

Continuous nuclear envelope surveillance is required for DNA double-strand break repair.

Corresponding Author: Dr Félix Machín

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This work explores the roles of Msc1, a relatively uncharacterized protein in budding yeast, in nuclear envelope morphology and double strand break repair. There is an attempt to connect the phenotypes exhibited by *msc1Δ* cells to those exhibited by deletion of key ESCRT machinery genes and, in so doing, relate genome and nuclear integrity pathways. The data are, in most cases, extensively quantified but they do not make a case for a functional relationship between double strand break repair and nuclear envelope integrity. Indeed, the major conclusions are based on shared phenotypes but there is no compelling genetic or biochemical data presented that links Msc1 to the ESCRT pathway.

Major Point:

Although deletion/inhibition of ESCRTs does phenocopy some elements of the *msc1Δ*, there is no evidence that they genetically or physically interact. There are growth assays presented in Figure 5c that might hint that the growth of *msc1ΔSNF7-AID* strains grow a little slower than *msc1Δ* alone (suggesting a synthetic fitness defect), but these data are extremely difficult to interpret due to the strange protective effect of the IAA drug. The other knockouts of ESCRT-III had no synergistic effect and the *snf7Δmsc1Δ* strain was not assessed. This is also problematic as although the use of AID is, in principle, a more elegant approach, tagging of ESCRTs is always fraught and one must be careful to assess functionality. For example, often tagged ESCRTs show gain of function phenotypes. There is no evidence that the authors assessed the functionality of their ESCRT fusions and in Figure 8, the Snf7-GFP localization resembles class E compartments, as opposed to endosomes, which are aberrant structures that arise due to loss or gain of function of ESCRT proteins.

Points to consider to improve the figures and text:

- 1) Throughout the text there is a reference to “late M cells” or “late M cultures”. It is rarely clear (and certainly is not at all clear in the figures) whether the authors are cherry-picking late-M cells out of an asynchronous population or whether they are using the *cdc15-2* strain at the non-permissive temperature. The labeling of *cdc15-2* as “WT” in the figures is extremely misleading, for example. WT should only be used for wildtype yeast strains. A *cdc15-2* strain, by definition, cannot be WT. This is even more confusing when DNA damaging assays are used on top of the *cdc15-2* arrest as DNA damage would also be predicted to lead to mitotic arrest on its own, likely before the *cdc15*-induced arrest. How would this influence the quantification of this assay?
- 2) Figure 1: These data are interesting, but it is confusing why the authors choose to examine the localization of the split-GFPs only under conditions of mitotic arrest. It would be more helpful to address the topology without adding this confounding variable. Ideally, the authors would also examine N-terminal fusions as well to round out the assay.
- 3) Figure 2: How do the authors differentiate between two G1 cells close together and late-M cells when quantifying a lack of nuclear envelope bridge?
- 4) Figure 4: The sensitivity of the ESCRT deletions to phleomycin is intriguing. To connect more clearly to the nuclear envelope, the authors should include Chm7 as this is the only known nuclear-envelope specific ESCRT.

Reviewer #2

(Remarks to the Author)

The manuscript presents some interesting observations on how a luminal NE protein and a membrane remodelling complex correlate with increased sensitivity to DNA damage as well as specific defects in nuclear shape and nuclear separation during mitosis that may contribute to the phenotype. Models based on nuclear compartmentalization of repair proteins and retrograde transport of sister chromatids are proposed. The principal limitation of the paper is that the interpretation is highly speculative and no direct examination of DSB repair, DSB persistence, or repair protein localization is performed. In the absence of such experiments that directly examine potential mechanisms, the study only convincingly demonstrates a correlation with defects in DSB repair.

Rather than statistical measures of phenotypes from different time points, more mechanistic data would be obtained by performing time-lapse.

Adding a DSB reporter system where the break persistence is marked by a repair protein would also be helpful in assessing repair deficiencies.

