Cell Biology
Chromosomes and Gene Expression

Dissociation of the nuclear basket triggers chromosome loss in aging yeast

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eLife Assessment

This **fundamental** study reveals that aging in yeast leads to chromosome mis-segregation due to asymmetric partitioning of chromosomes, driven by disruption of the nuclear pore complex and pre-mRNA leakage. The findings are **convincingly** supported by carefully-designed experimental data with a combination of genetic, molecular biology and cell biology approaches.

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Abstract

In many organisms, aging is a clear risk factor for chromosome mis-segregation, the main source of aneuploidy. Here, we report that old yeast cells lose chromosomes by partitioning them asymmetrically to their daughter cells together with the pre-existing (old) spindle pole body (SPB, centrosome equivalent in yeast). Strikingly, remodelling of the nuclear pore complex (NPC) and the displacement of its nuclear basket triggered these asymmetric chromosome segregation events. Simultaneously, nuclear basket displacement caused unspliced pre-mRNAs to leak into the cytoplasm. We show that removing the introns of three genes involved in chromosome segregation was sufficient to fully suppress chromosome loss in old cells. Promoting pre-mRNA leakage in young cells also caused asymmetric chromosome partitioning and loss through the same three introns. Therefore, we propose that basket displacement from NPCs and its consequences for pre-mRNA quality control are key triggers of aging phenotypes such as aneuploidy.

Introduction

Cellular aging results in conserved phenotypes, including chromosome mis-segregation, fragmentation of mitochondria, accumulation of damaged or misfolded proteins, and alteration of nuclear morphology[1–3]. Importantly, the nuclear pore complex (NPC) is remodelled in aging across evolutionary lineages, as shown in fungi, worms, and mammals [4–6]. As the primary gateway between the nucleus and cytoplasm, the NPC controls mRNA export and protein distribution between these two cellular compartments. The most prominent change in the NPCs of old cells is the loss of their nuclear basket, which lies on the nucleoplasmic side of the NPC. Nuclear basket loss is observed both in dividing cells and upon expression of the progeric variant of Lamin A, progerin[8–9]. The protein TPR (Mlp1 and Mlp2 in *S. cerevisiae*) is the main structural component of the nuclear basket in mammals. The nuclear basket serves as a critical processing hub for mRNA export and nuclear retention of pre-mRNA, protein quality control and gene regulation[10–15]. Altering the structure or function of the NPC or its nuclear basket could have diverse and wide-ranging effects on cellular function given the NPC's broad range of functions and central position in eukaryotic cellular information flow. Despite this and the fact that the reorganization of NPC architecture with age is prominent in a wide range of organisms, little is known about whether and how NPC remodelling contributes to aging.

The unicellular fungus *Saccharomyces cerevisiae*, budding yeast, is a remarkably useful system for studying the role of NPCs in cellular aging[1,3]. Budding yeast undergoes replicative aging, during which the mother cell produces a finite number of daughter cells before dying[16]. After the mother cell enters senescence, the rate of cell division slows. Importantly, several studies have emphasized that the yeast NPC is remodelled during replicative aging[4–6], but the exact consequences of its reorganization are not fully understood.

Extrachromosomal DNA circles (also called eccDNAs) accumulate in the mother cell nucleus and are one of the key drivers of aging in the budding yeast[7–9]. These DNA circles form during the cell's lifetime through excision of genomic segments, generally by recombination between repeated sequences[10]. The vast majority of these circles (>99%) stems from the highly repetitive rDNA locus and are referred to as extrachromosomal rDNA circles (ERCs)[7]. As the cell age, these circles accumulate exponentially because they are replicated in S-phase and retained in the mother cell at mitosis[7,11]. By the end of their life, yeast cells contain about 1000 ERCs, which represents roughly the same amount of DNA as the rest of the genome[11]. Importantly, their retention in the mother cell relies on their anchorage to NPCs via the acetyltransferase complex SAGA[5,12]. These NPC-ERC units are then confined to the mother part of the dividing nucleus by a lateral diffusion barrier forming in the nuclear envelope in the future plane of cleavage[12–14]. Yeast cells undergo a closed mitosis, meaning that they keep their nuclear envelope intact throughout mitosis. Remarkably, anchorage of ERCs to NPCs results in the dissociation of their nuclear basket, which is a direct result of SAGA-driven acetylation of nucleoporins on the nucleoplasmic side of the NPC, including Nup60[12]. Consequently, most of the NPCs of old yeast cells lack a nuclear basket[5]. However, it is unknown whether nuclear basket displacement has consequences for the physiology and survival of the cell or contributes to the aging process.

