RESEARCH ARTICLE

ZC3HC1 has functions distinct from TPR and is dispensable for TPR localisation to the nuclear basket

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Abstract

Background

The nuclear basket is a 'fishtrap'-like structure on the nucleoplasmic face of the nuclear pore complex which has been implicated in diverse functions including RNA export, heterochromatin organisation, and mitosis. Recently, a novel component of the nuclear basket, ZC3HC1, has been described. The localisation of ZC3HC1 to nuclear pores has been reported to occur reciprocally with TPR, a major structural component of the nuclear basket.

Methods

Using siRNA-mediated knock down, immunofluorescence and RNA sequencing we compare the consequences of depleting two proteins of the nuclear pore basket – TPR and ZC3HC1.

Results

We show that in human fibroblasts, although ZC3HC1 localisation to nuclear pores is TPR-dependent, TPR localises to pores regardless of the presence of ZC3HC1. We demonstrate that knockdown of TPR and ZC3HC1 produce distinct transcriptional profiles.

Conclusions

Our results suggest that there is little overlap in function between these two nuclear basket proteins in human diploid fibroblasts.

Plain language summary

Nuclear pores are structures critical for the regulating how molecules

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get into and out of the nucleus of our cells. They provide a channel through which selected cargoes are transported. These cargoes include the messenger RNAs that carry the information from DNA in the nucleus to make proteins in the cell cytoplasm. On the nuclear side of the nuclear pore, there is a poorly understood structure called the nuclear basket. This paper explores two proteins called TPR and ZC3HC1 that localise at the nuclear basket. Exploring the function of these two proteins, this paper shows that whilst TPR is important for the export of certain classes of messenger RNA, ZC3HC1 is not. This suggests that these two proteins of the nuclear basket have quite different functions. In addition, we show that, whilst ZC3HC1 depends on TPR for its localisation at nuclear pores, the reverse is not the case - TPR localisation appears to be independent of ZC3HC1.

Keywords
histone mRNA export, mRNA export. nuclear pore

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Introduction

The nuclear pore complex (NPC) is a large transmembrane complex consisting of around 30 different proteins known as nucleoporins (Nups), organised into a cylindrical assembly with eightfold symmetry (Petrovic *et al.*, 2022). The core structure consists of the inner ring, which lines the lumen of the nuclear pore, and outer rings sitting on each side of the nuclear envelope. On the cytoplasmic face of the pore, eight filaments extend into the cytoplasm, and on the nuclear side, eight nucleoplasmic filaments are joined to a double nuclear ring, forming a structure known as the nuclear basket (Singh *et al.*, 2024).

The nuclear basket was first described as a 'fishtrap'-like structure attached to the NPC (Maul, 1976). Although there are near-atomic structures of the rest of the NPC, until recently the position of proteins in the nuclear basket had only been coarsely approximated (Allegretti *et al.*, 2020; Kim *et al.*, 2018; Niepel *et al.*, 2013). However, cryo-electron tomography (cryo-ET) and integrative structural modelling have now provided unprecedented understanding of how the nuclear basket docks on to the double nuclear rings of the mammalian NPC (Singh *et al.*, 2024) (Figure 1A).

The nuclear basket has a range of functions, many of which have been attributed to TPR, a 267-kDa basket Nup which is anchored to the NPC by its interaction with NUP153 (Hase & Cordes, 2003). TPR is required for the specific export of short or intronless mRNAs by the TREX-2 complex (Aksenova *et al.*, 2020; Lee *et al.*, 2020; Zuckerman *et al.*, 2020). TPR also has a role in heterochromatin organisation – it is necessary for the exclusion of heterochromatin at nuclear pores, and for the formation of senescence-associated heterochromatic foci (SAHF) in senescent cells (Boumendil *et al.*, 2019; Krull *et al.*, 2010). During oncogene-induced senescence, TPR is also necessary for inflammatory signalling, which we recently showed is due to the role of TPR in the generation of cytoplasmic chromatin fragments (Bartlett *et al.*, 2024; Boumendil *et al.*, 2019).

Until recently the metazoan nuclear basket was thought to consist only of TPR and the NUP153 and NUP50 anchors to the nuclear ring (Krull *et al.*, 2004; Lin & Hoelz, 2019) and the relative position of these proteins is confirmed by the recent molecular structure study (Singh *et al.*, 2024) (Figure 1A). More recently, another component, ZC3HC1, was found in isolated *Xenopus* oocyte nuclear envelopes, and subsequently confirmed to be part of the nuclear basket in other cells, including human cell lines, by electron microscopy (Gunkel *et al.*, 2021). ZC3HC1, also known as NIPA, was previously shown to be part of a SCF E3 ubiquitin ligase which promotes the degradation of cyclin B1 during the cell cycle (Bassermann *et al.*, 2005). TPR and ZC3HC1 are reported to show reciprocally dependent localisation to the NPC (Gunkel *et al.*, 2021), with two pools of TPR in the nucleus; one which depends on ZC3HC1 for localisation to the nuclear pore and one which is ZC3HC1 independent (Gunkel & Cordes, 2022). ZC3HC1 interacts with the NPC via its nuclear basket interaction domain, which is made up of two zinc-finger containing modules (Gunkel *et al.*, 2023). A yeast homolog of ZC3HC1, known as Pml39, has a nuclear basket interaction domain with a similar structure to the human protein, but a low degree of sequence similarity (Gunkel *et al.*, 2023). Pml39 is involved in the retention of improper messenger ribonucleoparticles in the nucleus (Palancade *et al.*, 2005), suggesting that ZC3HC1 could play a role similar to TPR in regulating mRNA export. The precise position of Pml39/ZC3HC1 in the nuclear basket has yet to be determined (Singh *et al.*, 2024)