While increased DNA damage sensitivity is demonstrated, no repair defect is demonstrated

Reviewer #3

(Remarks to the Author)

Manuscript Number: COMMSBIO-24-4151

Corresponding Author: Dr Machín

This paper describes the role of Msc1 and ESCRT-III complex in nuclear envelope homeostasis in the budding yeast, *S. cerevisiae*. In the previous report, the author's group identified Msc1 as a possible key player in the repair of DNA double-strand breaks (DSBs) during the M-phase, which is associated with so-called sister chromatid approximation. In this new study, the authors extended their previous characterization of Msc1 and found that Msc1, which is located in the nuclear envelope (NE) with most of the C-terminal portion in the lumen of NE, is critical for the maintenance of NE morphology. Moreover, the authors also showed that ESCRT-III components, Snf7, Did4, and Vps24 are critical for the NE homeostasis as Msc1. The authors also reported a bit complicated genetic interaction of the *msc1* with the ESCRT-III mutant including the AID allele of SNF7; mainly these mutants share similar defects in NE morphology both in the absence or presence of DSBs. The experiments were well-designed and were carried out in good shape. Most of the results are convincing although the effect of the mutations was not drastic. The role of NE in chromosomal events such as DSB repair is one of the fields we have not known much about. Thus, the results in the paper are fascinating and provide a new idea of how NE homeostasis controls various chromosomal events in different cell cycle stages in mitosis. However, since the text was a bit difficult to read as shown below, the authors need additional words to rewrite. In addition, prior to the publication, the authors need some additional experiments to clarify their conclusions.

Major points:

1. The authors analyzed NE morphology of the *msc1* and ESCRT-III mutants in late anaphase mainly as well as G2/M arrest (Figure 7). However, the authors are recommended to provide more detailed results on the NE morphology of these mutants in a normal cell cycle (without DSBs). Do the mutants show abnormal NE in G1, S, or G2 phases? If so, what kind of defects, which are similar in those in late anaphase?
2. In this paper, it seems that the authors wanted to say the "direct" role of Msc1 in the DSB repair in late anaphase. However, as they acknowledged (e.g. Figure 9b), the effect of the mutations on the DSB repair might be indirect. In this line, the main text should be modified so that the authors focus on the role of Msc1 and ESCRT-III in NE morphology under normal conditions if the authors see NE defects in other cell cycle stages (#1 comments).
3. In Figure 5C, the authors checked the sensitivity of *msc1* and *msc1* SNF7-AID cells to Phleomycin (Phle). In the absence of IAA, *msc1* SNF7-AID cells are sensitive to Phle. On the other hand, in the presence of IAA (third and fourth panels; without OstIR1 such as 4th lane in the third panel is obvious), the cells are more resistant to the drug, indicating a partial suppression of the *msc1* defect by the SNF1 depletion or dosage effect. However, the authors did not mention this clearly and should discuss this implication on the functions of MSC1 and ESCRT-III in NE morphology. If the suppression is true, the authors need to check other data in the double mutant in this line.

Minor points:

1. line 25, NE health: Please define the word or use a more familiar word in the Abstract.
2. Line 136-: In this section, the authors need to explain their experimental system how they introduced DSBs such as by HO (Galactose-induced system is used, please mention when they added gal to media after *cdc15* arrest). A schematic figure for the timeline of each manipulation (times of temperature shift, the addition of phleomycin and sampling) should be presented in Figure 2.
3. Figure 2b, image of Sec61-GFP: for three images, please add a tracing of the GFP signal in each image, as shown as a representative on the top to explain what "partition" is in the paper (see above).
4. Line 148: Like partition, please show the typical image of the bulge (with the tracing) in Fig.2. Please explain how the bulge is different from the partitioned compartment (with a clear definition).
5. Line 174-179: Is the localization of Msc1-GFP to the nuclear bulge seen even in the absence of DSBs? Please mention this clearly and show representative images like Fig. 2e. It is important to avoid misunderstanding that the bulge is induced by the DSB treatment.

6. line 200: the sentence seems to be incomplete.
7. line 209: What do the authors mean by NE septation? Please define the word in the first place.
8. Figure 4b-g: Please add msc1 deletion in these assays as the comparison.
9. Figure 4d: For class "d", please add statistical comparison relative to the wild-type as shown in class "c".
10. Figure 5d-f: Please add the data of wild-type with or without Phleomycin as a control
11. Figure 5d, e: please add a statistical comparison between -iaa phle and +iaa phle.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Overall, the authors have been responsive to my comments and have thoughtfully revised the manuscript. My only minor point is the authors should not use the term "cytosolic NPC" as it implies the entire NPC is mislocalized as opposed to a single or a subset of nucleoporins/nups, which is more likely the case.

Reviewer #2

(Remarks to the Author)

I am satisfied with the revisions. The mechanism of regulating genomic stability is indirect but is significant.