One way to approach the question of whether nuclear basket displacement from the NPCs contributes to the aging process is to determine whether other aging phenotypes depend on basket displacement and if so, how. In this study, we focused on chromosome instability in old yeast cells as a case study of an aging phenotype. In many metazoans, including humans, aging results in increasing frequency of chromosome mis-segregation and aneuploidy, correlating with increasing risk of cancer[15,16]. Our understanding of the molecular mechanisms of chromosome

segregation errors in old cells remains rudimentary. Here, we report evidence that NPC remodelling during yeast aging leads to chromosome loss from old mother cells and characterize the underlying mechanisms.

Results

Aging yeast cells lose chromosomes

To examine whether aging affects the karyotype of yeast cells, we visualized and followed the fate of individual chromosomes in replicatively aging mother cells, using either TetO-labelled chromosome II or chromosome IV as reporters. Chromosome IV is the second longest chromosome, while chromosome II is of average size. Both chromosomes were labelled with an array of 256 TetO repeats in the vicinity of their centromere[17,18]. These chromosomes were visualized by expressing fluorescently tagged versions of the TetR protein, which binds to TetO repeats (TetR-GFP or TetR-mCherry; **Figures 1A-B**). We then monitored budding yeast mother cells throughout their entire replicative lifespan using live-imaging microscopy combined with a microfluidics platform[5,19]. The replicative age of each mother cell was measured by counting the number of daughters that it budded off over time, defining how many budding events they have already completed at a given time point (completed budding events, CBE). Bud size and the number and position of the spindle pole bodies (SPBs, labelled with mCherry) were used to identify the cell cycle stages at which the cells were imaged (**Figure 1A**).

Analysis of these images showed that all young mother cells (~0-3 CBE; t=0h) and their daughters contained a fluorescent dot, documenting the presence of the labelled chromosomes in all of them. However, when reaching the age of 18 to 22 CBE (t=24h), 10-15% of these now old mother cells had lost their dot (**Figures 1B-C**). This was observed irrespective of which chromosome was labelled, and which fluorescent tag was used. Fluorescence of the labelled SPB was not affected, indicating that dot loss was not caused by fluorophore instability in old cells. A chromosome dot could be lost from aging mother cells in three possible ways: 1) Chromosome mis-segregation during anaphase 2) Migration of the entire nucleus into the daughter cell 3) Through the loss of a TetO array. The SPB is located in the nuclear envelope, so the presence of the SPB but the absence of the chromosome points to chromosome loss during anaphase, rather than the mis-segregation of the entire nucleus into the daughter cell (**Figure 1A**). Nuclear mis-segregation into the daughter cells was previously reported during replicative aging and is marked by the segregation of both SPBs to the daughter compartment (**Sup Figure 1A-B**)[20]. While we do observe whole nuclei mis-segregation in ~3% of the cells, this is a distinct event from chromosome loss, which occurs in ~10-15% of final replicative divisions.

To test if the loss of fluorescent chromosomal foci was due to the loss of the TetO array specifically or of the entire chromosome, we included a second label (an array of 256 repeats of the LacO sequence) in the middle of the long arm of chromosome IV (**Figure 1B**) and asked whether the cells that lost the TetO array lost the LacO repeats as well. Analysis of these images showed that 85% of the cells lacking the TetO array had indeed lost the LacO array (50/59). Thus, these cells had probably lost the entire chromosome. Supporting this notion, loss of the TetO array was fatal to the cells. More than 99% (145/146 chromosome II loss events) of the dot-less cells failed to divide further, as expected for a haploid cell losing an entire chromosome. Thus, chromosome loss took place specifically in the last division of old mother cells and likely caused their death.

Finally, we examined whether the loss of a reporter chromosome points to general chromosome instability in old cells. To assess this possibility, we quantified how often aging cells simultaneously lost both chromosome II and chromosome IV (**Figure 1B-C**). As expected, the co-loss rate (~5%) was lower than the individual loss rates (~10-15%), but notably higher than the ~1% expected by chance alone. Therefore, the loss of a single chromosome does not necessarily accompany the loss

