization data for Cdc14-YFP are of insufficient quality. I am not convinced that Cdc14-YFP is at the SPB. Please show phase contrast; Cmn67 is normally only at SPBs - here the cell is full with Cmn67 signal (see Fig. 3B for how it should look like); Cdc14 also associates with kinetochores that cluster at SPBs; controls (only YFP, Cdc14-YFP without DSB) are missing. Why would Cdc14 be at SPBs in Raf (40%) in a pre-anaphase cell? This contradicts any publication of the last 20 years on the localization and cell cycle behaviour of Cdc14. A control, no DNA damage, is missing in Fig. 2C. Authors should show enlargements of the Cdc14-GFP signal. The authors should perform SIM analysis to discriminate SPB from kinetochore localization of Cdc14-YFP.

3. Husiman et al. does not describe astral microtubules phenotypes. It describes phenotypes of the nuclear microtubules. The lack of astral microtubules in Fig. 3A does not fit together with a regulation of Spc110. Spc110 localizes at the nuclear side of the SPB were it is organizing the nuclear microtubules. The protein Spc72 takes over the function of Spc110 at the cytoplasmic side of the SPB. Phosphorylation of Spc110 affects nuclear microtubules but not astral microtubules.

Thus, the phenotypes described here do not fit together with the dephosphorylation of Spc110 by Cdc14. It is important to check astral microtubule length without DSB. Do Wt and cdc14-1 cells have similar aMT lengths? It could be that the copy number of the GFP-TUB1-TRP1 affects microtubule behaviour (how was it checked?).

4. Fig. 3C: the Ddc2 signal is 500 nm away from SPBs. Most likely this is simply a reflection of the interaction of the CEN3 with SPB-organized nuclear microtubules. The authors should measure the distance of the MAT locus (lacO arrays) from SPBs. D is problematic because the authors measure max. projection and distance of 16 Nuf2 signals to Ddc2 (nicely demonstrated in Fig. 3E).

5. Line 217: the authors talk about SPB integrity (also in other parts of the paper). What does this mean? Nocodazole does not affect the integrity of SPBs.

6. The authors suddenly use spc110-220 mutant cells. As T. Davis has shown, spc110-220 cells have defective SPBs and nuclear organization. These cells accumulate intranuclear microtubule organizers that disrupt nuclear architecture. Thus, everything breaks loose! This figure belongs to the Supplement.

7. Fig. 6A shows the analysis of spc110 S36-91A cells - this makes perfect sense. The authors have to show that the spc110 S36-91A phenotype is complemented by Wt SPC110. Fig. 6C: statistics?

Minor points

1. The authors should test whether just Gal addition without DSB formation is inducting this Net upshift. Galactose dramatically changes the physiology of cells. It is important to control for this at some point in this paper.

2. The conclusion on p.9, middle (line 219-221) is not logical: Nup84 is not at the SPB. Only a fraction of Mps3 is at the SPB.

3. Fig. 3B: are the bars SD or s.e.m? The authors should provide statistical analysis.

Referee #2:

In this manuscript, Villoria and colleagues explore a new role for Cdc14 in tethering DSB to the SPB and in promoting HR. They initially show that Cdc14 contributes to viability of cells exposed to different DNA damaging agents. They further show that repair of a single HO cut in different contexts (intra or inter-chromosomal) is compromised in a cdc14-1 mutant. Their results indicate that Cdc14 acts downstream the DNA damage checkpoint and likely not at the level DSB resection. Strikingly, they find that Cdc14, in addition to its strong nucleolar localization, appears as foci that colocalize with the SPB after generation of single DSB indicating that Cdc14 is present at the SPBs and is part of the DDR. Consistent with these observations, they show that the FEAR is activated by the HO cut. By screening proteins being hyper-phosphorylated after DNA damage in the context of the cdc14-1 ts allele, they identify the spindle pole body protein Spc110 as one of the target of Cdc14 (among many others). Supporting these results, they provide direct evidence that Spc110 is phosphorylated after HO induction and that Cdc14 actively dephosphorylates Spc110 during the DNA damage response. Along the same line, they further show that inactivation of Cdc14 impairs astral microtubule formation and increases oscillatory SPBs movements during the DDR. Remarkably, they show quite convincingly that under the same experimental conditions, the DSB marked by Ddc2 foci is also recruited to SPBs and that the proximity of the DSB with the SPB is compromised in cdc14-1 cells suggesting that Cdc14 contributes to tether the DSB to SPBs. They
rule out indirect effects such as chromatin compaction as an explanation for this new function of Cdc14. Finally, the importance of the phosphorylation state of Spc110 in this process is attested by the fact that the Cdk phospho-deficient spc110S36-91A mutant exhibits defects in localizing the DSB to SPBs and in repairing the DSB. In parallel, similar results were found with a ts allele of Spc110 or by treating the cells with nocodazole.

In summary, this works reveals a new function of the Cdc14 phosphatase in the repair of DSB that is correlated to the relocation of Cdc14 to the SPB, dephosphorylation of Spc110 and stabilization of the metaphase spindle.

This is a great work with many new observations that have to be integrated in the general context of persistent DSB that have been shown to be relocated either to the NPC or the nuclear membrane protein Mps3. Beyond the new function of Cdc14, this work has the very strong merit to correlate new aspects of relocation of DSB with functional repair assay of DSBs. The experiments are of high quality and well presented. I have very few concerns about the results. Maybe the only relative weakness of the MS is the discussion. I list here some questions that may help to improve the discussion.

**Results**

1) At the experimental level, Ddc2-RFP is used as a marker for DSB. One could ask whether monitoring the colocalization between the DSB and SPB with a tagged HO locus would give the same results?

2) One the open question is the connection between the canonical DNA damage and the Cdc14 related events describes in this work. Does the rad53K227A affects the DSB/SPB interaction?

**Discussion**

1) Work from the Jaspersen's group shows that the SUN protein Mps3 is required for spindle pole body insertion into the nuclear membrane and SPB duplication (Friederichs et al. PLOS genetics, 2011). Therefore, to what extend, the relocation to DSB to SPB is related to the relocation of persistent DSB to Mps3 described by the Gasser and Peterson groups. This point is poorly discussed.

2) Cdc14 is a phosphatase known to counteract Cdk phosphorylation in normal cell cycle. The authors should better discuss how are Cdk and Cdc14 activities connected to the canonical response to DNA damage.

3) The authors propose that Cdc14 is localized at the SPBs to dephosphorylate Spc110 in response to DNA damage. However the results show that both phosphorylation and dephosphorylation of Spc110 are important for the DSB-SPB interaction, stabilization of the metaphase spindle, and HR. Therefore, the results suggest that during the DNA damage response, a tight regulation of phosphorylation and dephosphorylation of Spc110 mediated by Cdk and Cdc14, respectively, promotes DSB-SPB interaction and HR. This point should be better discussed.

4) Could the authors comment why stabilization of the metaphase spindle improves the kinetics of DSB repair.

**Minor comments:**

1) In the introduction, cite the appropriated references after the sentence:"Recently, it has been shown that the Cdk controls the decision to use NHEJ or HR depending on the stage of the cell cycle at which the lesion occurs". They are cited further in the MS.

2) At the end of the introduction [While some groups have argued for a role of Cdc14 in DNA damage checkpoint activation, other groups have suggested a function directly at the DNA repair level (Bassermann et al, 2008; Mocciaro et al, 2010)] write a sentence that summarizes the main findings of this work.