Here we show that, in primary human fibroblasts, although ZC3HC1 localises at the NPC and that this localisation depends on TPR, the localisation of TPR to nuclear pores is not ZC3HC1 dependent, contrary to previous reports. Furthermore, knockdown of ZC3HC1 produces a very different transcriptional signature to TPR knockdown, suggesting that the two proteins have different functions.

Methods

Cell culture and siRNA transfection

Human IMR90 cells, infected with pLNC-ER:STOP retroviral vectors to produce neomycin resistant control cells (Acosta *et al.*, 2013), were cultured in DMEM with 10% FBS, 100 nM 4-hydroxytamoxifen and 1% penicillin/streptomycin in a 37°C incubator with 5% CO_2.

siRNA knockdown was carried out as previously described (Bartlett *et al.*, 2024; Boumendil *et al.*, 2019). Briefly, 9×10^5 STOP IMR90 cells (except for imaging experiments, which used 1.5×10^5 cells) were transfected using Dharmafect transfection reagent (Dharmacon) with a 30nM final concentration of control (siCTRL, D-001810-10-59) or TPR (siTPR, L-010548-00) or ZC3HC1 (siZC3HC1, L-016879-02) siRNA pools (Dharmacon). Transfections were carried out in the presence of 4-OHT and were repeated at day 0, 3 and 6 and cells fixed for imaging on day 8 (Figure 1B). For RNA-seq, cells were harvested after 3 days after siRNA transfection.

Immunofluorescence

Cells grown on coverslips were fixed with 4% paraformaldehyde and blocked with 1% bovine serum albumin (BSA) as previously described (Bartlett *et al.*, 2024). Coverslips were then incubated with primary antibody diluted in 1% BSA at the dilutions detailed in Table 1, for 45 mins in a humid chamber. After washing three times with PBS, coverslips were then incubated for 30 mins with fluorescently labelled secondary antibodies (Life Technologies, Table 1) followed by two washes in PBS. PBS with 50ng/ml DAPI was added for 4 mins, before a final wash with PBS and mounting onto slides with VectaShield (Vector Laboratories).

Epifluorescence images were acquired as previously described (Bartlett *et al.*, 2024). Super-resolution images were acquired by Instant Sim microscopy (Azuma & Kei, 2015) using a Nikon SoRa™ system. Imaging was carried out using a SR HP Plan Apo λS 100x 1.35NA Silicone lens (Nikon Instruments). The CMOS cameras used for acquisition were Teledyne Photometrics Prime 95B 488 / 561nm laser lines. Step size for Z stacks was