Reviewer #3

(Remarks to the Author)

The authors nicely addressed my previous comments. There are some presentation issues that could be clarified.

Minor points:

1. Fig. 1a: To clarify the length of the N-terminal region of Msc1, it would be nice to have residue numbers on the TM domain in blue.
2. Fig. 1b: For a schematic presentation of Msc1, since Msc1 is a membrane protein, it should be depicted as it is. If possible, the location of the N- and C-terminal should be described too.
3. Fig. S2 and S3: Please switch Fig. S2 and S3 since Fig. S3 (line 160) appears earlier than Fig. S2 (line 169).

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COMMSBIO-24-4151

“Continued nuclear envelope surveillance is required for DNA double strand break repair.”

Rebuttal Letter.

We would like to thank the reviewers for taking time to thoroughly revise our work. We have considered their suggestions carefully and tried our best to respond to them point by point. As a result, a number of new experiments have been performed, new figures and figure panels have been added to the revised version, and several panels have been moved to supplementary figures to make room for the new data in the main figures:

Table of correspondence between old and new figures:

New Fig / Panel	a	b	c	d	e	f	g	h
1	1a	1b	1c	-	-	-	-	-
2	2b	2b	2c	2d	2e	new	new	new
3	3a	3b	3c	3d	3e	3f	-	-
4	4a + $\Delta chm7$	4c + $\Delta chm7$	4d + $\Delta chm7$	4e	-	-	-	-
5	new	new	new	new	-	-	-	-
6	6a + additional example	6b + $\Delta msc1$, $\Delta chm7$ and $\Delta msc1 \Delta snf7$	new	new	-	-	-	-
7	new	new	7a	7b	7c	7d	7e	-
8	new	new	new	new	-	-	-	-
9	9a	new	-	-	-	-	-	-
S1	new	new	new	new	-	-	-	-
S2	new	-	-	-	-	-	-	-
S3	2a	S1a	S1b	-	-	-	-	-
S4	S2a	S2b	S2c	S2d	-	-	-	-
S5	S3a	S3b	S3c	S3d	new	-	-	-
S6	S4a	-	-	-	-	-	-	-
S7	new	new	-	-	-	-	-	-
S8	new	new	-	-	-	-	-	-

S9	S5a	S5b	S5c	-	-	-	-	-
S10	S6a	-	-	-	-	-	-	-
S11	new	-	-	-	-	-	-	-
S12	S7a	S7b	-	-	-	-	-	-
S13	S8a + $\Delta chm7$	4b + $\Delta chm7$	S8b + $\Delta chm7$	-	-	-	-	-
S14	5a	new	new	5b	S12	-	-	-
S15	S11a	S11b	S11c	S11d	-	-	-	-
S16	new	new	new	-	-	-	-	-
S17	new	-	-	-	-	-	-	-
S18	new	5d	5e	5f	-	-	-	-
S19	6c	-	-	-	-	-	-	-
S20	S13a	S13b	-	-	-	-	-	-
S21	new	8a	8b	8c	8d	-	-	-
S22	new	new	new	new	new	new	-	-

Our point-by-point response to the reviewer's concerns is in blue text below.

#Editor

In particular, please note that the following revisions would be necessary for us to contact our referees again:

1) Please clarify the definitions used in the manuscript (per R1 and R3).

We have clarified the definitions in the manuscript by either defining key terms or opting to use more widely accepted terminology to ensure consistency and clarity (e.g., partitions instead of septation, blebs rather than bulges, etc). We believe these modifications will improve the understanding of the concepts presented in the manuscript.

2) Please examine N-terminal fusions for Figure 1 and include Chm7 for Figure 4 (per R1).

We have forced the N-terminal of Msc1 to reside in the lumen through a chimera with a signal peptide. Interestingly flipping Msc1 orientation renders a non-functional protein (new Figure S1). Chm7 deletion mutant was included for Figure 4 and the related Figure S13. Additionally, we have added data on the $\Delta chm7$ strains in Figures 5, 6 and 8.

3) Please obtain more mechanistic data by performing timelapse (per R2).

We performed time-lapse experiments with the strains containing fluorescently labeled Nup49 and Sec61 to study the stability of the segmented nuclei phenotype in the $\Delta msc1$ mutant with respect to the *MSC1* strain (Figure 2f-h, Figures S7 and S8). The $\Delta msc1 \Delta snf7$ double mutant was also examined (Figure 5d). This is in addition to the time lapse images of Msc1 patch dynamics and telomere reapproximation already presented before (Figure 2e, Figure S6 and Figure 3a-c).