Referee #3:
The premise of this ms. is that cdc14 mutants show sensitivity to DNA damage. This notion leads to an overall effort to shoehorn an explanation for all of the data into the view that Cdc14 plays a role in DNA repair. There is also an attempt to show some co-localization between components of the spindle pole body and Cdc14. The co-localization shown in Fig. 2A is not very convincing. In addition, the phleomycin and benomyl sensitivity shown in Fig. 1A is not very convincing.

Experiments with a mutant spc110 that cannot be phosphorylated by CDK presents some interesting results, but the authors are convinced that this gene is the main cause of the cdc14 effects studied at the beginning of the ms. Unfortunately, these results are only correlative and not proof of their notion that Spc110 is the target for the effects seen in cdc14 mutant cells.

The authors go on to do some mating type cutting experiments with several different MAT assays. Rad53 phosphorylation is examined in two experiments, but with differing results (one is a haploid and the second is a diploid). It is not exactly clear if the differences observed are due to ploidy.

In another series of experiments, they triangulate the two SPBs and the DSB. When the distance increases between the closer of the two SPBs to the DSB, it is interpreted to mean that the movement is more random. It is hard to understand how the arbitrary choice of the SPBs actually informs anything about closeness to the nuclear periphery. I was very confused by that argument.

It is well known that cdc14 mutants have pleiotropic effects on the cell cycle. It is important not to underplay the cell cycle effects of these mutations. For example, in Fig. 4B: Yes, there is a delay, but it may be related to the fact that the cells are being held in G2 and therefore did not have the opportunity to finish recombination instead of a direct effect on spindles etc. as postulated. This same reasoning holds for the delays postulated in Fig. 5C and 6D. The 1c/2c profiles lag behind about 1.25 hrs well explaining the shift seen MAT repair (and DSB persistence) in 5C, while the shift is even great in the S36-91A mutant (6D).

Pg. 8, ln. 186-188: This sentence is quite strong. How much residual Cdc14 phosphatase activity is there at the semi-permissive temperature?

Typos, et al.

Pg. 3, ln. 32: best reference?
Ins. 42-44: left out reference

Pg. 9, ln. 221: change to: ...that unrepairable DSBs...

Pg. 14, ln. 360: references are missing

Reviewer #1:

The reviewer finds that our contribution in the model of how the release of Cdc14 from the nucleolus in response to DNA damage is attractive and the data on the dephosphorylation of Spc110, the role of Cdc14 in DNA repair and the spc110^S36-91A mutant is quite convincing. In fact, he/she

Resubmission - authors' response 13 May 2016

First of all, I would like to thank you for your previous correspondence regarding our manuscript EMBOJ-2015-93540R-Q. I would also like to specially thank the reviewers for their time and their constructive criticisms. We are grateful to see that all referees have appreciated our work and have recognized the general interest on the topic and the potential importance of our data in the field of DNA repair. I have revised the manuscript taking into consideration the referee’s comments. We have now fulfilled their requirements, as detailed in our point-by-point response to the reviewer’s comments.

Reviewer #1:

The reviewer finds that our contribution in the model of how the release of Cdc14 from the nucleolus in response to DNA damage is attractive and the data on the dephosphorylation of Spc110, the role of Cdc14 in DNA repair and the spc110^S36-91A mutant is quite convincing. In fact, he/she
specifically suggests that the work may have a story worth for publishing in EMBO J. However, the referee raised a few issues that we have already addressed:

General suggestions:

1) Reviewer suggests repeating Southern blots to have a more accurate measurement and include a complete statistical analysis.

   We have now repeated every single Southern blot in the manuscript three times. We have also included a full statistical analysis of all quantifications using a 2-tailed unpaired Student t test. We have also extended this analysis to any quantification reported in the manuscript. The P values from three independent experiments have been included in all the figures.

2) Reviewer comments that the data presented here are not in line with previous published data by Ling and Wang.

   In this article the authors propose that the Chk1 kinase negatively regulate Cdc14 release by inhibiting both FEAR and MEN pathway. There is a fundamental technical difference between Ling’s experiments and ours. While these authors used a telomeric specific cdc13-1 allele as source of DNA damage, we have directly induced DSBs (generated by the HO endonuclease or phleomycin treatment). This may suggests that different genotoxic stresses could elicit multiple and different responses to deal with the DNA lesion. Supporting this suggestion, it has been shown that activation of MEN-Cdc14 is only inhibited (by the canonical MEN pathway inhibitors Bfa1 and Bub2) when telomeres are damaged but not when using other sources of DNA damaged (Valerio-Santiago et al, 2013). Accordingly, in Ling’s experiments Net1 is not phosphorylated in cdc13-1 backgrounds.

   One important feature showed in Ling’s article is that Net1 phosphorylation is required to promote Cdc14 activation. Accordingly, we have shown that in response to DSBs generated by the HO or phleomycin, Net1 becomes hyper-phosphorylated (Fig.3C and 3F). Taking into account these observations we have analysed in detail the localization pattern of Cdc14 in response to DSBs (Fig. 3). We have observed that upon phleomycin treatment there is a partial and transient Cdc14 release from the nucleolus concomitant with Net1 phosphorylation (Fig.3A and 3C). This nucleolar release is independent of the cell cycle, as cells previously blocked in metaphase by using nocodazole show the same transient exclusion pattern when phleomycin is added to the media (Fig.3D). Moreover, Cdc14 nucleolus/nucleoplasm shuttling is also observed in cells expressing a single DSB generated by the HO endonuclease (Fig.3E).

   In line with these results, several genotoxic stresses promote Clp1 (orthologous of Cdc14 in the fission yeast) phosphorylation and relocation to the nucleoplasm in a Chk1 dependent manner (Broadus & Gould, 2012). Similar results have also been reported by Dr. Bueno’s group (Diaz-Cuervo & Bueno, 2008). Given that mammalian Cdc14B is also mobilized from the nucleolus upon DNA damage (Bassermann et al, 2008; Mocciaro et al, 2010), our result may provide insight towards a conserved function of the phosphatase during the DNA damage response.

3) Reviewer suggests addressing if the DSB-SPB interaction observed is attained through the interaction with the spindle component Mps3.

   We agree that, taking into account the new insights provided by Dr. Gasser’s group in this respect, is a valuable experiment for this work. We have now investigated the effect of Mps3 in recruiting DSBs generated by the HO endonuclease to the SPBs. We have included these data into a full new section in the manuscript. We have demonstrated that, together with its nuclear membrane distribution, Mps3 is also located at the SPBs in response to DNA damage (Supplementary Fig.4A). Moreover, DSBs generated by the HO endonuclease are mainly recruited to the Mps3 fraction placed at the SPBs (Supplementary Fig.4B).

   Furthermore, we have taken advantage of previous reports demonstrating that the N-terminus domain of Mps3, while is not essential for viability or SPB duplication, presents defects in tethering DSB to the nuclear envelope. We have engineered strains expressing two truncated versions of the N-terminus domain of Mps3 (mps3Δ2-64 and mps3Δ75-150) as the sole source of the protein. By measuring D1, D2 and D3 distances in these mutants we have observed that only the lack of the
aminoacids 75-150 produces a substantial lack in DSB-SPB interaction (Fig.4D). We have also checked for the sensitivity of these mutants to DNA damage agents. In line with previous data form Gasser’s and Peterson’s groups, an mps3Δ75-150 mutant is not sensitive to MMS. However, in agreement with the lack of DSB-SPB interaction observed in mps3Δ75-150 mutant, only this background (but not the mps3Δ2-64) is sensitive to phleomycin (Supplementary Fig.4C).