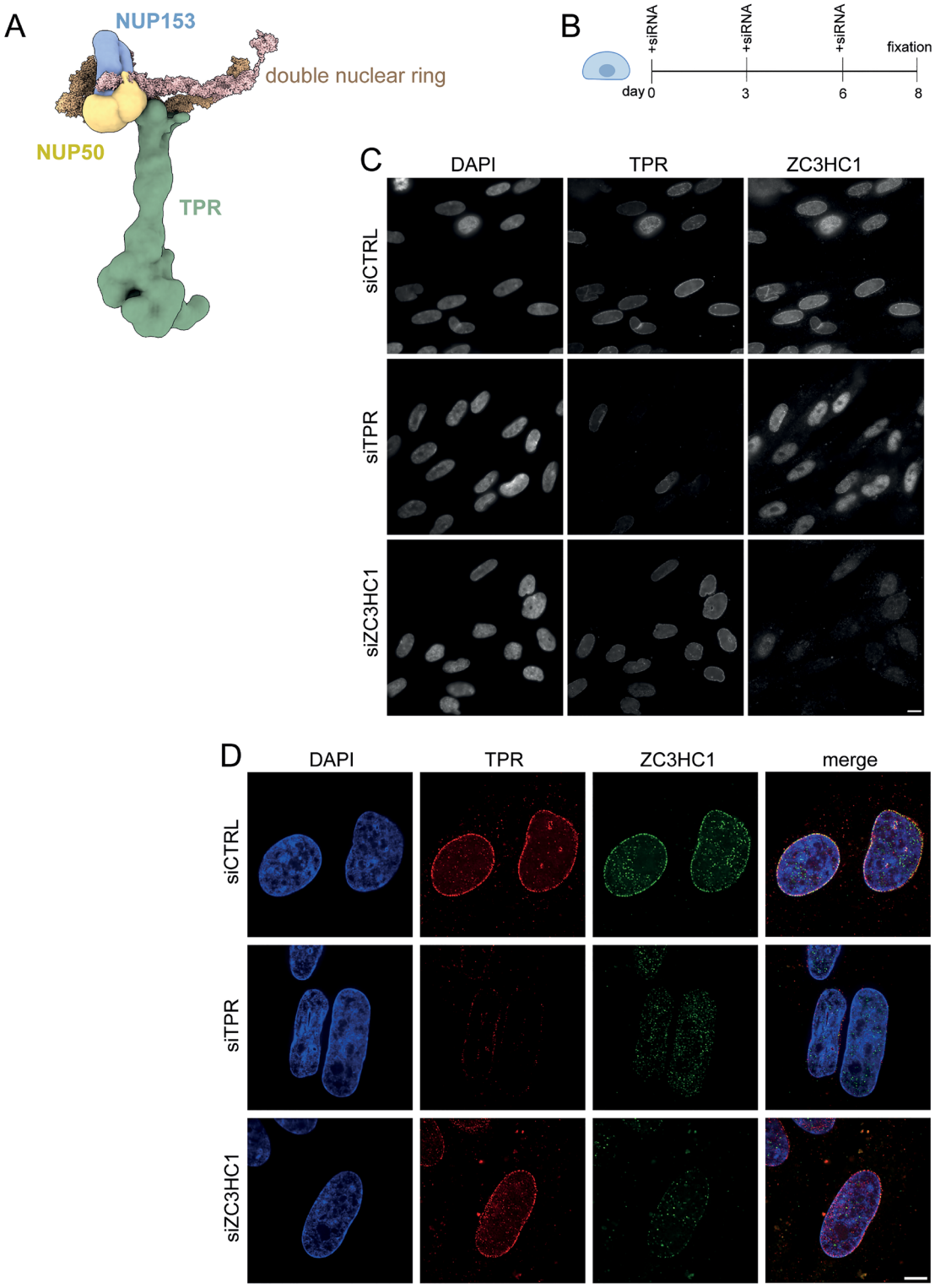

Figure 1. ZC3HC1 localisation to nuclear pores is dependent on TPR but ZC3HC1 knockdown does not delocalise TPR. A) Model of the mammalian nuclear pore basket showing the position of TPR (green), NUP50 (yellow), NUP153 (blue) and the double nuclear ring (brown). Adapted, with permission from Singh *et al.*, 2024. **B**) Schematic of experimental protocol for ZC3HC1 knockdown in IMR90 cells. **C**) TPR and ZC3HC1 immunostaining in DAPI-stained IMR90 cells after treatment for 8 days with control (CTRL) siRNA or with siRNAs targeting TPR or ZC3HC1. Immunostaining was carried out with antibodies detecting TPR (Abcam ab84516) or ZC3HC1 (Santa Cruz sc-365058). **D**) As in (**C**) but imaged on a SoRa spinning disk confocal microscope. Immunostaining was carried out with antibodies detecting TPR or ZC3HC1 that were a gift from Volker Cordes and Philip Gunkel (Table 1). Scale bars: 10μm.

Table 1. List of antibodies. Antibodies used in immunofluorescence (IF) experiments with their corresponding dilutions. RRIDs are from https://www.rrids.org

	Source or reference	Identifiers	Dilution
anti-TPR (rabbit polyclonal)	Abcam. Raised against residues 2300–2349	ab84516 RRID:AB_1861454	IF (1:500)
anti-TPR (mouse monoclonal)	Gift from Volker Cordes. Raised against residues 1462–1500		IF (1:100)
anti-ZC3HC1 (mouse monoclonal)	Santa Cruz. Raised against residues 1–300	sc-365058 RRID:AB_10847677	IF (1:100)
anti-ZC3HC1 (guinea pig polyclonal)	Gift from Volker Cordes. Raised against residues 307–355		IF (1:200)
anti-mouse IgG (H+L) secondary, Alexa Fluor 568 (donkey polyclonal)	Invitrogen	A10037 RRID:AB_11180865	IF (1:1000)
anti-rabbit IgG (H+L) secondary, Alexa Fluor 488 (goat polyclonal)	Invitrogen	A11034 RRID:AB_2576217	IF (1:1000)

set to 0.120µm as required by manufacturer's software. Acquisition of images and deconvolution was carried out using the Nikon NIS Elements Advanced Research software (https://www.microscope.healthcare.nikon.com/products/software/nis-elements/software-resources).

RNA-seq library preparation and analysis

Total RNA was extracted from a 10cm tissue culture plate using the RNeasy mini kit (Qiagen). Library preparation, sequencing and data quality control were carried out as previously described (Bartlett *et al.*, 2024).