Please address all the other concerns raised by the reviewers. Although adding a DSB reporter system where the break persistence is marked by a repair protein in assessing repair deficiencies to demonstrate DNA repair defect (R2) would enhance the impact of the study, these experiments would not be necessary for further consideration at the journal.

We cite a previous article in iScience (doi: 10.1016/j.isci.2024.110250) where we explored these aspects in depth in cells lacking Msc1. In addition, we have now added an experiment to visualise Rad52 repair factories in the $\Delta snf7$ and $\Delta chm7$ mutants (new Figure 8).

Reviewer #1 (Remarks to the Author):

This work explores the roles of Msc1, a relatively uncharacterized protein in budding yeast, in nuclear envelope morphology and double strand break repair. There is an attempt to connect the phenotypes exhibited by *msc1Δ* cells to those exhibited by deletion of key ESCRT machinery genes and, in so doing, relate genome and nuclear integrity pathways. The data are, in most cases, extensively quantified but they do not make a case for a functional relationship between double strand break repair and nuclear envelope integrity. Indeed, the major conclusions are based on shared phenotypes but there is no compelling genetic or biochemical data presented that links Msc1 to the ESCRT pathway.

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Indeed. We initially opted for the *aid** degron for the ESCRT-III components because of the previous negative genetic interaction with Msc1 orthologs in *S. pombe*. However, it is now clear that depletion of ESCRT-III in the *Δmsc1* background does not affect fitness. This was already evident from the spot assays of *Δmsc1 (ESCRT-III)-aid* in IAA (old Fig 5b, now Fig S14d and S16), but we have now checked it out directly through spore dissection (new Fig S17). Thus, we have generated the double mutants as requested and checked them in the presence of phleomycin. This way, we have ensured the complete loss of function of both ESCRT-III and Msc1 as well as getting rid of the unexpected chemical interaction between IAA and phleomycin. As a result, the figure for the *Δmsc1 SNF7:AID** mutant has been moved to supplementary material (new Fig S14), and has been replaced by a figure for the *Δmsc1 Δsnf7*, *Δmsc1 Δdid4*, *Δmsc1 Δvps24*, and *Δmsc1 Δchm7* double deletion mutants (new Fig 5). The slight synergism between *Δmsc1* and ESCRT-III's *Δsnf7* prevails.

For example, often tagged ESCRTs show gain of function phenotypes. There is no evidence that the authors assessed the functionality of their ESCRT fusions and in Figure 8, the Snf7-GFP localization resembles class E compartments, as opposed to endosomes, which are aberrant structures that arise due to loss or gain of function of ESCRT proteins.

Functionality assays of the Snf7 C' tags have been performed (spot assay and growth curves). The *Snf7-aid** is indeed slightly hypomorphic (new Fig S14b and c), but this is not a problem because genetic interactions with *Δmsc1* are now mainly studied with the double *Δmsc1 Δsnf7* mutant. On the other hand, the reviewer is right about Snf7-GFP: Our tests show it is largely non-functional, so the figure has been moved to supplementary (new Fig S20) and discussed accordingly.

Points to consider to improve the figures and text:

1) Throughout the text there is a reference to “late M cells” or “late M cultures”. It is rarely clear (and certainly is not at all clear in the figures) whether the authors are cherry-picking late-M cells out of an asynchronous population or whether they are using the *cdc15-2* strain at the non-permissive temperature. The labeling of *cdc15-2* as “WT” in the figures is extremely misleading, for example. WT should only be used for wildtype yeast strains. A *cdc15-2* strain, by definition, cannot be WT. This is even more confusing when DNA damaging assays are used on top of the *cdc15-2* arrest as DNA damage would also be predicted to lead to mitotic arrest on its own, likely before the *cdc15*-induced arrest. How would this influence the quantification of this assay?

In all the referred cases, cells are arrested in late-M at 34 °C (non-permissive temperature for *cdc15-2*) before generating DSBs, and the arrest is maintained throughout. Only in two figures cells were collected at the permissive temperature (25 °C) for assessing (i) the effect of DSB when they arrest in G2/M by the DNA damage checkpoint, and (ii) the cell cycle distribution of abnormal NE phenotypes in a subset of mutants and double mutants (Fig 7). And to examine the localization of Msc1 within the NE using the Split-GFP system in both conditions (Fig S1).