Specific points:

1) Better explanation for the genomic situations of the mating type locus in previous figure 1B.

I agree with the referee that the diagram explaining the different assays to analyse DNA repair using the HO was very concise. Because of the size, previous Figure1 has been split into Figures 1 and 2. A better diagram, including DNA fragment size information, has been included for all approaches used (Fig.1C, Fig.2A and Fig.2B). As commented before, we have now repeated all Southern blots for three times and a complete statistical analysis for each one has been included. See figure legend for details.

2) Poor quality figure 2A.

I agree that previous figure 2A was not clear enough due to the background fluorescence on the Cnm67-CFP cyan channel. To sort out this problem, we have constructed new strains containing Cdc14-GFP, Cnm67-RFP and Ddc2-CFP. A new photograph using this strain has been included in figure 3E. Additionally, to fully corroborate Cdc14 localization upon expression of the HO endonuclease, we have created a Cdc14-YFP, Cnm67-RFP and Ddc2-CFP. A representative picture for this strain has been placed at supplementary figure 2A.

Reviewer comments that 40% of Cdc14 at SPBs in pre-anaphase cells contradicts the previously described behaviour of the phosphatase. We acknowledge the referee to realise about this detail. In agreement with this observation, the new strains generated in the previous point do not have Cdc14 located at the SBP before inducing the HO endonuclease. We have eliminated this strain and any results generated with it from the manuscript. It is important to remark that we have now included a full characterization of Cdc14 localization in response to genotoxic stress, including a quantification of nucleolar/nucleoplasm presence of the phosphatase in order to demonstrate Cdc14 activation in our experimental conditions (Fig.3).

Reviewer asks for an undamaged control showing the localization of Cdc14. We have included photographs of asynchronous Cdc14-YFP cells prior generating DNA damage in figures 3A and 3D.

Reviewer suggests that the Cdc14 foci observed during the expression of the HO endonuclease could be due to the signal of Cdc14 at the kinetochores. In our experimental condition, kinetochores appear distributed alongside the two SPBs disposition during the induction of the HO endonuclease (using Nuf2-GFP as reporter) (Supplementary Fig.3B). We believed that the distinct foci formed in Cdc14-GFP cells colocalizing at the SPBs (Fig.3E) do not account for the Nuf2-GFP distribution. This indicates that, at least in our experimental conditions, Cdc14 is not present at the kinetochores.

3) Reviewer comments that the article of Huisman et al. does not describe astral microtubules phenotypes in spc110 mutants.

In figure 8C of this article, the authors show that in cells expressing Spc110D13-S36-91A, both SPBs were marked by the Dyn1p-GFP fusion (Dyn1p-GFP marks only the old SPB and is associated with astral MTs projected into the daughter cell) during separation. Additionally, with this lack of intrinsic asymmetry, both SPBs established dynamic astral MT interactions with the bud cortex. These experiments support the model that Cdk phosphorylation of Spc110 is indeed controlling the spindle polarity at the level of astral microtubule organisation.

In any case, we agree that due to the continuously high spindle rotatory movements observed in cells lacking Cdc14 activity, directly measurements of astral MTs could be subjected to inherent errors. To avoid this problem, we have substituted previous astral MTs measurement for the Dyn1 assay described above (Huisman et al, 2007) in response to a DSB generated by the HO.
endonuclease. Using this methodology, we have incorporated detailed data about spindle misorientation and Dyn1 symmetric loading onto astral MTs emanating from both mother and daughter SPBs (new and old SPB respectively) in cdc14-1 and spc110S36-914 mutants (Fig.6B and 6C). Moreover, we have included a section in the discussion speculating about how changes in the inner SPB plaque could contribute to microtubule behaviour at the outer cytoplasmic SPB side. We have also incorporated in this section some bibliography works to explain this possible mechanism.

We have also followed the reviewer’s recommendation to check for the Tub1-GFP protein levels to confirm the equal amount of tubulin in both wild-type and cdc14-1 mutant. A Western blot showing the similar levels of the protein has been included in Fig.6A.

4) Reviewer argues that the Ddc2 signal is 500 nm away from SPBs. He/she suggests that this could be a reflection of the interaction of the CEN3 with the SPBs during the induction of the DSB.

To rule out this possibility we have analysed other HO sites located at different locations and in different chromosomes (Supplementary Fig.2). Supplementary figure 2C shows a DSB generated at the PES4 locus at chromosome VI. Supplementary figure 2D shows four different DSBs generated simultaneously at MAT, LEU2, PES4 and TRP1 loci. In all these conditions, the DSB-SPB distance was very similar, indicating that DSB recruitment to SPBs does not depend on the specific localization of the MAT locus on chromosome III. Supporting this observation, DSBs randomly generated by Phleomycin treatment were also recruited to the SPBs with the same efficiency (Supplementary Fig.2E). It is important to remark that the 400 nm DSB-SPB length is an average distance and does not reflect the maximum proximity of the DSB to the SPB. Accordingly, previous works from Gasser’s group have shown that DSB interaction with the nuclear envelope present a complete overlap only in 10% of the cells, while the majority of the cells are grouped into what they call “touching state”, a situation where DSBs are quite close to the nuclear rim but not in fully contact (please, see Fig.2C in Horigome et al. for details) (Horigome et al, 2014).

Nonetheless, I really appreciate the recommendation to use a DSB-SPB measurement using a tagged HO locus instead of a Ddc2-RFP as DSB reporter. We have added this experiment into the manuscript. In figure 4C we have used a strain containing a TetO array fused to an I-SceI recognition site. Moreover, to fully demonstrate our previous results using Ddc2 as DSB reporter, we have included a Rad52-CFP in the same strain to analyse D1, D2 and D3 using both TetI-RFP and Rad52 proximity to a Cnm67-GFP as SPB reporter. Samples have been taken before and after expressing the I-SceI endonuclease. As expected 2.5 hours after expressing the endonuclease, the D2 distance measure using the TetI-RFP was only reduced in wild-type cells but not in cdc14-1 mutants when comparing with undamaged cells (Fig.4C). Accordingly, and confirming previous results, D2 measurement using Rad52-CFP signal in a wild-type strain was also shorter than in cdc14-1 mutants (Fig.4C).

We agree that measurement of DSB to kinetochore is problematic. We have repeated the experiment three times and we have used a completed statistical analysis to be sure of the measurements. In any case, we have decided to relocate the figure into the supplementary (Supplementary Fig.3A).

5) We apologize for the expression “SPB integrity” when working with the microtubule depolymerizing drug nocodazole. We have changed it for metaphase spindle integrity along the paper.

6) Reviewer suggests that spc110-220 mutants have defective SPBs and nuclear architecture problems.

We agree that the defects observed in this mutant could be a consequence of a nuclear organization problem due to faulty SPBs rather that a direct effect. However, independently of the reason behind this observation, we wanted to connect SPB integrity and genome stability during the DNA repair process. Under my point of view, the key point of this experiment is to demonstrate that stability of the metaphase spindle is required to fulfil DNA repair. Nevertheless, we agree to transfer this figure to the supplementary (Supplementary Fig.5). Again, we have repeated the experiment a third time and a statistical analysis has been included.