Differential expression analysis was carried out using DeSeq2 (Love *et al.*, 2014). Gene ontology analysis was carried out using clusterProfiler (Wu *et al.*, 2021). Volcano plots were rendered using ggplot2 (Wickham, 2016) and Venn diagrams rendered using VennDiagram (Chen & Boutros, 2011). A list of intronless genes was obtained from the UCSC hg19 GTF file (Nassar *et al.*, 2023) by sorting for genes with a single exon. The list of histone genes was obtained from HGNC (Braschi *et al.*, 2019).

Statistics

Statistical analysis was performed using R and the specific statistical tests used are described in the relevant text and Figure legends. p-value significance is denoted as follows: * < 0.05, **< 0.01, *** < 0.001.

Data availability

RNA-seq data for TPR and ZC3HC1 are available from NCBI GEO under Accession numbers GSE264387 and GSE286436 respectively.

Results

ZC3HC1 localisation to nuclear pores is dependent on TPR but ZC3HC1 knockdown does not delocalise TPR

We first sought to verify the presence of ZC3HC1 at nuclear pores. We used siRNAs to deplete TPR or ZC3HC1 in human IMR90 fibroblasts (Bartlett *et al.*, 2024) over an 8-day period (Figure 1B). Immunofluorescence and wide-field epifluorescence microscopy showed both TPR and ZC3HC1 present at the nuclear periphery in cells transfected with control siRNA (Figure 1C). Depleting TPR caused ZC3HC1 to move away from the nuclear periphery and into the nucleoplasm as previously reported (Gunkel *et al.*, 2021). However, TPR remained localised at the nuclear periphery in cells depleted of ZC3HC1. Super-resolution microscopy showed TPR and ZC3HC1 colocalised at nuclear pores in cells treated with the control siRNA (Figure 1D). Although TPR knockdown caused ZC3HC1 to move away from the nuclear periphery, TPR remained visibly localised to nuclear pores when ZC3HC1 was knocked down (Figure 1D).

The transcriptional signatures of ZC3HC1 and TPR knockdown are distinct

To better differentiate the functions of ZC3HC1 and TPR, we examined whether the transcriptional changes upon ZC3HC1 knockdown are similar to those that we have reported upon knockdown of TPR in IMR90 cells (Bartlett *et al.*, 2024). We carried out RNA-seq on IMR90 fibroblasts treated with either control (siCTRL) or ZC3HC1 siRNAs for three days, using the same protocol as for TPR knockdown (Bartlett *et al.*, 2024).

ZC3HC1 knockdown led to more extensive changes in gene expression than knocking down TPR (Bartlett *et al.*, 2024), with 1272 genes upregulated and 1374 downregulated upon ZC3HC1 knockdown (Figure 2A). The most downregulated gene was *ZC3HC1*, confirming successful knockdown. Gene ontology analysis showed significant differences between genes whose expression changes in response to either ZC3HC1 or TPR knockdown (Figure 2B and C). Loss of ZC3HC1 led to upregulation of genes associated with chromosome segregation and mitosis, consistent with the known role of ZC3HC1 in degrading cyclin B1 (Bassermann *et al.*, 2005). Genes associated with angiogenesis, skeletal system development and extracellular matrix organisation were downregulated upon ZC3HC1 knockdown (Figure 2B). TPR

