

Dynamins maintain nuclear envelope homeostasis and genome stability

Redactions – unpublished data

Corresponding Author: Professor Ira Milosevic

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)

In the manuscript "Dynamins maintain nuclear envelope homeostasis and genome stability," Aveleira et al. describe a novel role for dynamins (essentially dynamin 2 in this work) in maintaining nuclear envelope integrity. The authors clearly demonstrate that different and abnormal nuclear morphologies result from the complete lack of dynamins 1/2/3 or when dynamin function is impaired using a targeted mutant or an inhibitor. They further show that cells lacking dynamins have decreased lamin levels, increased DNA damage and a deficiency in DNA repair proteins. Interestingly, some of these defects observed in dynamin TKOs are rescued when the cells are treated with nocodazole. Together, these results suggest a novel role for the dynamins in preserving nuclear envelope integrity and genome stability, suggested through interaction with microtubules. This is a new set of functions for the dynamins and, if substantiated, would be of broad interest to the cell biological community generally. The authors clearly demonstrate nuclear abnormalities by IF and cryo-ET. However, a number of control and clarifying experiments or descriptions are suggested that would enhance the findings in this work.

Major comments:

The authors should absolutely report the results of 4-OHT treatment of control mouse fibroblasts, using the same regime as used to eliminate dynamins 1-3. The authors should show nuclear morphology and γ -H2AX staining under these conditions - and it would be necessary to check if tamoxifen/4-OHT by itself causes DNA damage (see small point below).

Figure 1b: More clarity on the category of nucleus that is considered dysmorphic? Is it all of the phenotypes described for dynamin TKO in Figure 1a? Are these nuclei analyzed by eye and binned into different categories?

Figure 1a: Are the representative nuclei shown in 1a age- and passage-matched to the control nucleus? Nuclear morphology is known to deteriorate with aging.

When Dyn2 is reintroduced to the Dynamin TKOs, is there a complete rescue of all nuclear morphology phenotypes? From figure 1g and extended figure 6, Dyn2 expression in the TKOs still seem to have nuclear invaginations. Is a figure 1d-like analysis performed on the Dyn2 rescues in the TKOs? How long is Dyn2 or Dyn2K44A expressed in cells before performing the experiments?

The authors need to include a positive control for K44A - it has well known effects on the membrane and endocytosis which could perhaps be used for this purpose.

Some authors have shown that K44A-type mutants in dynamin family members result in self-assembly into stable helices that can themselves drive membrane morphology changes - the use of only K44A therefore is a limit. Many assembly-deficient dynamin mutants are known and the authors could show that assembly activity is required for dynamin's role at the NE.

Figure 5: Do the dynamin K44A mutants have defective nuclear repair, similar to TKO cells?

Is the cell viability affected in the TKOs and the K44A mutants, given that these cells have increased DNA damage and reduced repair?

Is there more nucleophagy in these cells? More lysosomal activity to clear these damages? How does this change with CPT treatment?

Dynasore may have an effect on dynamins but it has very well known off-target effects also (PMID: 24046449 and PMID: 25889964). Fig. 4K may not be due to dynamin inhibition at all.

Minor comments:

Figure 1a,1f: How do authors differentiate between micronuclei and vesicles? Is there a size cutoff used in the analysis?

Figure 2: Can the authors comment on the significance of the cleaved caspase-3 data shown in Figure 2?

Figure 2: What is causing the difference in levels of cleaved caspase-3 observed with imaging vs. western blot analysis? Is it possible to show a positive control that increases levels of cleaved caspase-3?

Figure 2 and extended figure 6: Image representation in extended figure 6a seems easier to visualize the retention of dynamin in the nuclear envelope compared to the ones shown in figure 2g. Is the dynamin localization in the CTRL and Dyn TKO+Dyn2 cells similar? The authors suggest dynamin retention in the nuclear envelope when Dyn2 K44A is introduced in the TKOs or the control cells. It would be useful to have timestamps similar to those shown in extended figure 6a to make this more convincing. Quantifying how long these dynamin are in the nuclear envelope under different conditions/rescues would be helpful.

Figure 3: Please provide a color code for the tomograms.

Figure 3: Is the same budding phenomenon observed when the dyn K44A mutant is expressed in WT cells?

Figure 4: How is H2AX intensity quantified? Is the nucleus segmented based on Lamin A/C and then quantified for H2AX signal? Is the H2AX quantification provided in the figures, per nucleus? It would be more appropriate to normalize the H2AX intensities to nuclear area since the TKO cells have a larger nuclear area compared to control cells.

Figure 4: Why is the lamin staining different in Figure 4d from the rest of the lamin stainings in this panel (Figure 4a, 4g, etc)

Extended figure 8: Label extended Fig 8g as control and h as TKO for clarity

Authors show nocodazole rescue of dynamin TKO defects. Do they see similar effects of nocodazole in the dyn K44A mutant? Does nocodazole rescue the dynamin retention/stalled vesicles phenotype observed in the Dyn K44A mutant?

Small points:

The authors occasionally use tamoxifen and 4-OHT interchangeably. They have quite different effects on the genome: Tamoxifen has been shown to itself cause DNA damage (e.g., PMID: 10825130), so this inconsistency should be addressed.

Reviewer #2

(Remarks to the Author)

This work examines a potential role for dynamin GTPases in controlling some element of nuclear envelope remodeling that is also linked to DNA damage. As there is considerable interest in mechanisms at the intersection of nuclear and genome integrity, the central focus of the work should be of broad interest. The study leverages published cell lines where all three dynamins can be conditionally knocked out with the foundational observation being that there is considerable nuclear dysmorphism in the TKO cell line including interesting blebbing off the nucleus. In this way, the phenotypes are similar to those observed in contexts of lamina dysfunction and/or cell senescence. That DNA damage appears to be triggered alongside a downregulation of the DNA damage response is very interesting particularly as this damage often appears captured in the nuclear envelope blebs (that may reflect nuclear ruptures, see below). The authors propose a model where dynamins are required for outer nuclear membrane fission during a selective nuclear autophagy pathway (i.e. nucleophagy) that clears DNA damage from the nucleus. This is very exciting but both concepts i.e. that dynamins function to drive outer nuclear membrane fission and that nucleophagy can remove some DNA damage in nuclear buds have been recently published by other groups (PMIDs 39920277 and 39265577) limiting the potential impact. Nonetheless, demonstrating that dynamins also function at the nuclear envelope in mammalian cells would be an important advance.