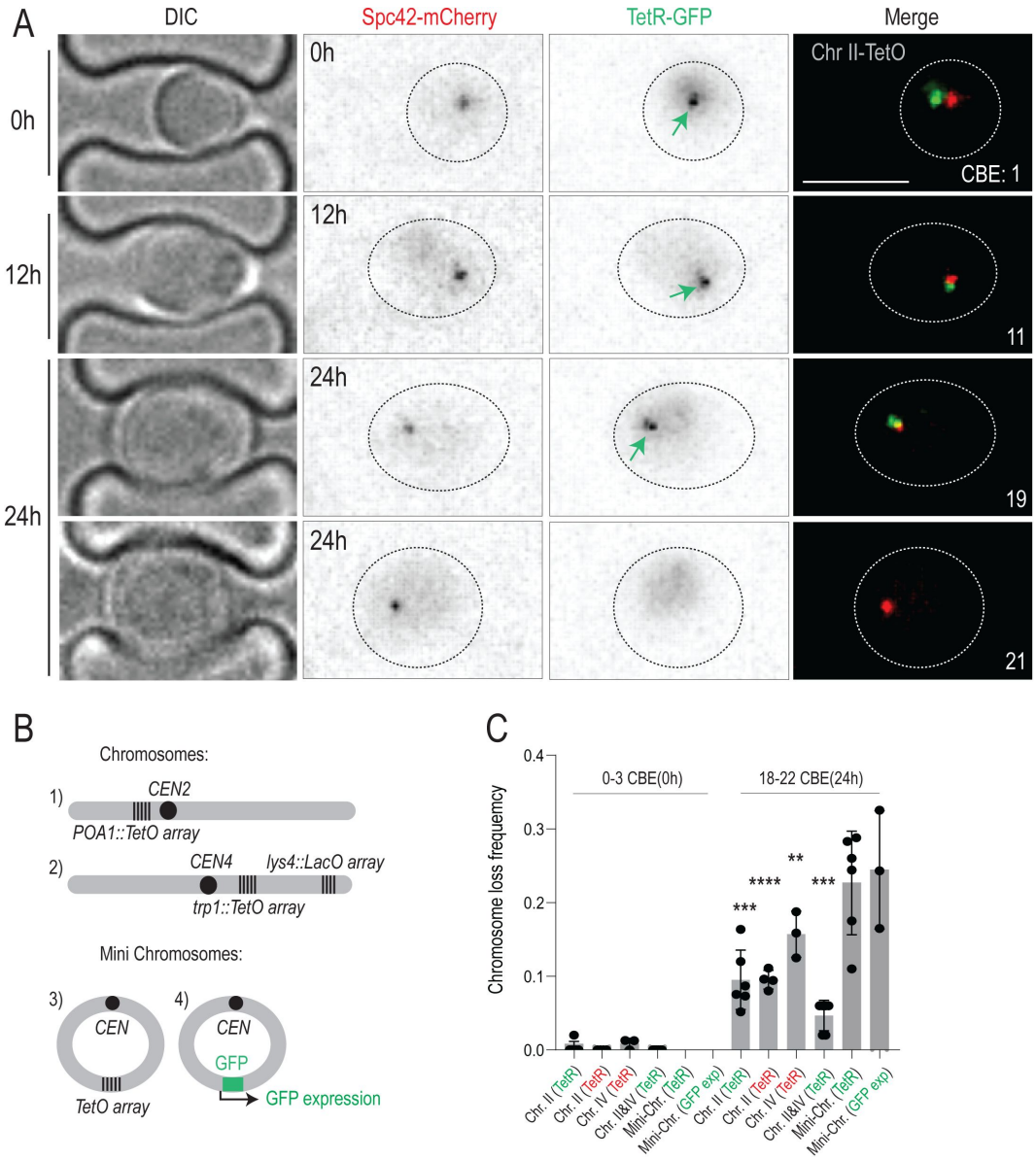

Figure 1

Old cells lose chromosomes

A) Cells carrying labeled chromosome II (Chr II) and imaged at different ages on the microfluidic chip. Time of acquisition is indicated on the left. Fluorescent markers are indicated on top. Replicative age of imaged cells (completed budding event – CBE) is indicated in merged images. Scale bar (upper right panel) is 5μm. Green arrow marks the TetR-GFP foci **B)** Schematic representation of the chromosome and minichromosome labels used in this study. **C)** Chromosome loss frequency of indicated chromosomes after 0h (~0-3 CBE) and 24h (~18-22 CBE) of imaging. The mode of chromosome visualization is indicated in parenthesis, i.e., TetR-GFP (green), TetR-mCherry (red) or mini-chromosome encoded GFP (GFP exp). Chromosome loss is defined as the absence of the label in the mother cell in G1 shortly after the final cell cycle, or the final anaphase. Each data point represents frequency of chromosome loss in a cohort of ~50 cells. Unpaired T-test (*<0.05, ** <0.005, ***<0.0005).

of another, but the high rate of simultaneous mis-segregation points to general chromosome instability in old cells. Considering that the budding yeast haploid genome has 16 chromosomes, the high loss frequency of a single chromosome and the fact that individual chromosomes are lost in not fully independent manners we estimate that 35-70 % of aging mother cells likely lose several chromosomes in their final division, whereas the other cells die with an intact karyotype. We concluded that much like many other organisms, yeast undergoes a burst of chromosome instability in old age (**Figures 1A, C**).