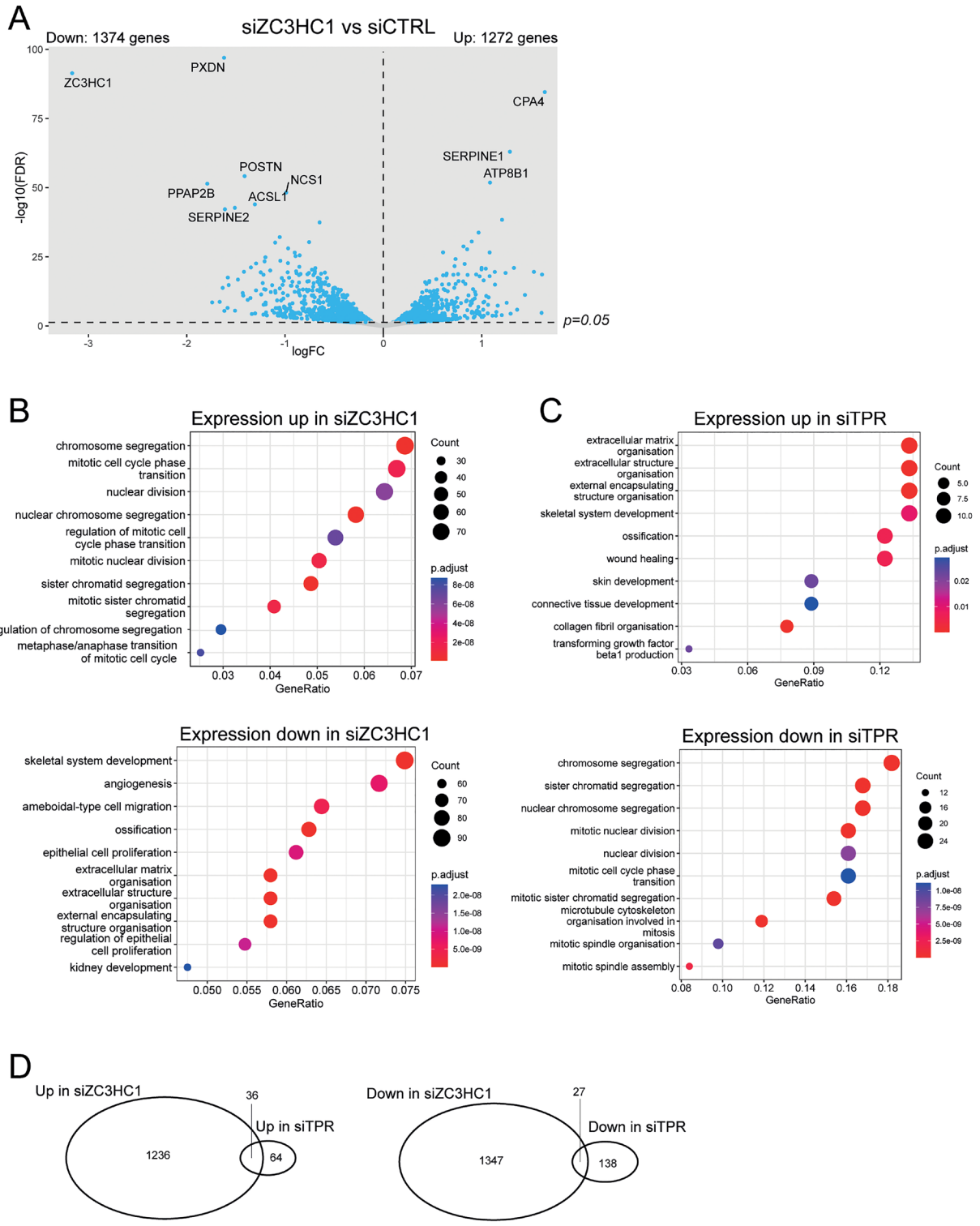

Figure 2. TPR and ZC3HC1 knockdown have distinct transcriptional signatures. A) Volcano plots showing differential expression analysis comparing siZC3HC1 and siCTRL samples. IMR90 cells were treated with the indicated siRNAs for 3 days. Blue dots indicate differentially expressed genes (adjusted p-value (FDR) < 0.05). The dashed horizontal line indicates an adjusted p-value of 0.05 and the dashed vertical line indicates a logFC of 0. The 10 genes with the most significant p-values are labelled. **B** and **C**) GO analysis carried out using clusterProfiler (Wu *et al.*, 2021) for genes which increase or decrease in expression when (**B**) ZC3HC1 or (**C**) TPR is knocked down. RNA-seq data for TPR knockdown is taken from (Bartlett *et al.*, 2024). **D**) Venn diagrams representing the overlaps between significantly up or downregulated transcripts upon TPR or ZC3HC1 loss in IMR90 cells.

knockdown showed the opposite: mRNAs for genes involved in chromosome segregation were downregulated and those associated with extracellular matrix organisation were upregulated (Figure 2C).

To investigate whether the two nuclear basket proteins have any shared functions, we examined how many genes change in expression upon both TPR knockdown and ZC3HC1 knockdown. Thirty-six (2.8%) of the genes upregulated upon ZC3HC1 knockdown were also upregulated on TPR knockdown, while 27 (2.0%) of the genes downregulated upon ZC3HC1 knockdown were also downregulated on TPR knockdown (Figure 2D). The limited overlap in differentially expressed genes between the two knockdown experiments suggests that TPR and ZC3HC1 have distinct functions.

ZC3HC1 is not required for the export of mRNAs from intronless genes

Consistent with the known role of TPR in the nuclear export of short and intronless mRNAs via interaction with the TREX-2 complex, TPR knockdown causes significant downregulation of mRNAs from intronless and histone genes (Aksenova *et al.*, 2020; Bartlett *et al.*, 2024; Lee *et al.*, 2020; Zuckerman *et al.*, 2020). We investigated whether ZC3HC1, by localizing TPR at the nuclear basket, might play a similar role in mRNA nuclear export. Fisher's exact tests showed that, in contrast to TPR knockdown, there were no more mRNAs for intronless genes significantly downregulated upon ZC3HC1 knockdown than would be expected by chance (p=0.93) (Figure 3A). There were also no more histone mRNAs downregulated upon ZC3HC1 knockdown than would be expected