Major Points:

1) No evidence for direct engagement of dynamins with the nuclear envelope. Although there is some microscopic data presented that a dynamin GTPase mutant localizes close to the nuclear envelope, the lack of colocalization with any nuclear envelope marker limits the conclusions possible particularly in lieu of any biochemical link between dynamins and any nuclear envelope protein. Images as those shown in Figure 2g would be more compelling if the Dyn2-K44A mutant colocalized with lamins, for example. Quantification of the prevalence of the localizations shown in Figure 2g is also essential (in addition to controls with the WT dynamin protein). The authors could also interpret why there doesn't seem to be any colocalization between the dynamin and lamin staining in Extended Figure 6. Another suggestion would be to leverage data in the budding yeast study (PMID 39920277) where the dynamin is recruited by the autophagy protein Atg11. The orthologue of Atg11 is thought to be FIP200.

2) No link to autophagy. Despite the published work and the proposed model, there is no investigation of autophagy in the TKO. At a minimum, examining the localization of LC3 and TEX264 in the TKO could be valuable. Further, examining the impact of bafilomycin treatment on the prevalence of the lamin or DNA-damage containing buds could also provide a more definitive link to autophagy to strengthen this conclusion.

3) The relationship between loss of nuclear envelope integrity and DNA damage is well established even as the mechanisms remain controversial. The authors have not ruled out that an indirect consequence of dynamin dysfunction in (for example) mitosis leads to losses of nuclear integrity, or, that it is involved in a nuclear envelope repair mechanism that is triggered after a rupture in the nuclear envelope. At a minimum, the authors should address the integrity of the nucleus by localizing a fluorescent reporter like an NLS-GFP.

Minor points:

1) The downregulation of lamins is not addressed in any way. It is possible that the observed phenotypes are directly related to loss of lamin levels and have nothing to do with dynamins. In lieu of stronger data linking dynamins to the nuclear envelope, this caveat should at least be mentioned in the text.

2) The cryo-ET analysis is very nice but could benefit from quantification. The reader needs to understand how many tomograms were examined and the number of observed structures segmented - statements like "very rarely detected" should be reflected by numbers. Further, it is curious that the narrow membrane necks appear between the outer nuclear membrane and what appears to be an expanded ER cisternae. These data might suggest that dynamins contribute to ER morphology and/or to maintaining connections between the ER and the nuclear envelope as these connections are thought to contain a unique, but so far unknown, set of proteins. See, for example, PMID 38877171. Further, the double-membrane structures that contain nuclear fragments are not connected to the nuclear envelope. Thus, fission has already occurred. How do the authors reconcile these data with their model? Have the authors examined ER morphology more broadly?

3) In cryo ET description in text: "nuclear intermembrane space" is an atypical description of the more commonly termed perinuclear space or nuclear envelope lumen. Further, the authors suggest there are "ruptures" of the inner nuclear membrane but these aren't really discussed. To my knowledge, this would be a unique phenotype and some consideration of how this might arise might be worth considering in the discussion. For example, an inner nuclear membrane rupture would presumably leak luminal components into the nucleus.

4) In the discussion, the authors seem to draw a distinction between selective autophagy of TEX264 and "nucleophagy" in yeast. Nucleophagy is, by definition, selective autophagy of the nucleus. Thus, the TEX264 pathway is also nucleophagy.

5) The authors should consider also referencing other papers on nucleophagy for example: PMIDs: 26524528; 37118512

Reviewer #3

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have made commendable efforts to address the questions raised by the reviewers. The inclusion of the well-characterized assembly-deficient dynamin2-R361S and dynamin2-R399A mutants has significantly enhanced the study of nuclear envelope dysmorphisms. We also appreciate the efforts to characterize autophagosome formation in the KO lines. These additions have provided necessary clarity to the mechanism. Furthermore, the additional controls have enhanced the impact of the manuscript.

Regarding the point raised by Reviewer 2 concerning the need for direct proof of dynamin engagement with the nuclear envelope (via colocalization with dynamin GTPase), we acknowledge the validity of this point but also recognize the technical limitations preventing this experiment. We are satisfied with the current level of evidence and encourage the authors to explore this particular interaction further in future studies. We support the publication of the manuscript in its revised form and commend the authors on an exciting study.

Reviewer #2

(Remarks to the Author)

I appreciate the efforts by the authors to address my criticisms both in the introduction of new data and new discussion. I think overall they make a compelling case for the role of dynamins in impacting nuclear envelope homeostasis even if the mechanism remains a bit uncertain. With the latter in mind, the authors are also thoughtful in their discussion about the limitations of their conclusions, which is also appreciated. I am supportive of publishing this work asap.

Reviewer #3

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

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We thank reviewers for their time and insightful comments, which we used to improve our manuscript. We also thank the editor for inviting resubmission.

Reviewer #1 (Remarks to the Author):

In the manuscript "Dynamins maintain nuclear envelope homeostasis and genome stability," Aveleira et al. describe a novel role for dynamins (essentially dynamin 2 in this work) in maintaining nuclear envelope integrity. The authors clearly demonstrate that different and abnormal nuclear morphologies result from the complete lack of dynamins 1/2/3 or when dynamin function is impaired using a targeted mutant or an inhibitor.