7) Reviewer suggests a complementation experiment in spc110S36-914.
We believe that the third time repetition of the Southern blot and the statistical analysis applied to the data is enough to demonstrate the diminished DNA repair efficiency by homologous recombination in spc110<sup>966-914</sup> mutant cells (Fig. 8D). Nevertheless, we agree to include this experiment if the referee still considering it necessary.

Minor points:

1) Reviewer suggests checking Net1 phosphorylation after Gal addition in non-DNA damage conditions.

*We agree that this control must be included in the article. We have transfer cell from raffinose to galactose in a non-HO background to demonstrate that Net1 phosphorylation is specific of DNA damage (Fig. 3F).*

2) *We apologize for the confusing paragraph in lines 219-221. We have corrected along the text the notion that only Mps3 and not Nup84 is located at the SPBs.*

3) *Reviewer asks for information about the statistical analysis of previous figure 3B. The information has been incorporated in the figure legend (relocate to Fig. 4A).*

**Reviewer #2:**

The reviewer is very supportive and considers our work of a high quality at the experimental level. He/she is quite fascinated about the merit to correlate relocation of DNA lesions to the SPBs with DSB repair. However, he/she proposes a few experiments to improve the quality of the work:

1) As reviewer #1, he/she suggests to measure a tagged HO locus instead of the Ddc2-RFP signal provided in this work.

*I am totally agreed to improve the quality of the paper by measuring DSB-SPB distance using a TetO array to verify the recruitment of a DNA lesion to the SPBs. We have included a full analysis of DSB-SPB recruitment using a TetO array nearby an I-SceI cut site. Moreover, we have introduced in the same strain a Rad52-CFP as DSB reporter to compared both TetI-RFP and Rad52-CFP distances to Cnm67-GFP as SPB marker (Fig. 4C). Please see details in point 4) to the response to reviewer 1.*

2) Reviewer open the question about the connection between the canonical DNA damage and the Cdc14 related events and suggests analyse DSB-SPB interaction in cells lacking a proficient DNA damage checkpoint activity.

*I am totally agreed that this experiment is important to demonstrate if DSB-SPB tethering is dependent of the well-established DNA damage pathway. We have now analysed DSB-SPB recruitment by comparing D1, D2 and D3 distances between wild-type, smi1Δ mutant and double smi1Δ rad53Δ mutants. We have demonstrated that DSB-SPB interaction depends on an efficient DNA damage checkpoint since smi1Δ rad53Δ but not smi1Δ mutant cells are affected in recruiting DNA lesions to the SPBs (Fig. 4E).*

3) *Reviewer suggests developing certain aspect of the discussion. He/she provides a few very important key points that must be addressed.*

*I am agreed to include all suggestions to improve the quality of the discussion. We have implement the discussion using all suggestion recommended by the referee.*

A) Improve the discussion regarding DSB recruitment to specific Msp3 located at the SPBs. *We have now extended the discussion in this respect, providing new results included in the new version of the manuscript and new bibliography references (Page 18, lines 489-507. Page 20, lines 535-543).*
B) Improve the connection between Cdc14 and CdK in the canonical response to DNA damage. We have implemented the discussion linking previous results about the CdK role in the DDR with new outcomes generated by the new experiment suggested by the reviewer #2 about the function of the canonical DNA damage checkpoint in promoting DSB-SPB tethering (Page 21, lines 555–575).

C) Increase discussion relating the observation that both cdc14-1 mutants and spc110<sup>926,91A</sup> (hyper or hypo-phosphorylated Spc110 respectively). We have extended the discussion regarding this specific suggestion as request by the reviewer (Page 17-18, lines 442-488).

D) Reviewer suggests explaining why stabilization of the metaphase spindle improves the kinetics of DSB repair. We have incorporated new ideas to explain the role of Cdc14 in promoting metaphase spindle stability and DNA repair (Page 17, lines 456-488. Page 19, lines 508-534).

Minor comments:

1) Reviewer suggests including a reference in the introduction after a sentence regarding the role of CdK in controlling the decision to use NHEJ or HR.

I am totally agreed to include a cite regarding the subject. An article from Lee`s lab has been included into the bibliography (Zhang et al, 2009) (Page 3, lines 47-49).

2) Reviewer suggests to clarify in the introduction the different result found in the literature about the role of Cdc14 during the DDR activation.

We have now described in the introduction the different points of view about the Cdc14 function in the DNA damage response between Bassermann et al, 2008 and Mocciaro et al, 2010 (Page 4, lines 71-82).

Reviewer #3:

While reviewer appreciate the overall effort to established the role of the Cdc14 phosphatase in the DNA damage response, he/she raises a few concerns about some experimental procedure.

1) As reviewer #1, he/she shows concern about the quality of figure 2A.

I totally agree that the quality of figure 2A was not appropriate due to the high background fluorescence generated when using a Cnm67-CFP fusion protein. We have now created a new strain containing Cdc14-GFP, Cnm67-RFP and Ddc2-CFP to check for their localization in response to a DSB generated by the HO endonuclease (Fig.3E). Moreover, we have generated a Cdc14-YFP, Cnm67RFP and Ddc2-CFP to validate Cdc14 localization in response to DNA damage (Supplementary Fig. 2A).

Reviewer also suggests improving the quality of the drop assay reported in previous figure 1A (bottom panel) showing the sensitivity of cdc14-1 mutants to phleomycin and benomyl. We have now improved the differences in drug sensitivity between the wild-type and the cdc14-1 mutant. For benomyl sensitivity, we have repeated the same concentration as used before. However, we have increased phleomycin concentration to better appreciate the differences in cell sensitivity between the wild-type and the cdc14-1 mutant background (Fig.1B).

2) Reviewer is not convinced that the activity of Cdc14 over Spc110 is the only function of the phosphatase during the activation of the DNA damage response.

I agree that being the CdK one of the most important kinases during the execution of the DDR, and Cdc14 the only described phosphatase to counterbalance its phosphorylation, it is reasonable to think that this phosphatase could account for many dephosphorylation events during the damage response. In fact, this suggestion correlates with our mass spectrometry data, where multiple DNA damage proteins have been isolated. Moreover, elimination of DSB-SPB interaction using a truncated version of the N-terminus domain of Mps3 (Fig.4D) showed sensitivity to phleomycin but not to MMS while cdc14-1 mutants cells are sensitive to both of them (Supplementary Fig.4C). This
is clearly suggesting that Cdc14 is accounting for other non-relating DSB-SPB tethering roles that are also important for DNA repair. Nevertheless, during the discussion I have dedicated a full paragraph to speculate about this possible scenario. I apologise for any misunderstanding generated during the writing of the manuscript regarding this issue. We have now tried to make this subject clearer along the text in the new manuscript.

3) Reviewer argues that the differences of Rad53 phosphorylation between previous Fig.1C and Fig.1D are due to the fact that while in the first experiment an haploid strain has been used, a diploid strain has been used in the second.

This is obviously a misunderstanding as all experiments reported in this work have been done using haploid backgrounds. It has been previously well established that the distinctive pattern of Rad53 phosphorylation between both experiments is the result of the execution of different DNA repair pathways. While DNA repair by SDSA (Synthesis Dependent Strand Annealing) represented in Fig.1C does not activate Rad53 (Kim et al, 2011; Pellicioli et al, 2001), repair by the canonical Holliday junction resolution pathway in previous Fig.1D (new Fig.2A) required fully checkpoint activation throughout Rad53 phosphorylation. This is now explained and referenced in lines 126-129 (Page 6) of the manuscript. I apologize for any misunderstanding regarding this issue.