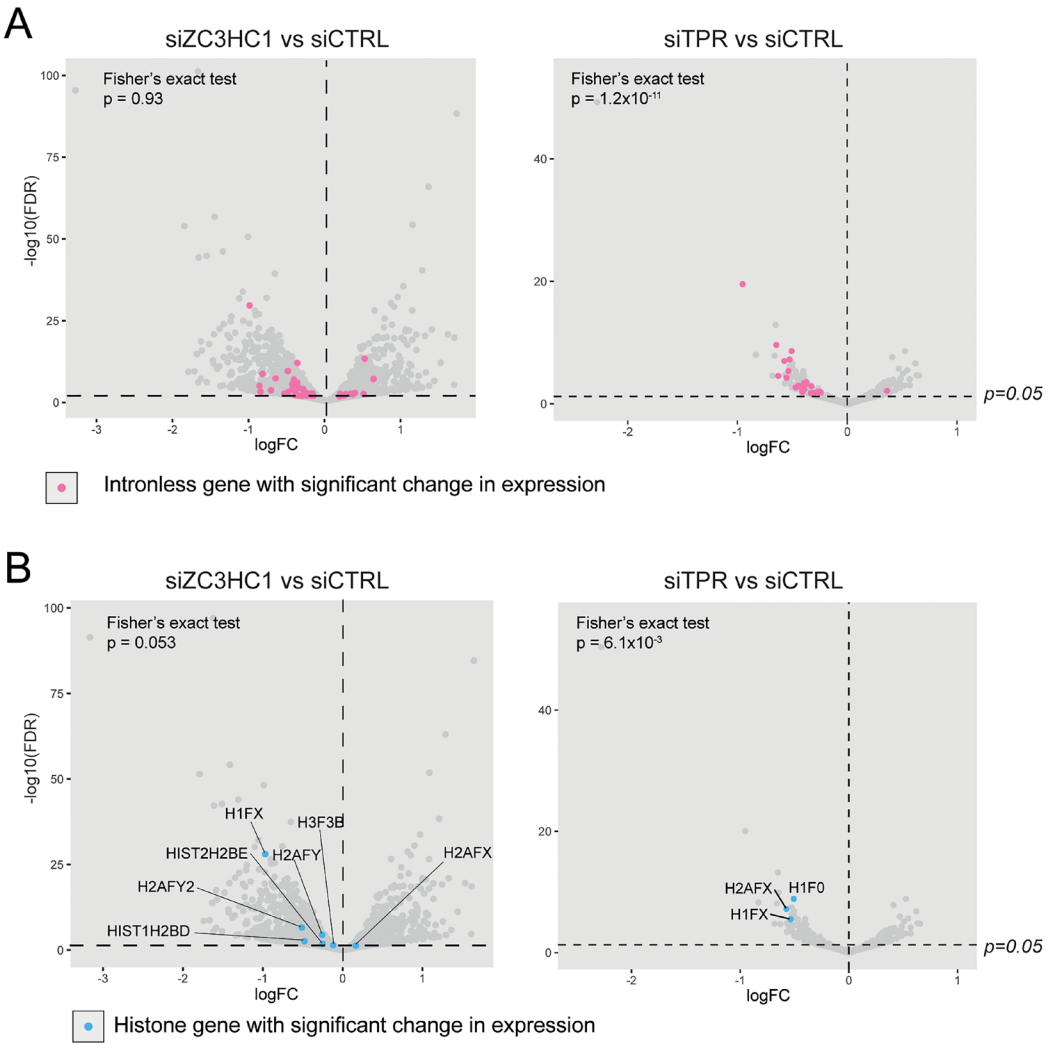

Figure 3. ZC3HC1 knockdown does not affect the expression of intronless or histone genes. A) Volcano plots of differential expression analysis of IMR90 cells treated with CTRL vs ZC3HC1 siRNAs (left), or CTRL vs TPR siRNAs (right). Intronless genes are labelled in pink. The horizontal dashed line indicates an adjusted p-value (FDR) of 0.05 and the vertical dashed line indicates a logFC of 0. Fisher's exact tests were carried out to determine whether the number of downregulated intronless genes was greater than expected by chance. **B**) As in (**A**) but with histone genes labelled in blue. Fisher's exact tests were carried out to determine whether the number of downregulated histone genes was greater than expected by chance.

by chance (p=0.053) (Figure 3B). This suggests that, unlike TPR, ZC3HC1 does not have a role in the nuclear export of intronless mRNAs.

Discussion

We have confirmed that ZC3HC1 localises to the nuclear periphery, suggesting that it is a nuclear pore protein, as reported (Gunkel *et al.*, 2021). However, depleting ZC3HC1 in IMR90 cells did not affect the localisation of TPR to nuclear pores, as assessed by immunofluorescence, contrary to previous reports (Gunkel *et al.*, 2021; Gunkel & Cordes, 2022). This suggests that, at least in human fibroblasts, ZC3HC1 may not play the structural role in establishing interconnections between TPR polypeptides at the nuclear basket which has been reported in cancer cells (Gunkel & Cordes, 2022).