We thank the Reviewer for recognizing that our study clearly demonstrates the occurrence of distinct and abnormal nuclear morphologies upon complete loss or functional impairment of dynamins 1/2/3. This was an unexpected observation, and we therefore performed extensive control and additional experiments to rigorously validate and confirm this previously unrecognized role of dynamins.

They further show that cells lacking dynamins have decreased lamin levels, increased DNA damage and a deficiency in DNA repair proteins. Interestingly, some of these defects observed in dynamin TKOs are rescued when the cells are treated with nocodazole. Together, these results suggest a novel role for the dynamins in preserving nuclear envelope integrity and genome stability, suggested through interaction with microtubules. This is a new set of functions for the dynamins and, if substantiated, would be of broad interest to the cell biological community generally. The authors clearly demonstrate nuclear abnormalities by IF and cryo-ET. However, a number of control and clarifying experiments or descriptions are suggested that would enhance the findings in this work.

We thank the reviewer for the constructive feedback. We have addressed all comments with new experiments and additional data, which further support and strengthen our findings.

Major comments:

The authors should absolutely report the results of 4-OHT treatment of control mouse fibroblasts, using the same regime as used to eliminate dynamins 1-3. The authors should show nuclear morphology and γ -H2AX staining under these conditions - and it would be necessary to check if tamoxifen/4-OHT (not tomo) by itself causes DNA damage (see small point below).

Thank you for this insightful comment. We would like to clarify that throughout the study we consistently used **4-hydroxytamoxifen (4-OHT)**, not tamoxifen; this labeling error has now been corrected in the manuscript. Early in the study, we verified that the observed effects are not due to 4-OHT treatment. To ensure rigor, we performed a new set of experiments which also revealed that 4-OHT does not affect nuclear morphology or the circularity index, nor does it increase γ -H2AX levels. These new data are now included in **Extended Data Fig. 2e–f** (nuclear circularity examined in wild-type cells treated with 4-OHT) and **Extended Data Fig. 8a–c** (γ -H2AX protein levels in the nuclei and whole cell homogenates were examined in wild-type cells treated with 4-OHT). As we confirmed that 4-OHT treatment does not alter lamin-A or lamin-C expression levels, we have included these data as well (**Extended Data Fig. 5a**).

Figure 1b: More clarity on the category of nucleus that is considered dysmorphic? Is it all of the phenotypes described for dynamin TKO in Figure 1a? Are these nuclei analyzed by eye and binned into different categories?

We are happy to provide more clarity to this point. While systematically studying nuclear morphology in cells without dynamins, we observed several distinct nuclear phenotypes and classified them as presented in Figure 1a. Notably, no additional phenotypes were detected in dynamin TKO cells—every observed nucleus could be assigned to one of the six defined categories. We illustrated both the most common examples (e.g., **Extended Data Fig. 2g**, upper left panel; **Extended Data Fig. 2h**, bottom example) and some of the more striking nuclear dysmorphisms (e.g., **Extended Data Fig. 2g**, lower left panel, where unusually elongated “necks” can be seen).

While the circularity index was quantified using image analysis software, nuclear morphology was assessed visually in a blinded manner. Three independent researchers, working in two separate institutions, performed these assessments. Although complete blinding was challenging given the distinct phenotype of dynamin TKO cells, the analyses were consistent across investigators. Furthermore, we consulted multiple experts in relevant fields—including dynamin biology, progeria/senescence, and nuclear structure—before finalizing the classification scheme.

To be classified as a lamin-A/C–positive vesicle, a structure had to be clearly separated from the nucleus and distinguished from micronuclei based on its interphase origin, higher abundance (typically >3 vesicles per cell), and smaller size ($\leq 1/100$ of the nuclear area in fluorescence images). In contrast, micronuclei typically arise during mitosis, are fewer in number (1–3 per cell), and are larger in size (up to $1/10$ of the nuclear area). Structures meeting the above criteria but remaining continuous with the nucleus were classified as nuclear buds.

Please note we have expanded the Methods section and added additional explanatory details to the relevant figure legends to ensure full transparency of our approach.

Figure 1a: Are the representative nuclei shown in 1a age- and passage-matched to the control nucleus? Nuclear morphology is known to deteriorate with aging.

We thank the reviewer for raising this important point. All cells shown in Figure 1a and throughout this study were carefully age- and passage-matched to their respective controls: we took particular care to maintain identical culture conditions throughout. We have now clarified this in the Methods section.

When Dyn2 is reintroduced to the Dynamin TKOs, is there a complete rescue of all nuclear morphology phenotypes? From figure 1g and extended figure 6, Dyn2 expression in the TKOs still seem to have nuclear invaginations. Is a figure 1d-like analysis performed on the Dyn2 rescues in the TKOs?

We appreciate the reviewer’s careful observation. Because visual assessment of nuclear morphology is highly time-consuming and (to some extent) subjective, our analysis primarily focused on the objective quantification of nuclear circularity, calculated for every dynamin-TKO cell expressing either wild-type (WT) dynamin-2, or one of the dynamin-2 mutants.

Upon re-examining the data for dynamin-TKO cells rescued with WT dynamin-2, we confirm the Reviewer's point that some of these nuclei still exhibit invaginations, albeit rarely. On average, these invaginations are less complex—predominantly type I—and less pronounced compared to those in non-rescued dynamin-TKO cells. This observation has now been noted in the Results.

How long is Dyn2 or Dyn2K44A expressed in cells before performing the experiments?

Both wild-type dynamin-2 and all dynamin-2 mutants were expressed for 24 hours. We have now also included this information in the Figure legends for clarity.

The authors need to include a positive control for K44A - it has well known effects on the membrane and endocytosis which could perhaps be used for this purpose. Some authors have shown that K44A-type mutants in dynamin family members result in self-assembly into stable helices that can themselves drive membrane morphology changes - the use of only K44A therefore is a limit. Many assembly-deficient dynamin mutants are known and the authors could show that assembly activity is required for dynamin's role at the NE.