4) Reviewer is confused by the method used to measured DSB-SPB distance.

Reviewer has interpreted that when the distance increases between the closer of the two SPBs to the DSB the movement is more random. I believe this is a misinterpretation, as we have not measured SPB or DSB movements at the space level to determine DSB-SPB proximity. We have just measured the distance between them. To support these measurements, we have included a new measurement to determine DSB recruitment to the nuclear rim by using the three concentric zones described by Gasser’s and Peterson’s groups (Supplementary Fig.2B). A deficient tethering of DSB to the nuclear envelope was observed in cdc14-1 cells when compared with a wild-type strain, corroborating our previous observation using the D1, D2 and D3 measures approach.

A whole detailed figure has been created only for demonstrating DSB-SPB interaction (Fig.4). Likewise, a full figure has been elaborated to show the information regarding spindle stability and orientation (Fig. 6).

5) Reviewer suggest that the delay in DNA repair observed in several of our experiments could be explained by the fact that under these circumstances the cells are blocked in G2 for longer, and thus they do not have the opportunity to finish recombination.

It is generally accepted that upon DNA damage, cells activate the DNA damage checkpoint in order to block the cell cycle in G2/M. This arrest gives time to the cells to repair the broken DNA prior entry into mitosis (Mathiasen & Lisby, 2014). Both the canonical DNA damage checkpoint kinase Mec1 and the Cdk activity have been reported to be essential in controlling both the cell cycle arrest and DNA repair (Trovesi et al, 2013). Moreover, in yeast DNA repair is preferentially achieved by homologous recombination during the G2/M block to ensure that nuclear segregation is not activated before the broken DNA has been fixed (Branzei & Foiani, 2008). Accordingly, we observed that wild-type cells start to repair the broken DNA when cells are in G2/M as showed by the FACS analysis (Fig.7B, Fig.8D and Supplementary Fig.5C). In this scenario, why holding the cell cycle in G2/M for longer in our experimental conditions would be in detrimental for DNA repair? We believe is more intuitive to think that the delay observed in the FACS profile is just simply mirroring the DNA repair problem that these cells are experiencing and thus the longer activity of the DNA damage checkpoint response. This conclusion is supported by the observation that Rad53 is phosphorylated (as measure for checkpoint activation) even during the SDSA execution (a situation where normally there is not DNA damage checkpoint activation) in the absence of Cdc14 activity (Fig. 1C).
6) Reviewer asks about the residual activity of Cdc14 under semi-permissive conditions during the measurements of Spc110 phosphorylation.

Unfortunately, we cannot account for the residual activity of the phosphatase under these experimental conditions. However, the effect in the levels of Spc110 phosphorylation when comparing a wild-type strain with cdc14-1 cells grown under these conditions are evident, indicating that this component of the SPB is a target of the phosphatase during the DNA damage response. I agree that a full cdc14-1 inactivation would probably give us even more pronounced defects, but we decided to design all experiments at semi-permissive condition to rule out any possible interference with other Cdc14 non-DNA damage related functions in the cell cycle. Note that using these conditions, cdc14-1 mutants are able to perfectly grow in the absence of DNA damage (Fig.1A).

7) Reviewer suggests a few typos and missed references in the manuscript that I have already corrected.

Reference in previous page 3 (line 32) has been implemented with two more cites (now in page 3, line 34). Two reviews from the group of professors Marco Foiani and Michael Lisby have been included to reinforce the sentence (Branzei & Foiani, 2008; Mathiasen & Lisby, 2014).

Reviewer suggests inserting references in lines 42-44 of the manuscript. There are already three cites included in that paragraph (new page 3, lines 45-47). Additionally we have as well included an extra cite (suggested also by reviewer 2) in previous page 3 (line 43) to reinforce the subject about the role of the Cdk in pathway choice decision (new page 3, line 49).

Reviewer suggests changing DSB for unrepairable DSBs when referring to DNA lesions that are recruited to the nuclear envelope (previous page 9, line 221). We have corrected the phrase to: “Unrepairable or DSB that are repaired slowly re-localize to the nuclear periphery...” (now in page 9, line 205).

Reviewer suggests including a reference in the discussion when mentioning that organization of DSBs into specialized repair centres may provide advantages to the cell (previous page 14, line 360). We have included a reference from professor Rothstein’s article to reinforce the sentence (Lisby et al, 2003) (now in page 19, line 492).

2nd Editorial Decision 18 July 2016

Thank you for your patience during the reconsideration of your manuscript on yeast Cdc14 and homologous recombination repair. It has now been reviewed once more by all three original referees, and I have also once more carefully read the study and discussed it with my colleagues. The conclusion from this reassessment is that in light of the major improvements to the study, we would be willing to now consider it further for The EMBO Journal, but only after several important additional revisions.

As you will see, referees 1 and 3 still raise several specific points that need to be addressed/responded to, as well as one major conceptual concern regarding the role of Spc110 and its phosphorylation in mediating Cdc14 effects on repair. While we appreciate that a detailed mechanistic understanding (e.g. by following up on the comments of referee 1) may be beyond the scope of the present manuscript, we consider it essential to provide deeper insight into the seemingly paradoxical role of Cdc14 and of Cdk phosphorylation sites in Spc110, both of which appear produce the same loss-of-function phenotypes (cf. referee 2’s point in the first round of review, and referee 3’s comment in this round; as well as referee 1’s original request for complementation of spc110-S36/91A with wt Spc110). It will therefore be important to not only conduct this complementation assay (as offered in your response letter), but also to back up the spc110-S36/91A and cdc14-1 data by at least analyzing the phenotypes of a phosphomimetic Spc110 mutant version, and by pointing out and discussing the apparent contradiction in more detail and with plausible explanations and potentially testable hypotheses.

In addition, there are also a number of editorial issues that would need to be taken care of. In
particular, language and writing of the manuscript will have to be significantly improved, and before resubmission the study should be proofread and edited in detail by a scientist with native English language proficiency. The manuscript also still lacks a proper closing paragraph to the introduction section, which would outline the question being addressed and summarize key findings. Furthermore, please carefully review the reference list for completeness and inclusion of all relevant primary citations (e.g. I notice that only Blanco et al 2014 but not Eissler et al 2014 is cited for Cdc14-Yen1 regulation). With regard to the figures, we note that some blot panels are overly bright/contrasted (e.g. Fig 1C, 2A, 2B) and should therefore be replaced with less adjusted images that still allow a direct assessment of their relation to the original data; in addition, please also upload source data files of the original, unmodified scans underlying these images and other key blot panels. Finally, please refer to our Guide to Authors (see below) for updated information on how to display supplementary data as Expanded View figures, and regarding the completion of an Author Checklist.

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REFEREE REPORTS

Referee #1:

Stabilization of the metaphase spindle by Cdc14 is required for recombination DNA repair

Villoria et al. analyse the role of budding yeast Cdc14, a conserved phosphatase, for a role in DNA repair. In essence, the authors conclude that Cdc14 contributes to inter-chromosome DNA repair through dephosphorylation of the gamma-tubulin complex receptor Spc110 that organizes the nuclear microtubules.