In contrast to chromosomes, extra-chromosomal DNA circles accumulate in the yeast mother cell with age rather than being lost[7,12]. Similarly, deleting the centromere from a chromosome causes the retention of both sister chromatids in the mother cell[18]. Thus, we wondered whether centromeres drive chromosome loss in old mother cells. DNA circles containing a centromeric sequence (*CEN*) were lost from aged mother cells at a similar rate to full-size chromosomes (~20% of old cells; **Figures 1B-C**). Removing the centromere of the exact same circle was shown in previous work to cause its retention in the mother cell rather than its loss[5,12,13]. Therefore, centromeres are necessary and sufficient for chromosomes and mini chromosomes to be lost from aging mother cells. To exclude the possibility that the TetO array used to label them is the cause of chromosome and mini-chromosome loss in aging, we also characterized the stability of a circular mini-chromosome expressing short-lived GFP as a marker instead of TetO repeats. Scoring of the GFP signal demonstrated that old mother cells lost these mini-chromosomes at essentially the same frequency as they lose the TetO labelled one (**Figures 1B-C**). We concluded that centromeres drive chromosome loss in old cells.

Aged cells asymmetrically partition both sister chromatids to the bud

Given the prominent role of the centromere in chromosome loss, we rationalized that the loss of chromosomes in old mother cells could be due to mitotic mis-segregation events. To determine whether it was the case, we next focused our analysis on cells imaged during mitosis in time-lapse recordings of aging cells (**Figure 2A**). Analysis of anaphases in old mother cells revealed a striking increase in the frequency of chromosome mis-segregation, using labelled chromosome II, IV and minichromosome as reporters (**Figure 2B**). In 600 total anaphase events captured in our movies we found that 66 (11%) of them co-segregated the labelled sister chromatids to a single pole with, 54/66 (82%) of the mis-segregation events resulting in asymmetric partition of the chromatids to the bud of these old mother cells (**Figure 2C**). This was virtually never observed in young cells (**Figure 2A-B**). Remarkably, the frequency of chromosome loss in the old mother cells and of sister chromatids segregating asymmetrically to the bud were in the same size order, irrespective of whether chromosome II or chromosome IV was labelled. Therefore, chromosome loss from old mother cells seems fully explained by a rise in sister chromatid non-disjunction with age, resulting in co-segregation of the sister chromatids asymmetrically to the bud. Next, we examined the mechanism behind this peculiar mode of directed chromosome mis-segregation.

In budding yeast, the pre-existing (old) SPB, which the cell inherited from the previous mitosis, segregates in a highly biased manner to the bud (~95% of mitoses)[21–23]. Consequently, labelling of stable SPB components, such as the core SPB protein Spc42, with mCherry causes the pre-existing SPB to shine bright and segregate in 95% of mitoses to the bud (**Figure 2A**, red arrowhead), while the new one is dim and remains in according proportions in the mother cell. This useful effect is a result of the folding kinetics of mCherry. In short, mCherry matures slowly (~45 minutes half-time) relative to the duration of yeast mitosis (~50 minutes from spindle assembly to completion of cytokinesis), and therefore the new mCherry-labelled SPB is dimmer than the old one. Given the mother-daughter bias of SPB inheritance and sister chromatid mis-segregation with age, we wondered whether the non-disjoining sister chromatids of old cells attach to and co-segregate with the old SPB. Thus, we examined the partition of the labelled chromosomes relative to the pre-existing and new SPBs in old mother cells (t=24h, **Figures 2D,**