We did confirm that TPR is required for the localisation of ZC3HC1 to nuclear pores in IMR90 fibroblasts (Figure 1). Even though ZC3HC1 is displaced to the nucleoplasm on TPR knockdown, only a small number of mRNAs which we previously reported changed in expression after TPR knockdown (Bartlett *et al.*, 2024) also change in expression upon ZC3HC1 knockdown (Figure 2). This suggests that most mRNAs that change in expression in response to reduction of ZC3HC1 do not depend on its localisation at the nuclear basket. Amongst the mRNAs downregulated upon ZC3HC1 depletion, we do not see an enrichment of mRNAs originating from intronless or histone genes, which are known to dependent on TPR localisation at the nuclear pore basket for their nuclear export (Aksenova *et al.*, 2020; Bartlett *et al.*, 2024; Lee *et al.*, 2020) (Figure 3). This further supports our conclusion that ZC3HC1 is not required for TPR localisation to the nuclear pore basket in IMR90 cells.

ZC3HC1 has been reported as a component of a nuclear SCF E3 ligase, which is required for the degradation of cyclin B1 and thereby regulates mitotic entry and exit (Bassermann *et al.*, 2005). Our RNA-seq data showing that levels of mRNAs involved in chromosome segregation and the mitotic cell-cycle phase are elevated on ZC3HC1 knockdown is consistent with a role for ZC3HC1 in the regulation of mitosis.

To distinguish which functions of ZC3HC1 are dependent on its localisation to the nuclear pore, RNA-seq could be repeated in cells expressing a version of ZC3HC1 which cannot localise to nuclear pores, for example by making a single amino acid substitution in ZC3HC1 (C429S) which abolishes its interaction with TPR (Gunkel & Cordes, 2022).

Ethics and consent

Ethical approval and consent were not required

Data availability

All quantitative data associated with this manuscript are been deposited in NCBI GEO and is freely accessible with no restrictions on use or distribution.

Source data

NCBI GEO: RNA-seq data following TPR knockdown in IMR90 cells. GSE264387;

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE264387 (Bartlett *et al.*, 2024)

Underlying data

NCBI GEO: RNA-seq data following ZC3H1 knockdown in IMR90 cells. GSE286436; https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE286436 (Bartlett & Bickmore, 2025)

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Open Peer Review

Current Peer Review Status: ? ? ✓ ?

Version 1

Reviewer Report 29 May 2025

https://doi.org/10.21956/wellcomeopenres.26159.r122284

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Richard W. Wong
Kanazawa University, Kanazawa, Japan

Reviewer Comments (Major Revision Required):

The manuscript by Bartlett et al. presents a careful comparative study on the nuclear basket proteins ZC3HC1 and TPR, showing that ZC3HC1 requires TPR for nuclear pore localisation, but not vice versa, and that their knockdowns result in distinct transcriptional profiles. The work is well conducted and addresses an important question in nuclear pore complex (NPC) biology. However, I have several concerns that must be addressed before this manuscript can be considered for acceptance.

1. Depth of Discussion on TPR Multifunctionality:
While the manuscript focuses primarily on TPR's role in mRNA export and heterochromatin exclusion, TPR's broader functions, especially its connections to mitotic regulation, autophagy, and tumorigenesis, are not sufficiently acknowledged. Several previous studies have demonstrated that TPR interacts with mitotic regulators (e.g., Aurora A kinase), is involved in sustaining mitosis via dynein interaction, and plays critical roles in autophagy regulation and cancer cell survival. These aspects are highly relevant to the findings on ZC3HC1 and mitosis-related gene expression changes. Therefore, I request that the authors integrate and cite the following foundational studies on TPR functions:

- Bartlett B, et al. (2024) - **Ref 1**
- Dewi F, et al. (2021) -**Ref 2**
- Singh D, Soni N, et al. (2024) - **Ref 3**
- Dewi F, Domoto T, et al. (2024) - **Ref 4**
- Gunkel P, et al. (2023) - **Ref 5**
- Rajanala K,et al. (2022) - **Ref 6**
- Kobayashi A, et al. (2015) - **Ref 7**
- Li Y, et al. (2021) - **Ref 8**
- Funasaka T, et al. (2021) - **Ref 9**

- Kosar M, et al. (2021) - **Ref 10**
- Nakano H, et al. (2010) - **Ref 11**
- Lee E, et al. (2020) - **Ref 12**

Discussing these studies would provide a broader biological context for the distinct mitotic and autophagy-related signatures observed upon ZC3HC1 depletion, especially with Volker Cordes's group published ZC3HC1 findings.

2. Further Statistical Validation:
As another reviewer already noted, the observed overlap in gene sets between TPR and ZC3HC1 knockdowns should be statistically evaluated against random expectation. Please apply appropriate statistical tests (e.g., hypergeometric test) to rigorously assess whether the overlap is truly non-significant.

3. Clarification of ZC3HC1 and TPR Knockdown Efficiency:
While immunofluorescence data suggest efficient knockdown, an immunoblot for ZC3HC1 (and ideally TPR) should be provided. Even residual low levels could potentially sustain partial nuclear basket architecture, impacting interpretation.

4. Expansion of mRNA Characteristic Analyses:
I encourage the authors to perform additional analyses on the characteristics (e.g., GC content, length, intron content) of the mRNAs affected by ZC3HC1 depletion, as suggested by previous reviews. This could shed light on whether ZC3HC1 loss preferentially affects certain classes of transcripts.