We thank the Reviewer for this helpful comment. In this study we focused on dynamin-2, as it represents the predominant dynamin expressed in fibroblasts. In designing these experiments, we consulted with Dr. Jenny Hinshaw (NIH) and built upon the previous work of Dr. S. Schmid (Ramachandran R, Surka M, Chappie JS, Fowler DM, Foss TR, Song BD, Schmid SL. The dynamin middle domain is critical for tetramerization and higher-order self-assembly. EMBO J 26, 559-66 (2007)) to select two additional mutations: dynamin2-R361S and dynamin2-R399A that disrupt the tetrameric structure of dynamin in its unassembled state, thereby impairing its ability to stably bind to membranes and nucleate higher-order self-assembly. We performed cloning as detailed in Methods, and experiments as for dynamin2-K44A. Of note, no rescue of nuclear envelope dysmorphisms in dynamin-TKO cells was observed upon expressing of dynamin2-R361S-mRFP or dynamin2-R399A-mRFP mutants in these cells (Fig. 1i, Extended Data Fig. 3d). The corresponding data are now presented in **Figure 1i** and **Extended Data Fig. 3d**. Please note that we did not combine these results with earlier datasets, as the new experiments were performed using different microscope settings.

Figure 5: Do the dynamin K44A mutants have defective nuclear repair, similar to TKO cells?

To address this point, we examined RAD51 protein levels and localization in dynamin-TKO cells expressing the dynamin2-K44A mutant. Our results show that the dynamin2-K44A mutant also exhibits lower nuclear RAD51 intensity, closely resembling the phenotype observed in TKO cells, and suggesting defective nuclear repair. These new data are presented in **Figure 6i** and **Extended Data Fig. 9c**, and the corresponding findings have been described in the Results section. Of note, use of 4-OHT does not affect the RAD51 expression levels (**Extended Data Fig. 7d**).

Is the cell viability affected in the TKOs and the K44A mutants, given that these cells have increased DNA damage and reduced repair?

We thank the reviewer for this important question. We have previously performed several cell viability and apoptosis assays, including a trypan blue exclusion assay, TUNEL and caspase-3 assays. We have now additionally assessed viability and apoptosis in dynamin-TKO cells using PI/Annexin V staining (new **Figure 3d**), and have relocated the TUNEL data from the Extended data to the main Figure. A positive control (H_2O_2 treatment) was included in most experiments but omitted from the main figure because its robust signal would mask the experimental effects. We further examined the incidence of cell death in cells expressing dynamin2-K44A and found no difference compared to other transfected cells. In sum, these various analyses indicate that cell viability is not significantly reduced under the conditions used in this study, despite the increased DNA damage and reduced repair capacity.

Of note, we observed that dynamin-TKO cells exhibit increased senescence (data provided below in **Figure R1**; these results require further investigation and follow-up before publication so we did not include them in the present manuscript). Importantly, during senescence, cells undergo metabolic and signalling changes that favour survival, not cell death. These findings further support data showing that overall cell viability is not compromised in dynamin-TKO cells.

Redacted

*Is there more nucleophagy in these cells? More lysosomal activity to clear these damages?
How does this change with CPT treatment?*

To address Reviewer's comment, we first set to determine if dynamin TKO cells are able to produce autophagosomes, and if autophagy flux is affected in this model. Hence, we investigated autophagy in control and dynamin-TKO cells, and observed increased LC3B-II levels (**Fig. 7a**). Upon expressing LC3B-mRFP in control and dynamin TKO cells, we noted accumulation of enlarged autophagosomes (**Fig. 7b-c**), suggesting reduced autophagic flux. These observations were further supported by elevated p62/SQSTM1 (**Fig. 7d**) and direct flux measurements (**Fig. 7e-f**). The reduction in flux is not due to decreased lysosomal mass, as LAMP1 levels were increased (**Fig. 7g**), though we cannot fully exclude functional lysosomal defects.

The camptothecin (CPT) treatment indirectly results in nucleophagy through the nuclear damage induction. Yet, when control and dynamin-TKO cells were treated with CPT, we do not observe changes in autophagosome biogenesis in dynamin TKO cells (see **Figure R2** below). We have now included these autophagy data in Results section and have extended a discussion of this matter.

Redacted

Dynasore may have an effect on dynamins but it has very well known off-target effects also (PMID: 24046449 and PMID: 25889964). Fig. 4K may not be due to dynamin inhibition at all.

We agree with the reviewer that dynasore, while affecting dynamins, is also known to have significant off-target effects. Accordingly, the effects shown in **Fig. 4k** and **Extended Data Fig. 4** may not be solely attributable to dynamin inhibition. We have now addressed this point explicitly in the Results and also in the Figure legends.

Minor comments:

Figure 1a, 1f: How do authors differentiate between micronuclei and vesicles? Is there a size cutoff used in the analysis?

While assessing nuclear morphology in dynamin TKO cells we observed a distinct NE phenotype characterized by the presence of several small lamin-A/C-positive vesicles. These vesicles were not connected to the nucleus, and were distinguished from micronuclei by their interphase origin, abundance (more than 3 per cell) and small size (in the fluorescent microscopy images, their sizes were defined up to 1/100th of the nuclear size). It is not challenging to differentiate lamin-A/C-positive vesicles from the micronuclei that arise during cell cycle, and there are usually just 1-3 per cell, with sizes up to 1/10th of the size of the main nucleus as in the wild-type mouse fibroblasts. Taking this into account, we set a cutoff size of 1/100th of the nucleus area to define lamin A/C-positive vesicles, and when there are more than 3 such structures per cell. These criteria are now detailed in Methods.