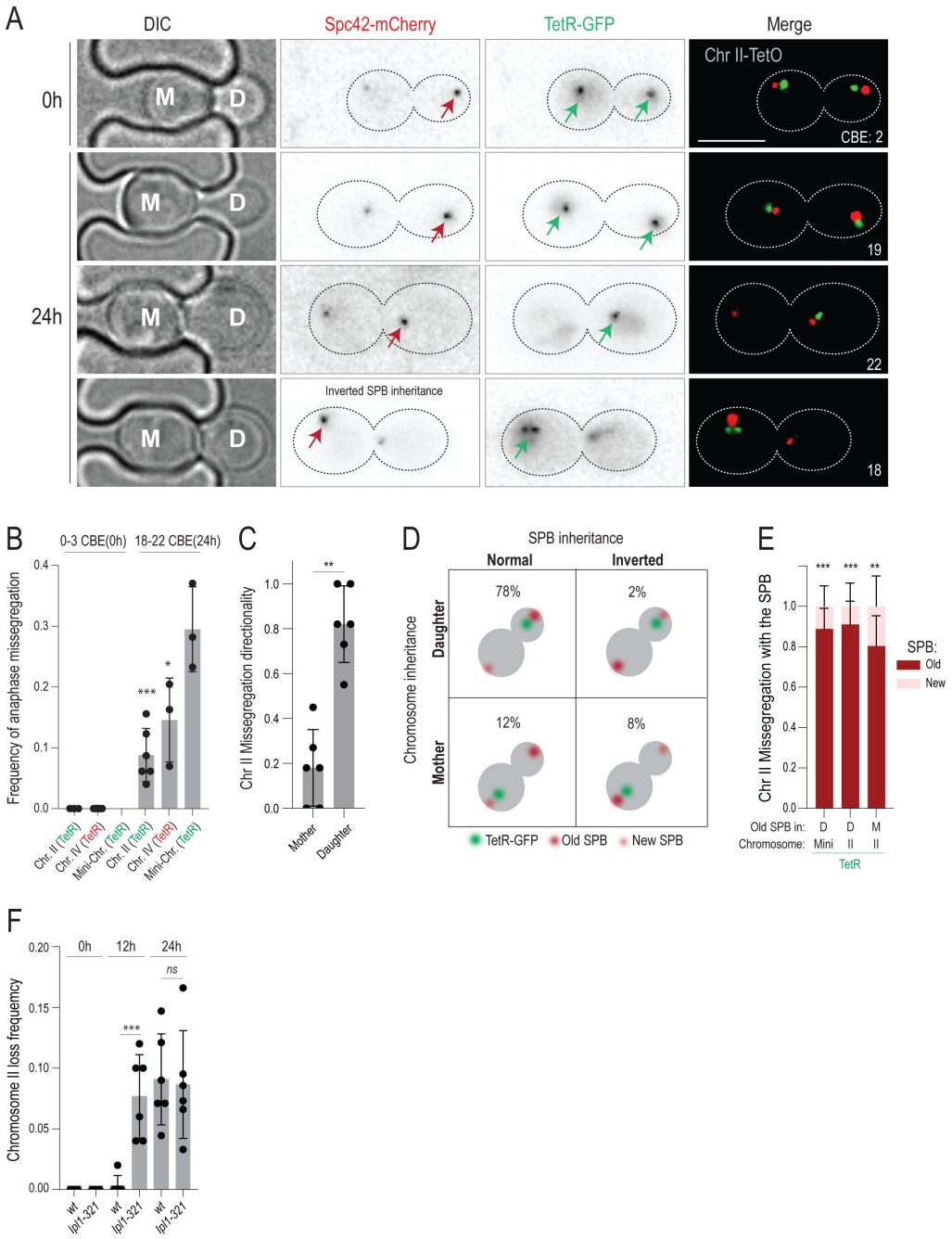

Figure 2

Old cells lose chromosomes by asymmetric sister chromatid partitioning

A) Images of anaphase cells carrying the labeled chromosome II as in **Fig. 1A**. Red arrows mark the old SPBs. **B)** Frequency of anaphase mis-segregation events where chromosomes are visible only in the mother or daughter cell (n>150) Bars are labelled as in **Fig 1C**. Each data point represents frequency of anaphase mis-segregation in the cohort of ~50 anaphases **C**) Fraction of anaphase mis-segregation events that lead to TetR-GFP foci being present only in mother or daughter cell (n=66, cohorts of 10). **D)** Graphical schematic of all observed anaphase mis-segregation events and their frequency. **E)** Fraction of anaphase mis-segregation events biased towards the old (red) or new SPB (pink) for mini-chromosome (n=30) and Chr II with normal (Daughter(D)) and inverted segregation of the old SPB (Mother (M)) (n=60 and 30). Cohorts of 10-20 missegregating anaphases **F)** Frequency of chromosome II loss in *wt*, *Ipl1-321*, at 0h (~0-3 CBE), 12h (~8-12 CBE) and 24h (~18-22 CBE) at 27°C. Each data point represents loss frequency in a cohort of ~50 cells. Unpaired T-test (*<0.05, **<0.005, ***<0.0005).

E). In about 10% of old cells, SPB inheritance is inverted, meaning that the old SPB goes to the mother cell instead of the daughter[21,22]. In a strong majority of these cases (80%), the non-disjoining sister chromatids followed the old SPB to the mother cell, instead of going to the bud (**Figures 2D, E**). We conclude that non-disjoining sister chromatids of old mother cells remained attached to the pre-existing SPB and partitioned with it to the bud, causing them to lose these chromosomes. In other words, SPB identity mattered more than mother-daughter identity in determining which cell inherited the mis-segregated chromosomes.