The first submission had a number of problems. Importantly, many experiments were only done twice and lacked statistics. Overall, the difference between WT and cdc14-1 mutant phenotypes is relatively small.

In the revised version of the manuscript, Villoria et al. have repeated critical experiments (Southern blots) a third time. Quantifications now show SD and statistical analysis. This is a major improvement of the manuscript. The differences between WT and cdc14-1 cells are with ca. 20% relatively small.

They further show that DNA damage causes release of Cdc14 from the nucleolus. The authors show in Fig. 4 that the DSB is recruited close to SPBs in dependence of Cdc14 activity. SIM analysis of the cells would have been beneficial. The authors improved this experiment including SceI induced DNA damage, mps3 alleles and NuF2 kinetochore control.

In subsequent experiments, the authors show that dephosphorylation of Spc110 at two residues within the N-terminus by Cdc14 is important for the role of Cdc14 in DNA repair by recombination. The data are significant and the MMS and phleomycin sensitivity of spc110-S36-91A mutant supports this conclusion. However, the mechanistic basis for this role of Spc110 remains in the dark. Moreover, although Spc110 has a well described function only in the nucleus, the spc110-S36-91A allele affects spindle orientation and dynein localization to the SPB on the cytoplasmic SPB side. My guess is that the altered microtubule nucleation activity in spc110-S36-91A cells on the nuclear side (see Lin et al. eLIFE 2014) affects microtubule nucleation on the cytoplasmic side indirectly by increasing cytoplasmic gamma-TuSC. The authors discuss the connection between SPC110 mutations and spindle orientation thoroughly.

Major point:
1. Figure S1B: this experiment was done at a semi-permissive temperature. Thus, cdc14-1 cells pause in anaphase because of a mitotic exit defect (seen by FACS and accumulation of large budded cells). Therefore, this experiment is inconclusive and does not allow concluding successful repair. I suggest removing this experiment.
2. The authors should indicate how often in Fig. 3E Cdc14, Ddc2 and Cnm67 co-localize.
Minor points:
1. p.2, line 2: "our cells are" ... "Cells are ...".
2. A role of Cdc14 in cytokinesis was first described by the Pereira lab. The authors should cite this work.

Referee #2:
The authors have done a great work to revise their manuscript. They have addressed my concerns. One or two sentences should be added at the end of the introduction that summarizes the paper. The paper should be published.

Referee #3:
The authors have responded well to many of the criticisms raised by all three reviewers. I, the notorious Reviewer #3, must once again be very negatively critical of the manuscript. I think that there is something useful that these authors have uncovered. However, the Cdc14 connection to the DNA damage response and to repair has already been uncovered in fission yeast and mammalian cells. This ms. adds to the field by showing a correlation between cdc14 deprivation and spindle attachments and may even have made a significant contribution concerning the interaction between Cdc14 and Spc110. However, the effects on mating type recombination are still rather minimal. In addition, there is one real contradictory result, namely the fact that the spc110S36-91A mutation, that cannot be phosphorylated by CDK, shows the same phenotype as failure to dephosphorylate Spc110. These situations are opposite. - A cdc14 defective cell will have more phosphorylated Spc110, not less. A direct correlation between a phospho-mimick would have been much more compelling. In fact, the authors really don't even point this fact out. In addition to this major problem, they are too strong in many of their conclusions in the discussion. Here are some, but not an exhaustive list:

In. 284-285: remove the last clause - too strong
In. 447: exerts this function by promoting... is too strong
In. 451: might contribute to, not "stimulates the stabilization of... " The phosphorylation-blocked mutant is the problem here with this overstatement.
In. 455: "essential" is too strong
Ins. 535-543: What is this paragraph doing here?
Ins. 557-558: again, the phosphorylation-blocked mutant counters their argument

Issues with the Figures:
Figure 1: Here and in the subsequent ones, the effect on recombination, essentially MAT switching is small.
Figure 3: What is the frequency of Cdc14 released and Net1 phosphorylation during a normal mitosis (no Phleomycin treatment)? 3E: What % of Cdc14 release is Ddc2 colocalized?
Figure 4: Is the distance increase due to release of tethering or increased mobility of the cut locus? Typo: in 4D Mps3 should be italicized and capital letters.
Figure 7: In 7A, the the D2 P value is listed as <0.0001. It is probably a typo and should not be there.

In general, the paper needs English editing, which I will not do.

2nd Revision - authors’ response  05 October 2016

I would like to thank you for your previous correspondence regarding our manuscript EMBOJ-2015-93540R1. I would also like to particularly thank the reviewers for their constructive criticisms on our work. I am very pleased to see that all referees have recognized the major improvements from the original version of the manuscript. We have now fulfilled all the requirements requested in the
previous correspondence. The details are included in our point-by-point response to the editorial board and reviewers comments.

Response to the editorial board comments.

A) General suggestions.

1) The editor suggests performing a complementation assay by expressing a wild-type copy of Spc110 in an spc110S36-91A background (proposed by reviewer #1 in previous revision).

To achieve this subject, we have PCR amplified and cloned the 5’ promoter region and the ORF of SPC110 into an integrative pRS306 plasmid. This construct has been linearized and integrated at the URA3 locus in a spc110S36-91A mutant background in order to express a wild-type version of SPC110 under its own promoter. We have used this new strain to check for the recovery of the spc110S36-91A sensitivity to phleomycin in drops assays (new Appendix Figure S1A). We have also used this strategy to perform physical analysis by Southern blot and compare the efficiency of DNA repair by HR in a wild-type, a spc110S36-91A mutant and a spc110S36-91A pRS306-SPC110 strain (Appendix Figure S1B). As predicted, the reintroduction of a wild-type copy of SPC110 in an spc110S36-91A mutant background bypass all DNA damage defects previously observed in both drop assays and physical DSB repair experiments.

2) Phenotypic analysis of a phospho-mimetic Spc110 mutant version.

In order to attain this subject we have created a phospho-mimetic version of Spc110 by mutating serines 36 and 91 to aspartic acid residues (spc110S36-91D) and a fully characterization of this mutant has been performed (new Figure 9).

First, we have analysed the sensitivity of a spc110S36-91D background to genotoxic drugs by performing drops assays. Interestingly, mutating both Cdk consensus sites to aspartic acid does not affect viability on MMS plates (in contrast to the increased sensitivity observed in cdc14-1 and phospho-mutant spc110S36-91A cells). However, the exposure of the spc110S36-91D to phleomycin drastically affects its viability in a similarly manner than in cdc14-1 and spc110S36-91A backgrounds (Figure 9A). It is worth mentioning that all the spc110S36-91D clones generated during this study present a mild growth defect in non-DNA damage conditions, arguing for an important function of S36-91 dephosphorylation during an unperturbed cell cycle.

Second, we have analysed in detail the effect in DSB-SPB recruitment and SPB dynamics of a constitutive phosphorylated Spc110. In agreement with the higher sensitivity to phleomycin previously observed, a spc110S36-91D mutant is affected in both DSB-SPB tethering and SPB stability when a single HO cut is induced (Figure 9 B and C respectively).

Third, we have performed physical Southern blot assays to characterize the DNA repair efficiency in a phospho-mimetic Spc110. In agreement with the phenotypes mentioned above, spc110S36-91D cells repair an HO-induced DSB with a slower kinetics than wild-type cells in an inter-chromosomal DNA repair assay (Figure 9D).