Figure 2: Can the authors comment on the significance of the cleaved caspase-3 data shown in Figure 2? What is causing the difference in levels of cleaved caspase-3 observed with imaging vs. western blot analysis? Is it possible to show a positive control that increases levels of cleaved caspase-3?

Our data show that cleaved caspase-3 levels are reduced in the dynamin TKO cells, which display a senescent phenotype (see **Figure R1**). These findings are consistent with the well-known resistance of senescent cells to apoptosis. During senescence, cells undergo metabolic and signaling changes that favor survival, including reduced activation of caspase-3. Persistent DNA damage signaling and cell-cycle arrest further reinforce this pro-survival state, limiting the ability of senescent cells to engage apoptotic programs even under stress conditions. Together, these features may explain the diminished caspase-3 cleavage observed in the dynamin-TKO cells. All that said, we still observe non-consistency between immunocytochemistry and Western blot data. We carefully examined both data sets, and decided to remove the Western blot data from this publication, as they require further investigation before they can be published.

Figure 2 and extended figure 6: Image representation in extended figure 6a seems easier to visualize the retention of dynamin in the nuclear envelope compared to the ones shown in figure 2g. Is the dynamin localization in the CTRL and Dyn TKO+Dyn2 cells similar?

Yes. We carefully examined this point and found that the dynamin-2 localization in control and dynamin-TKO cells rescued with dynamin-2 seems to be comparable i.e. dynamin-2 is found to be mainly cytosolic, present at endocytic pits and to transiently interact with nuclear surface.

The authors suggest dynamin retention in the nuclear envelope when Dyn2 K44A is introduced in the TKOs or the control cells. It would be useful to have timestamps similar to those shown in extended figure 6a to make this more convincing.

Due to change in Extended Data figure order, Extended figure 6 is now Extended figure 5. Of note, the dynamin-2 K44A mutant remained stably associated with membranes throughout duration of our experiments, which precludes meaningful time-resolved analyses. We are aware that such experiment was included in **Extended Data Fig. 5**; it was originally intended to illustrate that the dynamin-2 K44A mutant remains membrane-associated for at least one minute. In practice, however, the mutant persisted at the membrane throughout the entire

image acquisition period (several minutes, sometimes tens of minutes). We sometimes observed a progressive accumulation of the dynamin-2 K44A mutant signal in some instances (and tried to depict that in new **Figure 3h**), yet dynamin-2 K44A did not dissociate from membranes under the live-cell imaging conditions used in this study. This note is now added to in the figure legend.

Figure 3: Please provide a color code for the tomograms.

Thank you for this insightful note - a colour code for the tomograms is now added in the Figure 4 and its figure legend.

Figure 3: Is the same budding phenomenon observed when the dyn K44A mutant is expressed in WT cells?

Yes, we also observed the budding phenotype upon overexpression of the dynamin-2 K44A mutant in WT cells for 24 hours; however, it was less pronounced than in dynamin-TKO cells. Because we did not manually quantify this dataset (nuclear buds are rare and such analysis is extremely time-consuming — for example, we examined >2000 cells per condition for the control and dynamin-TKO datasets, and a comparable dataset was not available for dynamin-2 K44A expression), these observations are not quantified in the manuscript. Nevertheless, the presence of nuclear buds in dynamin-2 K44A-expressing cells can be seen in Figure 1j (indicated by the arrow).

Figure 4: How is H2AX intensity quantified? Is the nucleus segmented based on Lamin A/C and then quantified for H2AX signal? Is the H2AX quantification provided in the figures, per nucleus? It would be more appropriate to normalize the H2AX intensities to nuclear area since the TKO cells have a larger nuclear area compared to control cells.

Thank you for raising this point. We have revised the Methods section to increase clarity. Nuclei were segmented based on lamin A/C staining in **Figure 5a-b**, and γ -H2AX intensity was quantified per defined region of interest (ROI) given that dynamin-TKO cells have a slightly bigger nuclei than control cells. Specifically, z-stack confocal images of nuclei stained with lamin A/C and γ -H2AX were acquired, and maximum-intensity projections were generated and analyzed in FIJI. Nuclei in all images were automatically segmented, and ROIs of identical size were applied to both cell types. The mean γ -H2AX fluorescence intensity within each ROI was then determined using FIJI.

Figure 4: Why is the lamin staining different in Figure 4d from the rest of the lamin stainings in this panel (Figure 4a, 4g, etc)

The lamin signal shown in the former Figure 4d (now Figure 5d) differs from that in the other panels because it was acquired and presented differently. Specifically, Figure 5d was imaged using a custom-built spinning disc confocal microscope (see Methods for details), and a single optical section is shown, whereas the other panels (e.g., Figures 5a, g, i, k) were acquired using a standard confocal microscope and are presented as maximal projections. We have now clarified this distinction in the figure legend.

Extended figure 8: Label extended Fig 8g as control and h as TKO for clarity

We thank the reviewer for this careful observation. The respective labels have been added to the figure.

Authors show nocodazole rescue of dynamin TKO defects. Do they see similar effects of nocodazole in the dyn K44A mutant? Does nocodazole rescue the dynamin retention/stalled vesicles phenotype observed in the Dyn K44A mutant?

To address the Reviewer's comment, we performed new experiments in which control and dynamin-TKO cells were transfected with dynamin-2 K44A. Twenty-four hours post-transfection, the cells were treated with nocodazole as previously described. Unexpectedly, nearly all transfected cells failed to survive nocodazole treatment, or exhibited severely compromised morphology. We repeated the experiment with consistent results. Although the underlying cause of this increased cell death remains unclear, these technical limitations currently prevent us from further pursuing this specific analysis.

Small points:

Ther authors occasionally use tamoxifen and 4-OHT interchangeably. They have quite different effects on the genome: Tamoxifen has been shown to itself cause DNA damage (e.g., PMID: 10825130), so this inconsistency should be addressed.