In budding yeast, sister chromatids both initially attach to the pre-existing SPB, which is available first. These syntelic attachments are then dissolved by the aurora B kinase, allowing sister chromatids to reorient, reach bi-orientation and partition symmetrically in mitosis[24,25]. Failure to correct these initial attachments errors causes sister chromatids to co-segregate with the old SPB to the bud[26]. The similarity between this phenotype and the mechanism of chromosome loss in old mother cells suggested, therefore, that the error correction pathway might be impaired in old yeast cells. To test this possibility, we analysed the effects of dampening the function of aurora B (called Ipl1 in budding yeast) during aging. Young cells carrying the temperature sensitive *ipl1-321* mutant allele and grown at a permissive temperature (27°C) showed no detectable chromosome loss (**Figure 2F**). However, they started losing chromosomes early in age (**Figure 2F**), resulting in a shortened lifespan (**Sup Fig 1C**). Strikingly, the chromosome loss frequency of the old *ipl1-321* mutant cells (t=24h) was indistinguishable from that of the wild type cells, indicating that the effects of age and reducing Ipl1 activity were not additive (**Figure 2F**). This suggests that age and the *ipl1-321* mutation affect the same pathway. We concluded that old cells lose chromosomes due to defects in correcting chromosome attachment errors.

ERC accumulation drives chromosome loss in aging

To identify mechanisms behind the deterioration of mitotic error correction with age, we investigated whether chromosome loss is causally linked to other aging events, particularly to the displacement of the basket from NPCs[5]. Given that basket displacement is triggered by accumulating ERCs and their SAGA-dependent anchorage to NPCs[5,12,13], we first investigated whether ERC accumulation promotes chromosome loss. Mutant cells lacking the deacetylase Sir2, which inhibits recombination in the rDNA locus, form ERCs at an increased rate, accumulate them quicker, and their replicative lifespan is shorter than that of wild type cells[7,27]. Supporting the notion that ERC accumulation might promote chromosome loss, these mutant cells also showed a strong and premature loss of the reporter chromosome (**Figure 3A**). To further test the effect of ERC accumulation, we asked whether reducing their formation rate delays chromosome loss. Recombination in the rDNA locus is stimulated by the presence of a replication fork barrier between rDNA units[28]. While these barriers prevent the transcription machinery to collide with replication forks coming from neighbouring rDNA units, the stalling forks are fragile, causing high rates of double strand breaks. Therefore, removing these fork barriers, for example by inactivating the fork barrier protein Fob1, drastically decreases the rate at which ERCs form and accumulate, stabilizes the nuclear baskets of NPCs and prolongs the life span of the cells[5,28]. Strikingly, the *fob1*Δ mutant cells trapped in our microfluidics platform lost chromosome II at a substantially reduced frequency compared to wild type cells, even after dividing 20 times or more (**Figure 3A**). Introducing an artificial replicative DNA circle with a different sequence than an ERC into wild type cells also promoted chromosome loss, supporting the idea that DNA circles promote chromosome loss with age independently of their sequence (see below). Taken together, the chromosome loss rates seen in cells with artificial DNA circles, along with those in *sir2*Δ and *fob1*Δ cells, show that chromosome loss correlates well with the accumulation of extrachromosomal DNA circles. Since the primary known effect of DNA circle accumulation is to cause the displacement of the nuclear basket from NPCs, we wondered next whether this event was causally linked to chromosome loss. Supporting this notion, removal of the SAGA subunit Sgf73, which mediates ERC attachment to NPCs and the subsequent displacement of their nuclear basket, phenocopied the effect of the *fob1*Δ mutation[12]: the *sgf73*Δ mutant cells

failed to show any significant increase in chromosome loss frequency at any age (**Figure 3A**). Thus, our data suggested that ERC formation and anchorage to NPCs triggered chromosome loss in old yeast cells.

Nuclear basket remodelling drives chromosome loss

To investigate whether basket displacement promotes chromosome loss in some manner, we next perturbed the nuclear basket and measured whether this accelerated chromosome loss and aging. To do this, we disrupted the *MLP1* gene, encoding the major isoform of the structural basket protein TPR in yeast. In stark contrast to the *sgf73*Δ and *fob1*Δ single mutants and the wild type cells, the *mlp1*Δ single mutant cells exhibited a rapid increase in chromosome loss frequency as they aged, as determined by using our reporter chromosome II, along with a shortened replicative lifespan (**Figures 3A-B**). Thus, our data suggest that displacement of the basket from NPCs as the cells age promotes chromosome loss through some yet unknown mechanism.

Introns in genes of the aurora B pathway drive asymmetric chromosome partitioning in aging

Together our data suggested that the displacement of the nuclear basket from NPCs upon ERC anchorage weakened the molecular pathway that corrected chromosome attachment errors (**Figure 3A**). This impaired ability to correct erroneous chromosome attachments in turn caused old mother cells to lose chromosomes to their buds. To test this hypothesis, we next investigated how basket displacement could lead to asymmetric chromosome partitioning. The basket of the nuclear pore has been involved in various processes, such as mRNA export[29], protein quality control[30], docking of environmentally regulated genes to the nuclear periphery upon their induction[31], the retention of faulty mRNAs in the nucleus, such as unspliced pre-mRNAs[32,33], as well as docking Mad1 and Mad2, two key components of the mitotic spindle assembly checkpoint (SAC), to the NPC[34,35].