5. siRNA Information:
Full siRNA sequences must be listed in the methods section to ensure reproducibility, considering commercial catalog numbers may become obsolete.

6. Toning Down Some Statements:
Certain conclusions should be made more precise. For example, modify statements such as "TPR and ZC3HC1 have distinct functions" to "distinct functions in terms of gene expression and mRNA metabolism," unless additional evidence beyond transcriptomics is provided.

References

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Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 29 May 2025

https://doi.org/10.21956/wellcomeopenres.26159.r122278

Weidong Yang
Temple University, Philadelphia, USA

This manuscript focuses on the nuclear basket protein ZC3HC1. The authors demonstrate that ZC3HC1's localization within the nuclear basket depends on the presence of another nuclear basket component, TPR, whereas TPR's localization is independent of ZC3HC1. They further show that ZC3HC1 knockdown does not affect the expression of intronless or histone genes. Instead, they suggest that ZC3HC1 may play a role in regulating mitosis.

The study is relatively simple and straightforward, employing well-established methodologies. The data appear solid, and the conclusions are adequately supported.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cell Biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 10 May 2025

https://doi.org/10.21956/wellcomeopenres.26159.r122277

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Daniel Zenklusen
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ZC3HC1 and TPR have been described as structural components of the nuclear basket. However, whether ZC3HC1's function in basket biology is primarily structural or whether it has additional, and distinct, functions then TPR, has not been fully investigated. In this manuscript, Bartlett and coworkers use microscopy and RNomic approaches to propose that ZC3HC1 depletions in primary fibroblast don't affect the level of TPR localized to the nuclear envelope, and that it affects expression of a distinct set of RNA when compared to TPR, suggesting distinct functions for these two basket proteins.
Overall, the study is well designed, and their conclusions mostly supported by their data. The observation that TPR and ZC3HC1 depletions affect expression of different types of mRNA is intriguing and, as stated the authors, future mechanistic studies will be important to better understand the function of these two proteins at and off the NPC. However, the microscopy data in Figure 1 needs quantifications to allow the conclusions drawn by the authors, and/or a more balanced discussion with respect to previous studies.
The authors perform siRNA KDs for ZC3HC1 and localize TPR signal using immunofluorescence to conclude that in IMR90 cells, TPR localization is not altered after ZC3HC1 depletion. However, the authors do not provide quantification of their data. As mentioned by the authors, previous studies by the Cordes lab suggested that only a fraction of NPC bound TPR requires ZC3HC1 for NPC/nuclear periphery association. Images shown in Figure 1C seem to show different intensities for nucleoplasmic and nuclear periphery localized TPR, as well as various expression levels of ZC3HC1 in different cells, suggesting different KD efficiencies in individual cells. To better support their conclusion, the authors have either to provide quantification that convincingly shows that ZC3HC1 depletions does not alter NE associated TPR levels, or provide a more nuanced interpretation of their data. Moreover, in case these quantifications show that IMR90 cells indeed do not contain a ZC3HC1 dependent fraction of TPR at the nuclear periphery, the authors might want to shortly discuss whether this might suggest that there t exist different types of baskets in different cell types, and refer to the appropriate literature related to NPC/basket specialization.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: NPC, nuclear transport, microscopy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 26 April 2025

https://doi.org/10.21956/wellcomeopenres.26159.r122280

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Alexander Palazzo
Department of Biochemistry, University of Toronto, Toronto, ON, M5G 1M1, Canada

In this manuscript, Bartlett and co-authors invesigate the relationship between ZC3HC1 and TPR. They demonstrate that while the former requires the later for localization to the nuclear pore, the reverse is not true. They then analyze the effects of depleting each component on mRNA levels. The authors claim that these do not overlap, although some statistical assessment is warranted. I only have a few concerns:

1. The authors should provide an immunoblot of ZC3HC1 – perhaps a low level of protein is sufficient for TPR localization? Although the authors may feel that this is unlikely, this possibility should be stated.
2. At the end of the transcriptomics section the authors state “The limited overlap in differentially expressed genes between the two knockdown experiments suggests that TPR and ZC3HC1 have distinct functions.” This should be toned down. If the two proteins function in protein trafficking or NPC assembly, this may not be reflected in changes in the transcriptome. They should at least modify the sentence by adding “distinct functions in terms of gene expression and mRNA metabolism.”
3. Although the overlap in Figure 2D is low in absolute counts, about 1/3rd of the mRNAs upregulated by TPR-depletion are found in the ZC3CH1-dependent set. The authors should determine whether this is above what is expect from chance alone. The same for the downregulated mRNAs. This will depend on the overall number of mRNAs that were evaluated in both sets.
4. Some analysis of the mRNAs affected by ZC3HC1-depletion is warranted. Basic characteristics, such as GC-content, intron density, and mRNA length (as compared to the unaffected mRNAs) should be provided.

5. siRNA sequences should be listed – companies may stop listing items on their website and these sequences are important for reproducibility.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: mRNA nuclear export in mammalian cells.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.