We have now taken several actions in the MS to avoid confusion in *cdc15-2* arrests: (i) Y-axis labels have been changed from “% of cells in late-M” to “% of late-M (*cdc15-2*) cells” (i.e., % of whatever phenotype comes exclusively from late-M arrested cells); (ii) in the main text, we have replaced the term “WT” for “*cdc15-2*” and then have added the corresponding mutation afterwards (in some cases, we have highlighted the absence of the mutation; e.g., *cdc15-2 MSC1*); (iii) we have made these changes in the figures wherever possible, but note that there are a few instances where the number of mutants and mutant combination precluded us from adding *cdc15-2* before, or the text would be too large for the chart. As an alternative, we have used “*cdc15-2* (WT)” in the first mention, and define it further in the figure legends.

2) Figure 1: These data are interesting, but it is confusing why the authors choose to examine the localization of the split-GFPs only under conditions of mitotic arrest. It would be more helpful to address the topology without adding this confounding variable. Ideally, the authors would also examine N-terminal fusions as well to round out the assay.

C' tagging was also examined in asynchronous cells in the logarithmic phase of growth and in cells arrested in G2/M by activation of the DNA damage checkpoint after phleomycin treatment for three hours at the permissive temperature (25°C). We have added these results in the new Fig S1.

In addition, we examine N-terminal fusions that force an orientation flip of Msc1, so that the short N-terminal part now faces the lumen whereas all Msc1 motifs relocate to the nucleoplasm (and cytosol under the assumption that Msc1 resides in both the INM and the ONM as its *S. pombe* counterparts). We took the advantage of one of the Jaspersen's construct for this aim, which is based on a signal peptide (from Kar2) that precedes the GFP₁₋₁₀ that is then tagged at N-terminal of Msc1. Interestingly, this orientation flipping renders a non-functional Msc1 with regards to its role in DSB repair (Fig. S1)

3) Figure 2: How do the authors differentiate between two G1 cells close together and late-M cells when quantifying a lack of nuclear envelope bridge?

In these experiments, cells are arrested in late mitosis (late-M) due to the inactivation of the temperature-sensitive *cdc15-2* allele, ensuring that they do not progress to G1. Therefore, two closely positioned G1 cells are not expected in this context, as the arrest is tight and prevents cell cycle progression beyond anaphase/telophase.

4) Figure 4: The sensitivity of the ESCRT deletions to phleomycin is intriguing. To connect more clearly to the nuclear envelope, the authors should include Chm7 as this is the only known nuclear-envelope specific ESCRT.

We have included data on the Chm7 deletion mutant in Figure 4 and the related Figure S13. Additionally, this mutant has been incorporated into the analysis of NPC distribution (Figure 6) and tested in new experiments assessing nuclear envelope permeability and Rad52 localization (Figure 8). The absence of Chm7 unexpectedly does not lead to increased sensitivity to DSBs. This finding led us to discuss whether phleomycin sensitivity in core ESCRT-III mutants is actually independent of NE, or their effects on the NE are indirect.

Reviewer #2 (Remarks to the Author):

The manuscript presents some interesting observations on how a luminal NE protein and a membrane remodelling complex correlate with increased sensitivity to DNA damage as well as specific defects in nuclear shape and nuclear septation during mitosis that may contribute to the phenotype. Models based on nuclear compartmentalization of repair proteins and retrograde transport of sister chromatids are proposed. The principal limitation of the paper is that the interpretation is highly speculative and no direct examination of DSB repair, DSB persistence, or repair protein localization is performed. In the absence of such experiments that directly examine potential mechanisms, the study only convincingly demonstrates a correlation with defects in DSB repair.

Rather than statistical measures of phenotypes from different time points, more mechanistic data would be obtained by performing timelapse.

We have included several short time lapse series for the “segmented nuclei” phenotype. These allowed us to assess the stability of the partitions, particularly differentiating between *MSC1* and $\Delta msc1$ (Figure 2f-h and S7,S8), as well as in $\Delta msc1 \Delta snf7$ (Figure 5d) under both mock and phleomycin treatments. This expands upon our previous phenotype quantifications, confirming that partitions are not only more frequent in the mutants but also more stable. We define stability as their persistence throughout the entire time lapse series (5 min). In the *MSC1* strain, we observed NE motions similar to those previously described for Msc1 patches.