Together, our experiments hint that spindle stability is tightly regulated by the activity of Cdk/Cdc14 over Spc110 during the DNA damage response. Importantly, the steady state phosphorylation of Spc110 is not only important to promote spindle stability but also to stimulate DSB-SPB interaction and in turn recombinational DNA repair. Finally, and as suggested by the editorial panel, we have deeply discussed and explained the apparent contradiction between the similar phenotypes observed in both spc110S36-91A and spc110S36-91D mutants in the discussion section. We have included a complete paragraph with previous evidences supporting our observations, possible explanations for the paradoxical phenotypes found and testable hypothesis to verify these theories (Discussion lines 487-527).

B) Editorial issues.

1) The language and writing of the manuscript should be corrected by a scientist with native English language proficiency.
A native English language scientist has revised the manuscript in detail.

2) The manuscript should contain a closing paragraph at the introduction.

A paragraph summarizing the key findings has been added at the end of the introduction. We have taken advantage of the opportunity to highlight the importance of spindle stability in DSB-SPB recruitment and DNA repair, what I consider the most relevant conclusion of the article.

3) It is suggested to complete and include relevant citations.

We have examined the manuscript looking for new and miss-incorporated citations. As proposed, we have incorporated the work of professor Mark Hall when discussing about the role of Cdc14 in Yen1 regulation (Page 4, line 58).

4) It is recommended to use less contrasted images for the Southern blots depicted in Figures 1C, 2A and 2B.

We have now re-scanned the originals films to include non-processed images of the mentioned figures. Additionally we have included the original scans (that will be uploaded together with the manuscript) of any single Southern blot included in the article.

5) It is mentioned to updated information on how to display supplementary data and the completion of an Author Checklist.

We have modified the way to refer the supplementary data, including the Expanded View Figures and the Appendix Figures. We have also changed the name of the original files accordingly to the information provided in the Guide to Authors section of the EMBO Journal web page.

Response to referees comments.

In addition to the comments proposed by the editorial panel, there are other specific issues that have been raised up by the reviewers through the last revision. We have also decided to accomplish these matters and include them into the original manuscript as detailed in the following section.

Reviewer #1:

A) Major points.

1) Reviewer argues that the pause in anaphase observed in *cdc14-1* mutant cells is due to a defect in mitotic exit rather than problems in DNA repair (Supplementary Figure 1B).

I am agreed that the delay observed by FACS analysis together with the gradual accumulation of large budded cells could reflect a delay in exiting mitosis instead of a DNA repair problem. However, in the mentioned Supplementary Figure 1B it is scored (by DAPI staining) the ratio of mono-nucleated dumbbell cells between wild-type and *cdc14-1* mutant cells. The observation that the mono-nucleated dumbbell cells ratio is increased in *cdc14-1* mutants suggests that Cdc14 is required for a proficient DNA repair. A delay in exiting mitosis (as suggested by the reviewer) would have led to an increase in bi-nucleated and segregated nuclei instead. We have gone back to the manuscript and realized that while in the main text we specifically refer as to “increased in mono-nucleated G2/M cells”, this specification is missing at the figure legend in Expanded View Figure 1B (previously Supplementary Figure 1B). I guess this is the cause of the misinterpretation. I deeply apologize for this misunderstanding. In the new version of the manuscript we have mentioned at the figure legend of Figure EV1B that the graph specifically represents mono-nucleated G2/M cells scored by DAPI staining. Similar corrections have been incorporated at the legends of Figures EV1C and EV1D.

2) The reviewer asks for the percentage of cells with Cdc14-Cnm67-Ddc2 colocalization upon expression of a non-reparable HO break.
We have gone back to the original images and scored the number of cells with Cdc14-SPB-DSB colocalization in response to a single DSB induced by the HO endonuclease by measuring the physical interaction between Cdc14-GFP, Cmm67-RFP and Ddc2-CFP. We have determined that up to 40% of cells present interaction between these three proteins. A graph representing this quantification has been included in Figure 3D.

B) Minor points.

1) At page 2, line 2 contains a grammatical error. We have changed “Our cells are...” to “Cells are...” as proposed by the reviewer.

2) Reviewer suggests mentioning the work of professor Pereira regarding the function of Cdc14 in cytokinesis. We have now added two cites from Pereira’s work referencing the role of Cdc14 in cytokinesis at the introduction (Page 3, line 55).

Reviewer #2:

1) The reviewer suggests including a sentence at the end of the introduction to summarize the paper.

As recommended, we have included a complete paragraph at the end of the introduction highlighting the role of Cdc14 in DNA repair (lines 81-93). We have made a special effort to emphasize the connection between spindle integrity, DSB-SPB interaction and DNA repair.

Reviewer #3:

A) Major points.

1) Reviewer comments that the Cdc14 connection to the DNA damage response and repair has already been discovered in fission yeast and mammalian cells.

I agree that the original observation of a Cdc14 function in DNA repair was previously postulated in S. pombe and, later on, in mammalian cells. However, as I mentioned in the introduction, an evident controversy has been settled between different groups about the precise molecular function of Cdc14 during the activation of the DDR. Moreover, one characteristic that makes our work unique when compared with previous studies, is that we have taken advantage of the well characterized mating type HO system to fully describe the function of Cdc14 in the diverse features of the DDR (i.e. Intra- and Inter-chromosomal DNA repair and repair pathway choice). These results have led us to pinpoint its precise role executing different types of DNA repair pathways (SDSA and DSBR) and determine its importance in the resolution of DNA intermediates (i.e. Gene conversion vs crossover). This level of refinement it has never been previously achieved. Nevertheless, independently of the precise role of Cdc14 in the DDR, I consider that the principal outcome of our work is the concept that spindle integrity is an important feature of the DNA repair pathway. Additionally, the observation that spindle stability is important to promote the interaction of DSBs to the SPBs makes this work unique and original, and opens new avenues in the DNA repair field about the importance of the spatial regulation of DNA lesions. It is my belief that this last notion is the essential breakthrough of this work.

2) Reviewer argues that the defects on mating type recombination assays are rather minimal.

I agree that the physical repair assays to analyse HR efficiency did not show massive differences between the wild-type and cdc14-1 mutant. We have to bear in mind that because of the essential function of Cdc14 in the cell cycle, all experiments showed in this study have been performed using a semi-permissive temperature. One advantage to work under these conditions is that we can rule out any possible interference with other Cdc14 functions in the cell cycle and specifically attain the role of the phosphatase in DNA repair. However, one inconvenient when using this strategy is that we still contain a residual Cdc14 activity in our experiments. That would explain the relative small differences observed between the two strains. In any case, I differ in underestimating these defects. Small defects in DNA repair can render cells to a profound sensitivity to genotoxic stress. In fact, cdc14-1 mutant cells growing at semi-permissive conditions present a considerable loss of viability.
in response to drugs that induced DNA damage. This suggests that the DNA repair defects observed under these conditions are indeed important to maintain cell viability in response to DNA lesions.

3) Reviewer claims that cdc14-1 mutants present the same phenotype as spc110\textsuperscript{369-91A}.