We apologize for the confusion. Throughout the study, we used 4-hydroxytamoxifen (4-OHT), and any previous references to "tamoxifen" were incorrect. This distinction has now been clarified in the manuscript.

Reviewer #2 (Remarks to the Author):

This work examines a potential role for dynamin GTPases in controlling some element of nuclear envelope remodeling that is also linked to DNA damage. As there is considerable interest in mechanisms at the intersection of nuclear and genome integrity, the central focus of the work should be of broad interest.

Thank you for recognizing the significance of these findings. We devoted substantial resources and effort to validating these results, and we are excited to be able to share them with the community.

The study leverages published cell lines where all three dynamins can be conditionally knocked out with the foundational observation being that there is considerable nuclear dysmorphism in the TKO cell line including interesting blebbing off the nucleus. In this way, the phenotypes are similar to those observed in contexts of lamina dysfunction and/or cell senescence. That DNA damage appears to be triggered alongside a downregulation of the DNA damage response is very interesting particularly as this damage often appears captured in the nuclear envelope blebs (that may reflect nuclear ruptures, see below).

Thank you for raising this point. We are aware of previous studies reporting that DNA damage can induce nuclear blebbing and rupture. However, the phenotype observed in our study appears distinct from those reports. Our findings reveal a unique form of nuclear

envelope remodeling linked to dynamin function, not previously associated with canonical DNA damage-induced blebbing. As experts in membrane trafficking with over two decades of experience studying dynamins, we initially focused on the membrane remodelling aspects underlying this phenotype. In response to the Reviewer's insightful comments below, we performed additional experiments to further assess the potential link between DNA damage and the nuclear envelope defects observed in dynamin-TKO cells (see details below).

The authors propose a model where dynamins are required for outer nuclear membrane fission during a selective nuclear autophagy pathway (i.e. nucleophagy) that clears DNA damage from the nucleus. This is very exciting but both concepts i.e. that dynamins function to drive outer nuclear membrane fission and that nucleophagy can remove some DNA damage in nuclear buds have been recently published by other groups (PMIDs 39920277 and 39265577) limiting the potential impact.

We respectfully disagree with the comment suggesting limited impact. We acknowledge the novelty of the studies cited, including PMID: 39265577 that originates from our work (these two studies were developed around the same time). We are also familiar with the work of Lusk and King (PMID: 39920277), which we have followed for over two years. In budding yeast (*Saccharomyces cerevisiae*; model in 39920277), several dynamin-related proteins (DRPs) exist, each dedicated to specific membrane-remodelling processes. Unlike mammals, which express three classical dynamins (DNM1/dynamin-1, DNM2/dynamin-2, and DNM3/dynamin-3), yeast DRPs are “dynamin-like” GTPases that function in diverse organellar pathways rather than in endocytosis per se. Although dynamins and DRPs belong to the same superfamily, they are distinct proteins. The study by Lusk et al. investigates the role of Dynamin-like protein 1, known to have a role in mitochondrial and peroxisomal fission in budding yeast (of note, in mammalian cells, the ortholog of this protein is Drp1, not dynamin; the ortholog of dynamin in yeast cells is Vps1). Our study focuses on the classical dynamins in mammalian cells: their role in nuclear envelope remodelling and DNA damage has not previously been explored.

Nonetheless, demonstrating that dynamins also function at the nuclear envelope in mammalian cells would be an important advance.

Major Points:

1) No evidence for direct engagement of dynamins with the nuclear envelope. Although there is some microscopic data presented that a dynamin GTPase mutant localizes close to the nuclear envelope, the lack of colocalization with any nuclear envelope marker limits the conclusions possible particularly in lieu of any biochemical link between dynamins and any nuclear envelope protein. Images as those shown in Figure 2 g would be more compelling if the Dyn2-K44A mutant colocalized with lamins, for example. Quantification of the prevalence of the localizations shown in Figure 2g is also essential (in addition to controls with the WT dynamin protein). The authors could also interpret why there doesn't seem to be any colocalization between the dynamin and lamin staining in Extended Figure 6. Another suggestion would be to leverage data in the budding yeast study (PMID 39920277) where the dynamin is recruited by the autophagy protein Atg11. The orthologue of Atg11 is thought to be FIP200.

We agree with the reviewer that this study does not provide direct evidence for the physical engagement of dynamins with the nuclear envelope. We recognize the importance of this

point and have clarified this limitation in the revised Discussion. Despite the technical challenges in visualizing transient, low-abundance membrane interactions, several complementary experiments suggest that dynamins may transiently associate with the nuclear envelope.

First, we detect dynamin—a cytosolic protein that typically associates with cytoplasm-facing membranes—in the nuclear fraction (**Fig. 3g**).

Second, reintroduction of dynamin-2 into dynamin-TKO cells rapidly (within 6 h, the earliest time point analysed) rescues nuclear envelope dysmorphisms, suggesting a transient interaction that is difficult to capture once the phenotype is resolved. Consistent with this, we observe dynamin-2 transiently “visiting” the nuclear surface in live-cell imaging of dynamin-TKO cells (**Extended Data Fig. 5c**). These imaging sessions were conducted with a 4-min interval over 24–36 h due to acquisition limits, so we cannot comment on events occurring at shorter timescales. Lamin-A was co-expressed for reference; however, as lamin localizes beneath the inner nuclear membrane while dynamin likely interacts with the outer nuclear membrane, direct colocalization was not expected.

Third, expression of the GTPase-deficient mutant dynamin-2 K44A—which retains membrane-binding capacity through its pleckstrin homology domain but becomes ‘locked’ on membranes due to impaired GTP hydrolysis—leads to the accumulation of fluorescent signal near nuclear envelope buds and blebs (**Fig. 3h**). We intentionally avoided using outer nuclear membrane markers in these experiments, as overexpression of ONM components such as full-length nesprin (of the LINC complex) perturbed nuclear envelope organization and dynamics in our hands, complicating interpretation.