Adding a DSB reporter system where the break persistence is marked by a repair protein would also be helpful in assessing repair deficiencies.

In a previous work (doi: 10.1016/j.isci.2024.110250) which provides the basis for the current study, we assessed DSB reporters (Rad52 and Rfa1) for $\Delta msc1$ in late mitosis. We have now extended this analysis with microscopy-based visualization of Rad52 repair factories in $\Delta chm7$ and $\Delta snf7$ mutants, revealing a slightly higher (yet not significant) accumulation of these foci in $\Delta snf7$ (Figure 8).

While increased DNA damage sensitivity is demonstrated, no repair defect is demonstrated.

In our previous work (doi: 10.1016/j.isci.2024.110250), where we selected Msc1 from a proteomic abundance assay comparing HO-induced and phleomycin-induced damage to a mock control during late mitosis, we found compelling evidence of repair deficiencies in $\Delta msc1$ cells. Specifically, we characterized that $\Delta msc1$ cells exhibit a reduction in the overall rate of homologous recombination, which is reflected in increased Rad53 hyperphosphorylation, accumulation of RPA foci, decreased Rad52 repair factories, and delayed DSB repair. These findings demonstrate that the absence of Msc1 leads to impaired repair processes, consistent with the observed increased DNA damage sensitivity.

Reviewer #3 (Remarks to the Author):

This paper describes the role of Msc1 and ESCRT-III complex in nuclear envelope homeostasis in the budding yeast, *S. cerevisiae*. In the previous report, the author's group identified Msc1 as a possible key player in the repair of DNA double-strand breaks (DSBs) during the M-phase, which is associated with so-called sister chromatid approximation. In this new study, the authors extended their previous characterization of Msc1 and found that Msc1, which is located in the nuclear envelope (NE) with most of the C-terminal portion in the lumen of NE, is critical for the maintenance of NE morphology. Moreover, the authors also showed that ESCRT-III components, Snf7, Did4, and Vps24 are critical for the NE homeostasis as Msc1. The authors also reported a bit complicated genetic interaction of the *msc1* with the ESCRT-III mutant including the AID allele of SNF7; mainly these mutants share similar defects in NE morphology both in the absence or presence of DSBs. The experiments were well-designed and were carried out in good shape. Most of the results are convincing although the effect of the mutations was not drastic. The role of NE in chromosomal events such as DSB repair is one of the fields we have not known much about. Thus, the results in the paper are fascinating and provide a new idea of how NE homeostasis controls various chromosomal events in different cell cycle stages in mitosis. However, since the text was a bit difficult to read as shown below, the authors need additional words to rewrite. In addition, prior to the publication, the authors need some additional experiments to clarify their conclusions.

Major points:

1. The authors analyzed NE morphology of the *msc1* and ESCRT-III mutants in late anaphase mainly as well as G2/M arrest (Figure 7). However, the authors are recommended to provide more detailed results on the NE morphology of these mutants in a normal cell cycle (without DSBs). Do the mutants show abnormal NE in G1, S, or G2 phases? If so, what kind of defects, which are similar in those in late anaphase?

We incorporated new data about abnormal NE phenotypes in logarithmically growing cells (Fig 6a,b). We evaluated both the presence of aberrant NE morphologies and NPC aggregates in $\Delta msc1$ mutants, ESCRT-III complex mutants, and the $\Delta msc1 \Delta snf7$ double mutant. The results from asynchronous cultures of the $\Delta msc1$ mutant are very similar to those observed in cells arrested in either late mitosis by *cdc15-2* or G2/M by the DNA damage checkpoint activation. In ESCRT-III mutants and the double mutant, the effects on NPC aggregates were similar to those previously observed. However, "blurred nuclei" (category c) were only found in cells arrested in late mitosis, with the 'segmented nuclei' (category b) increasing in asynchronous cells of these mutants, though not reaching the frequency seen in the $\Delta msc1$ mutant.

2. In this paper, it seems that the authors wanted to say the "direct" role of Msc1 in the DSB repair in late anaphase. However, as they acknowledged (e.g. Figure 9b), the effect of the mutations on the DSB repair might be indirect. In this line, the main text should be modified so that the authors focus on the role of Msc1 and ESCRT-III in NE morphology under normal conditions if the authors see NE defects in other cell cycle stages (#1 comments).