The SAC components Mad1 and Mad2 bind Mlp1, Mlp2 and Nup60 at the basket of NPCs[35]. However, basket dissociation, which delocalizes Mad1/2 from the nuclear periphery, does not appear to interfere with their function[35]. Nevertheless, we investigated whether SAC dysfunction could account for aging phenotypes caused by basket dissociation and for the premature aging phenotype seen in *mlp1*Δ mutant cells. Arguing against this hypothesis, the *mad1*Δ and *mad2*Δ single mutant cells exhibited no change in replicative lifespan compared to wild type cells and did not phenocopy *mlp1*Δ mutant cells (**Sup Fig 2A**). Therefore, we searched for other mechanisms through which basket displacement could trigger chromosome loss.

While perusing the list of genes involved in chromosome segregation (**Figure 4A**) we noticed that only three of them contain an intron, *NBL1*, *MCM21* and *GLC7*. Surprisingly, these three genes all function with Ipl1/aurora B in preventing syntelic chromosome attachment. Nbl1/borealin is a component of the chromosome passenger complex of which aurora B/Ipl1 is the catalytic subunit. Mcm21/CENP-O is a docking receptor for Ipl1/aurora B at kinetochores. Glc7/PP1A is the protein phosphatase 1, which counteracts aurora B by dephosphorylating its targets at the outer-kinetochore[36–38]. Intrigued by this remarkable convergence, we wondered whether asymmetric chromosome partitioning in aging could stem from the presence of these three introns in error correction genes. We rationalized that displacement of the basket from NPCs could potentially affect the regulation, export and hence expression of intron-containing transcripts, specifically, thereby affecting the function of the *NBL1*, *MCM21* and *GLC7* genes.

To investigate the possibility that they contribute to the high rate of chromosome loss in old cells, we removed the introns of *GLC7*, *MCM21*, *NBL1* (-Δ*i* alleles) and imaged these mutant cells throughout their replicative lifespan, monitoring chromosome II segregation. Removing each intron individually or all three simultaneously (*3x*Δ*i* strain) had no detectable effects on

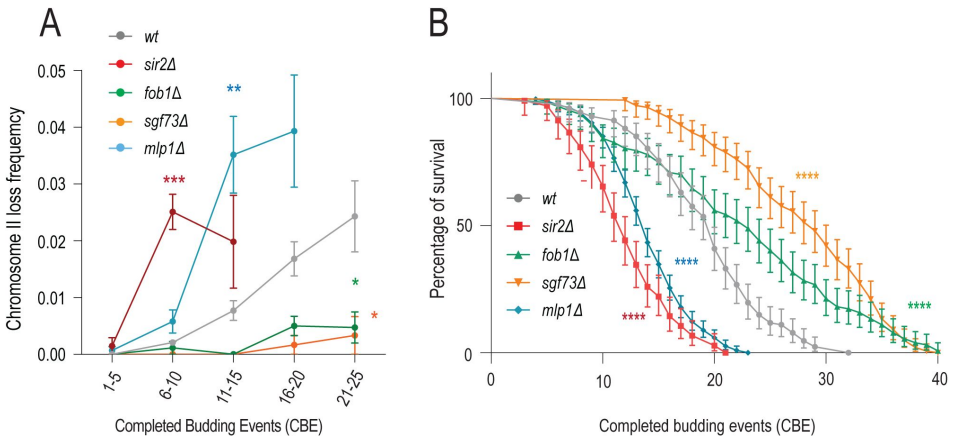

Figure 3

ERC formation and NPC remodeling drive chromosome loss and aging

A) Chromosome II loss frequency per CBE in strains of indicated genotype (divided into categories of 5xCBE)*(N, n(cells/divisions) wt=458/8600, sir2Δ=158/1779, fob1Δ=127/2681, sgf73Δ=142/3950 mlp1Δ=302/4270*).Loss frequencies in the same age category are compared to wt. Unpaired T-test (*<0.05, **<0.005, ***<0.0005). **B)** Replicative lifespan of listed genotypes (n>120 cells, Log-rank (Mentel Cox) test *<0.05, **<0.005, ***<0.0005).