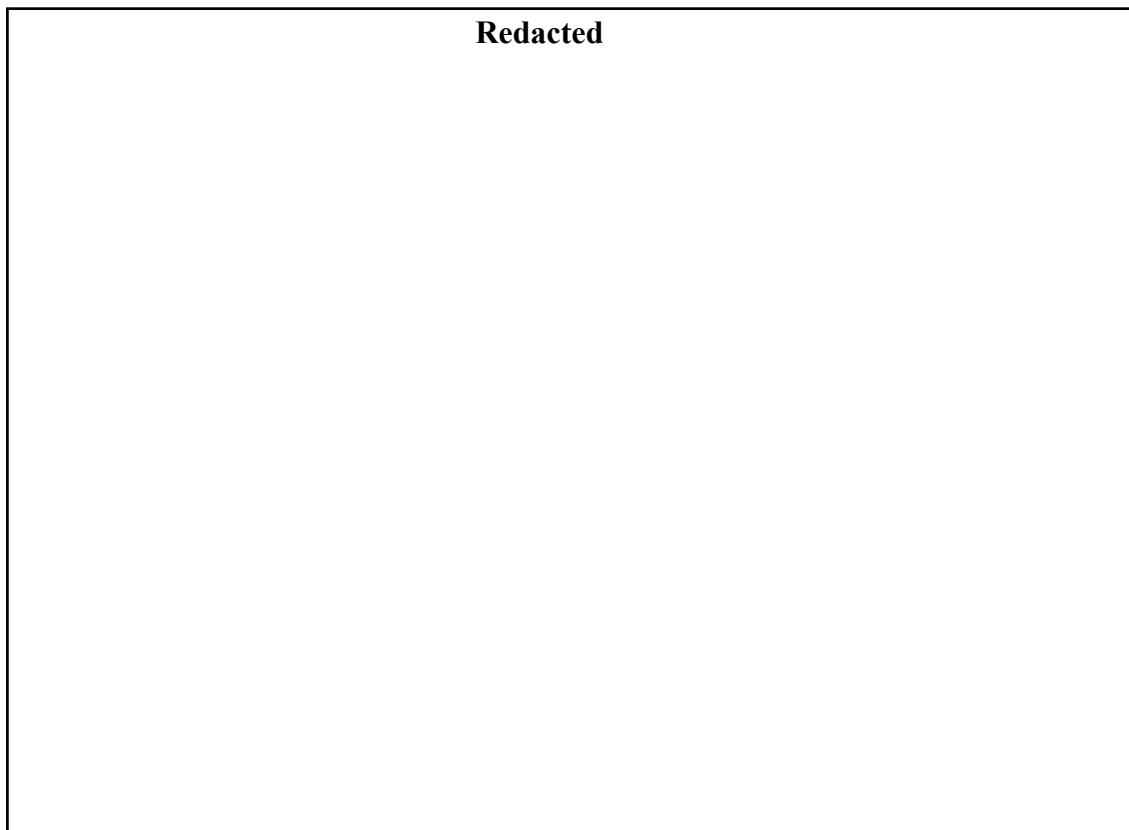
We thank the reviewer for the FIP200 suggestion. We consider that mixing two models, budding yeast and murine cells which possibly have different mechanisms, would bring complexity rather than clarity at this stage of research.

2) No link to autophagy. Despite the published work and the proposed model, there is no investigation of autophagy in the TKO. At a minimum, examining the localization of LC3 and TEX264 in the TKO could be valuable. Further, examining the impact of bafilomycin treatment on the prevalence of the lamin or DNA-damage containing buds could also provide a more definitive link to autophagy to strengthen this conclusion.

To address Reviewer’s comment, we performed a comprehensive analysis of autophagy in dynamin-TKO cells. We first assessed whether these cells can form functional autophagosomes. Immunoblotting revealed elevated LC3B-II levels in dynamin-TKO compared with control cells (**Fig. 7a**). Upon expression of LC3B–mRFP, we observed an accumulation of enlarged autophagosomes in TKO cells (**Fig. 7b–c**), consistent with impaired autophagic flux. This conclusion was further supported by increased p62/SQSTM1 levels (**Fig. 7d**) and by direct flux measurements (**Fig. 7e–f**). The reduction in autophagic flux does not appear to result from diminished lysosomal content, as LAMP1 levels were elevated (**Fig. 7g**), although subtle lysosomal functional defects cannot be excluded.

Following the Reviewer’s suggestion, we further examined the localization of LC3 (a marker of autophagosomes) and TEX264 (a transmembrane ER protein previously shown to mediate selective autophagy at the nucleus) in control and dynamin-TKO cells. Preliminary data are presented in **Figure R3**. We observed alterations in both LC3 localization and fluorescence intensity, as well as changes in TEX264 distribution and signal in dynamin-TKO

cells. While LC3 data have been incorporated into the current study (new **Figure 7**), we believe that a comprehensive analysis of TEX264's role in nucleophagy — both in general and specifically in dynamin-TKO cells — requires a more systematic investigation that exceeds the scope and timeline of this revision. Of note, we describe a previously unrecognized role for mammalian dynamin proteins at the nucleus for the first time, and hope to encourage further investigations beyond this initial study.



In addition, given that camptothecin (CPT) induces nuclear damage and can indirectly trigger nucleophagy, we also compared CPT-treated control and dynamin-TKO cells. Notably, CPT treatment did not alter autophagosome formation in dynamin-TKO cells (see **Figure R2**), indicating that the autophagic phenotype observed is intrinsic to the loss of dynamins rather than a secondary response to CPT-induced nuclear damage.