We fully acknowledge that the effects of Msc1 and ESCRT-III mutations on DSB repair must be "indirect" (i.e., Msc1 molecularly interacts with neither DNA nor HR proteins), as illustrated

in the final hypothetical model (Figure 9). This conclusion is primarily based on the lack of phenotypic differences on the NE between DSB-inducing (phle) and mock conditions, as well as the localization of Msc1's functional domains within the NE lumen. However, despite this indirect effect, sensitivity to DNA-damaging agents is evident, along with the accumulation of unrepaired DNA, impaired re-approximation of sister loci following DSBs in late mitosis, and defects in the completion of homologous recombination repair, as we previously described for $\Delta msc1$ mutants (doi: 10.1016/j.isci.2024.110250). We have carefully revised the manuscript to ensure our interpretation is not misrepresented and have explicitly clarified that the role of these proteins in the DSB response is strong but indirect.

3. In Figure 5C, the authors checked the sensitivity of *msc1* and *msc1* SNF7-AID cells to Phleomycin (Phle). In the absence of IAA, *msc1* SNF7-AID cells are sensitive to Phle. On the other hand, in the presence of IAA (third and fourth panels; without OSTIR1 such as 4th lane in the third panel is obvious), the cells are more resistant to the drug, indicating a partial suppression of the *msc1* defect by the SNF1 depletion or dosage effect. However, the authors did not mention this clearly and should discuss this implication on the functions of MSC1 and ESCRT-III in NE morphology. If the suppression is true, the authors need to check other data in the double mutant in this line.

The observed decrease in phleomycin sensitivity in the presence of IAA is attributed to an unexpected chemical interaction between IAA and phleomycin. This is clearly visible in the $\Delta rad52$ strain, which serves as a positive control for phleomycin sensitivity independent of the auxin-degron system, and in the fact that the effect is observed in strains both with and without OSTIR1. Given the potential for misinterpretation of these data and the fact that double deletion mutants of *msc1* and ESCRT-III complex proteins are viable in *S. cerevisiae*, we have moved the *msc1* SNF7:AID* mutant figure to the supplementary material (Figure S14) and replaced Figure 5 with new data from double deletion mutants ($\Delta msc1 \Delta chm7$; $\Delta msc1 \Delta did4$; $\Delta msc1 \Delta snf7$; and $\Delta msc1 \Delta vps24$). These new results suggest synergism between Msc1 and ESCRT-III proteins, except for Chm7. Additionally, we have incorporated observations on the NE-associated phenotypes and the partial reversion of chromosome segregation in response to late mitosis DSBs in the $\Delta msc1 \Delta snf7$ mutant, which shows the strongest synergistic effect. This mutant has also been used to visualize the NPCs distribution in both late mitosis and asynchronous cultures (Figures 6 and 7)

Minor points:

1. line 25, NE health: Please define the word or use a more familiar word in the Abstract.

We have replaced the term NE “health” with NE “homeostasis” which we consider more commonly understood.

2. Line 136-: In this section, the authors need to explain their experimental system how they introduced DSBs such as by HO (Galactose-induced system is used, please mention when they added gal to media after *cdc15* arrest). A schematic figure for the timeline of each manipulation (times of temperature shift, the addition of phleomycin and sampling) should be presented in Figure 2.

The system we used does not involve a carbon source shift. Instead, we employed the lexO-HO system, which allows precise control of gene expression via β -estradiol without metabolic constraints or toxicity. This is achieved through a chimeric transcription factor (LexA-ER-B112) that, upon estrogen binding, activates the synthetic PlexO promoter to express HO endonuclease. We have explicitly stated this information in the Materials and Methods section, where references to the original articles describing the system can also be found.

We have also added a mention in the suggested section, replacing “controlled expression of HO endonuclease” with “ β -estradiol-inducible expression of HO endonuclease” (line 186). Additionally, to enhance clarity, we have now included a schematic representation of the experimental timeline as Figure S2, detailing the timing of temperature shifts, phleomycin and β -estradiol additions, and sampling. Likewise, we have added a schematic for auxin experiments (Figure S15a).

3. Figure 2b, image of Sec61-GFP: for three images, please add a tracing of the GFP signal in each image, as shown as a representative on the top to explain what “partition” is in the paper (see above).

Although we have not included tracings of the GFP signal for each specific example due to space constraints and to preserve figure clarity, representative sketches for all categories—including the one corresponding to the shown examples—are clearly presented in the graph legend. Furthermore, we have clarified what “partitions” are in the main text by simplifying the use of synonyms throughout.