I am agreed that this result does not fit with the simple idea that Spc110 dephosphorylation is the only mechanism that controls spindle stability during the DDR activation. To clarify this issue, and as recommended by the reviewer, we have generated a phospho-mimetic spc110\textsuperscript{369-91D} strain and analysed the effect of a constitutive phosphorylation of Spc110 in cell viability and spindle stability under genotoxic stress. We have also characterized the importance of Spc110 dephosphorylation in the repair of a single HO-induced DSB by HR. We have generated a whole new Figure 9 with the data obtained. The details of the results are included at previous section A2 of the response to the editorial board. Briefly, a phospho-mimetic spc110\textsuperscript{369-91D} phenocopy the majority of the defects observed in cdc14-1 and spc110\textsuperscript{369-91A} mutant backgrounds. This observation implies that both Spc110 phosphorylation and dephosphorylation (by Cdk and Cdc14 respectively) are significantly important during the response to DNA damage to maintain the integrity of the metaphase spindle and thus facilitating the processing of DNA lesions. We have also improved the discussion by incorporating a full paragraph with possible explanations and testable hypothesis to explain these results (Discussion lines 487-527).

B) Minor points.

1) Reviewer suggests removing the sentence at previous line 284-285 “These data demonstrate that...” for a less strong sentence. We have changed it for “These data support a model whereby...” now in line 294.

2) Reviewer suggests removing the sentence at previous line 447 “Cdc14 exert this function by promoting intra and inter-chromosomal DNA repair” for a less strong sentence. We have changed it for “Cdc14 exert this function by stimulating intra and inter-chromosomal DNA repair” now located in line 479.

3) Reviewer suggests changing the sentence “stimulates the stabilization of...” at previous line 451 for “might contribute to the stabilization of...”. This sentence has been fixed accordingly and is now placed in line 482.

4) Reviewer suggests swapping “essential” in previous line 455 for a less strong term. We have changed it for “important”, now in line 487.

5) Reviewer disagrees for the localization of the paragraph at previous lines 535-543. We have now replaced the full paragraph in a more logical context at lines 562-569.

C) Figure Issues.

1) Figure 1. Reviewer argues that the effect on recombination is small. We have already explained this subject in previous section A2 of the response to reviewer #3.

2) Figure 3. Reviewer asks for the frequency of Cdc14 release and Net1 phosphorylation during an unperturbed mitosis. We have performed an alpha-factor G1 arrest and release in order to score both Cdc14 localization and Net1 phosphorylation along the cell cycle. These data are now located in Figure 3B. Briefly, the levels of Net1 phosphorylation in response to DNA damage resemble those observed in non-DNA damage synchronized cultures at the anaphase stage. Curiously, at this stage of the cell cycle Cdc14 is fully liberated from the nucleolus. This suggests that Net1 phosphorylation is not enough to
sustain a fully Cdc14 nucleolar release during the DNA damage response and evidencing that additional mechanism might be regulating Cdc14 localization during the execution of the DDR.

Reviewer also asks for the percentage of cells with Cdc14 localized with Ddc2 in response to DNA damage. As requested also by reviewer #1, we have scored the levels of Cdc14-Ddc2-Cnm67 colocalization and the data have been included in a graph in Figure 3D.

3) Figure 4. Reviewer questions if the increased DSB-SPB distance is due to release of the DNA lesion from the SPB or to increased mobility of the cut locus.

We believe that the effect is exclusively related to the capacity of Mps3 to retain the DSB to the SPBs. Firstly, because our data indicate that elimination of the N-terminus domain of Mps3 is enough to abolish DSB-SPB recruitment. Secondly, because preliminary data from our laboratory have shown that the mobility of DSB generated by the HO endonuclease is in fact more static in cells lacking Cdc14 activity than in wild-type control cells.

Reviewer suggests italicizing and capitalizing Mps3 in figure 4D. We have changed it accordingly.

4) Figure 7. Reviewer noticed a typo in the D2 P value at figure 7A.

We have corrected this mistake.

5) Reviewer proposes improving the English editing of the manuscript.

A native English scientist has proofread the manuscript looking for grammatical errors and non-standard English expressions.

Thank you for submitting your revised manuscript for our consideration.

I have now had a chance to look through it and to assess your responses to the comments raised in the previous round, and I am happy to inform you that there are no further objections towards publication in The EMBO Journal.
You must complete all cells with a pink background.

Please note that this checklist will be published alongside your paper.

Corresponding Author Name: Andrés Clemente Blanco
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2015-93540R1

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.
- 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 authorship 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 (e.g. 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/changed/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 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 z tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - Any tests one-sided or two-sided?
  - Any adjustments for multiple comparisons?
  - Exact statistical test results, e.g., P = value + a bold if P value < 0.05.
  - Definition of “center value” 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.

To the pink boxes below, please give 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

1. a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?

b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.

2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?

3. Were any steps taken to minimize the effects of subjective bias when deciding animal/samples to treatment (e.g. randomization procedure)? If yes, please describe.

4. a. For animal studies, include a statement about randomization even if no randomization was used.

b. 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.

5. a. For animal studies, include a statement about blinding even if no blinding was done.

b. For every figure, are statistical tests justified as appropriate?

6. In the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.

C. Reagents

1. Where an estimate of variation within each group of data?

2. If the variance similar between the groups that are being statistically compared?
D- Animal Models

18. Report the clinical trial registration number (at top right), such as "NCT00123456". This is strongly recommended for any datasets that are central and integral to the study; please include a statement confirming that consent to publish was obtained. See author guidelines, under "Reporting Guidelines". Please confirm that you have followed these guidelines.

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 structured repositories such as Dryad (see link list at top right) or figshare (see link list at top right)). See also: MIRIAM guidelines (see link list at top right).

20. You may use traditional controlled vocabularies, please refer to the MESH controlled vocabulary (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 that you have followed these guidelines.

E- Human Subjects

1. Identify the committee(s) approving the study protocol.

2. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the Declaration of Helsinki and the Department of Health and Human Services Belmont Report.

3. For publication of patient photos, include a statement confirming that consent to publish was obtained.

4. Report any restrictions on the availability (and/or on the use) of human data or samples. The relevant accession numbers or links should be provided.

5. When possible, standardized machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized formats (SRA, MIAL, CSV, XML) 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 BioModel (see link list at top right) or MIRIAM repository (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.

F- Data Accessibility

6. Provide accession codes for deposited data. See author guidelines, under "Data Deposition".


8. 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 that you have followed these guidelines.

9. For phase IV 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 that you have followed these guidelines.

10. We recommend consulting the ARRIVE guidelines (see link list at top right) (Public Edit: 30; e1000432, 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.

11. Identify the committee(s) approving the study protocol.

12. Provide accession codes for deposited data. See author guidelines, under "Data Deposition".

13. For publication of patient photos, include a statement confirming that consent to publish was obtained.

14. Report any restrictions on the availability (and/or on the use) of human data or samples. The relevant accession numbers or links should be provided.

15. Report the clinical trial registration number (at top right). The data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository database (http://www.ebi.ac.uk/pride) and assigned the identifier PXD000208.

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 that you have followed these guidelines.

17. For phase IV 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 that you have followed these guidelines.

F- Data Accessibility

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21. Dual use research of concern.

D- Animal Models

1. 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., antibodies (see link list at top right), Uniprot (see link list at top right).

2. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for infections, contamination.

3. For all hyperlinks, please use the table at the top right of the document.

E- Human Subjects

4. The mass spectrometry data from this publication have been submitted to the ProteinScape database.

F- Data Accessibility

5. The mass spectrometry data from this publication have been submitted to the ProteinScape database.

G- Dual use research of concern

6. 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.