Lastly, we performed additional set of experiments which revealed that bafilomycin A increased the prevalence of NE buds in dynamin TKO cells with a concomitant increase of the γ H2AX-containing buds, further supporting a role of autophagy on nuclear envelope remodelling and DNA damage accumulation. While we consider that preliminary data displayed in Figures R1-R3 are not yet developed for publication, we would be open to publish the dataset presented in Figure R4. We do not wish to bias follow-up studies on this topic, hence these data are presented as Figure R4 at present.

Redacted

3) The relationship between loss of nuclear envelope integrity and DNA damage is well established even as the mechanisms remain controversial. The authors have not ruled out that an indirect consequence of dynamin dysfunction in (for example) mitosis leads to losses of nuclear integrity, or, that it is involved in a nuclear envelope repair mechanism that is triggered after a rupture in the nuclear envelope. At a minimum, the authors should address the integrity of the nucleus by localizing a fluorescent reporter like an NL-GFP.

Following the Reviewer's suggestion, we assessed nuclear integrity in control and dynamin-TKO cells using a well-established NLS-mCherry probe. We observed no significant cytosolic red fluorescence in dynamin-TKO cells (**Fig. 2e**) and comparable numbers of cells with ruptured nuclei between conditions (**Fig. 2f**). We thank the Reviewer for this insightful recommendation, which provides a more robust approach than our initial assessment of the integrity of the outer nuclear membrane. Of note, we expressed the fluorescent nesprin-3, an outer nuclear membrane protein, in both control and dynamin-TKO cells. We observed that nesprin-3 localized to the ONM as expected, and revealed uniform signal (**Fig. 2g**). We also acknowledge the Reviewer's comment that indirect consequences of dynamin dysfunction,

such as during mitosis, may contribute to nuclear defects reported in this study: this point has now been incorporated into the Discussion.

Minor points:

1) The downregulation of lamins is not addressed in any way. It is possible that the observed phenotypes are directly related to loss of lamin levels and have nothing to do with dynamins. In lieu of stronger data linking dynamins to the nuclear envelope, this caveat should at least be mentioned in the text.

We thank Reviewer for raising the important point regarding lamin downregulation. To investigate the basis for the observed decrease in lamin protein levels, we performed several experiments. We first confirmed that 4-OHT treatment itself does not affect lamin expression (**Extended Data Fig. 5a**). We then found that *Lmna* transcript levels remain unchanged in dynamin-TKO cells (**Fig. 2c**), indicating that the reduction in protein levels is not due to transcriptional downregulation. Instead, our data show that proteasomal degradation contributes to the lower levels of lamin-A and lamin-C proteins (**Fig. 2d**). We agree that this aspect should be highlighted, hence we included the aforementioned data in the Figure 2.

2) The cryo-ET analysis is very nice but could benefit from quantification. The reader needs to understand how many tomograms were examined and the number of observed structures segmented - statements like “very rarely detected” should be reflected by numbers.

We examined our cryo-ET data again. We collected 38 tomograms total, among which 18 have the nuclear membrane and 7 are showing the ONM forming buds with the narrow neck. We measured the size of the neck at different distance from the ONM. The width is ranging from 7.7 nm up to 24 nm. These quantitative data are now included in the Results section of the manuscript, and the phrase ‘very rarely detected’ was removed.

Further, it is curious that the narrow membrane necks appear between the outer nuclear membrane and what appears to be an expanded ER cisternae. These data might suggest that dynamins contribute to ER morphology and/or to maintaining connections between the ER and the nuclear envelope as these connections are thought to contain a unique, but so far unknown, set of proteins. See, for example, PMID 38877171.

We agree with the Reviewer that even if these membrane compartments are still clearly connected by narrow necks to the ONM, they present ribosomes on their surface suggesting a link between NE remodelling and the ER. Yet at this stage, this mainly remains an observation. Despite we capture the buds linked by narrow neck only in dynamin TKO cells, further experiments are needed to provide direct mechanistic evidence that could inform on whether and how dynamin contribute to the maintenance of the nuclear envelope in physiological contexts. We added this remark in the figure legend of main Figure 4.

Further, the double-membrane structures that contain nuclear fragments are not connected to the nuclear envelope. Thus, fission has already occurred. How do the authors reconcile these data with their model? Have the authors examined ER morphology more broadly?

Dynamin act as catalysers of the fission, but even in their absence, the membrane fission can occur. For example, dynamins have been shown to be important in synaptic vesicle reformation but synapses of dynamin mutants contain synaptic vesicles (much less, but nonetheless some vesicles are formed in these mutants too, e.g., PMID: 24963135). Hence, we presume that membrane fission can occur from the nuclear envelope too, but it is rate-

limiting and dynamins will speed up this process multi-fold. A few sentences referring to this point were already present in the Discussion, yet we additionally highlighted this aspect in the Discussion and added a new reference.

3) In cryo ET description in text: “nuclear intermembrane space” is an atypical description of the more commonly termed perinuclear space or nuclear envelope lumen.

We now use the nuclear envelope lumen, thank you.

Further, the authors suggest there are “ruptures” of the inner nuclear membrane but these aren’t really discussed. To my knowledge, this would be a unique phenotype and some consideration of how this might arise might be worth considering in the discussion. For example, an inner nuclear membrane rupture would presumably leak luminal components into the nucleus.

We removed the reference to the ruptures in the inner nuclear membrane from the figure legend and the model. We re-examined the cryo-ET data again, and cannot rule out technical issues, in particular with automated segmentation. We have also redrawn the model (**Extended data Figure 10**).

4) In the discussion, the authors seem to draw a distinction between selective autophagy of TEX264 and “nucleophagy” in yeast. Nucleophagy is, by definition, selective autophagy of the nucleus. Thus, the TEX264 pathway is also nucleophagy.

We agree, and have rephrased the statement.

5) The authors should consider also referencing other papers on nucleophagy for example: PMIDs: 26524528; 37118512

Thank you: we added these references in the manuscript.

Reviewer #3 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Thank you!