4. Line 148: Like partition, please show the typical image of the bulge (with the tracing) in Fig.2. Please explain how the bulge is different from the partitioned compartment (with a clear definition).

Although we did not include a tracing of the bleb example in Figure 2 either, we have clarified in the text the conceptual difference between “blebs” (formerly referred to as “bulges,” which we have renamed for clarity) and “partitions” (also referred to as “segmented” or “septated” nuclei). Blebs are NE protrusions containing nucleoplasm that remain fully connected to the main nuclear compartment, whereas partitions represent NE septa that subdivide the nucleus into distinct sub-nuclear compartments. Additionally, we have incorporated a new panel in Figure 9 (hypothetical model) that visually illustrates the differences between normal nuclei, blebbed nuclei, and segmented nuclei.

5. Line 174-179: Is the localization of Msc1-GFP to the nuclear bulge seen even in the absence of DSBs? Please mention this clearly and show representative images like Fig. 2e. It is important to avoid misunderstanding that the bulge is induced by the DSB treatment.

We have removed the reference to “after DSB generation” from the main text, even though the image shown corresponds to a cell undergoing phleomycin treatment, since we also observed Msc1 enrichment at NE blebs in mock-treated cells.

6. line 200: the sentence seems to be incomplete.

We have slightly modified the sentence to avoid the impression that it is incomplete. The revised version now reads: “DSB generation by phleomycin reduced these percentages to 30%, although this reduction occurred equally in both strains.”

7. line 209: What do the authors mean by NE septation? Please define the word in the first place.

This point is addressed in our response to comment 5. Additionally, to avoid confusion, we now refer to them as “partitions” throughout to avoid confusion.

8. Figure 4b-g: Please add *msc1* deletion in these assays as the comparison.

To avoid excessive repetition, we did not include the $\Delta msc1$ strain in Figure 4. However, the values obtained with $\Delta msc1$ mutant can now be compared to those of the ESCRT-III mutant $\Delta snf7$ in the Figures 5b-d and S18, which also includes *MSC1 SNF7* and $\Delta msc1 \Delta snf7$ strains for direct comparison.

9. Figure 4d: For class “d”, please add statistical comparison relative to the wild-type as shown in class “c”.

It has been done.

10. Figure 5d-f: Please add the data of wild-type with or without Phleomycin as a control

We decided to move these data to the Supplementary Information (Figure S14-S16) and replaced them in the main figure with results from double deletion mutants, due to the complexity of interpreting the combined effects of auxin and phleomycin. The new Figure 5 allows for a clearer comparison between double mutants, single mutants, and WT (*cdc15-2*) strains processed in the same experimental batches.

11. Figure 5d, e: please add a statistical comparison between -iaa phle and +iaa phle.

It has been done.

“Continued nuclear envelope surveillance is required for DNA double strand break repair.”

Rebuttal Letter to R1 version.

We would like to thank the reviewers for taking the time to thoroughly revise our work twice. We have considered their new suggestions and responded to them point by point below in blue text.

Reviewer #1 (Remarks to the Author):

Overall, the authors have been responsive to my comments and have thoughtfully revised the manuscript.

Thank you very much.

My only minor point is the authors should not use the term "cytosolic NPC" as it implies the entire NPC is mislocalized as opposed to a single or a subset of nucleoporins/nups, which is more likely the case.

Indeed. We have changed the main text accordingly.

Reviewer #2 (Remarks to the Author):

I am satisfied with the revisions. The mechanism of regulating genomic stability is indirect but is significant.

Thank you very much.

Reviewer #3 (Remarks to the Author):

The authors nicely addressed my previous comments.

Thank you very much.

There are some presentation issues that could be clarified.

Minor points:

1. Fig. 1a: To clarify the length of the N-terminal region of Msc1, it would be nice to have residue numbers on the TM domain in blue.

These residue numbers have been added to the figure panel.

2. Fig. 1b: For a schematic presentation of Msc1, since Msc1 is a membrane protein, it should be depicted as it is. If possible, the location of the N- and C-terminal should be described too.

We have drawn a new schematic representation whereby Msc1 is better depicted, including the fact it is embedded in the NE through a unique TM domain.

3. Fig. S2 and S3: Please switch Fig. S2 and S3 since Fig. S3 (line 160) appears earlier than Fig. S2 (line 169).

The first citation of Fig. S2 occurs on line 132, and then again on line 169. Therefore, the order is correct.