A

Yeast genes encoding kinetochore-associated proteins

CBF3	CCAN	CPC	SAC
NDC10	*MIF2*	*IPL1*	*MAD1*
CEP3	*CSE4*	*SLI15*	*MAD2*
CTF13	*CTF19*	*NBL1*	*BUB1*
SKP1	*OKP1*	*BIR1*	*BUB3*
	MCM21	SPC105	*MPS1*
CBF1	*AME1*	*SPC105*	Motor
	CHL4	*YDR532*	*KIP1*
MIS12	*CNN1*	DAM1	*KIP3*
MTW1	*WIP1*	*ASK1*	*CIN8*
DSN1	*MHF1*	*DAD1*	*KAR3*
NNF1	*MHF2*	*DAD2*	MAPS
NSL1	*MCM16*	*DAD3*	*SLK19*
	CTF3	*DAD4*	*BIK1*
NDC80	*MCM22*	*DAM1*	Phosphatase
NDC80	*MCM19*	*DUO1*	*GLC7*
NUF2	*NKP1*	*SPC19*	*CDC14*
SPC24	*NKP2*	*SPC34*	
SPC25	*YBP2*	*HSK1*	

Adapted from Biggins et al 2013

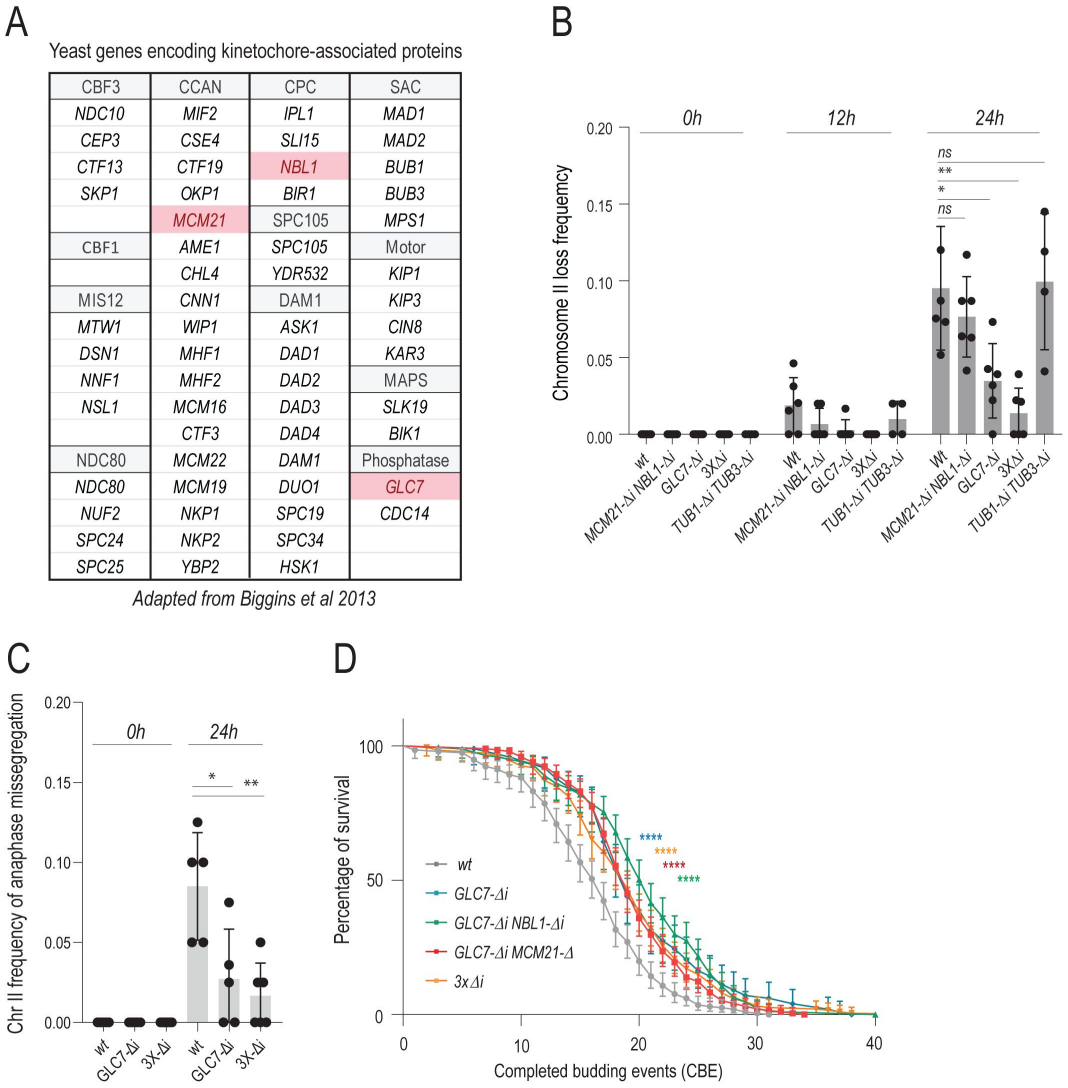

Figure 4

Introns drive asymmetric chromatid partitioning and chromosome loss in aging

A) A list of genes encoding kinetochore-associated proteins adapted from *Biggins et al 2013*. Intron containing genes are marked in red. **B)** Chromosome II loss frequency at indicated aging time (top) in cells of indicated genotype. *3xΔi stands for GLC7-Δi MCM21-Δi NBL1-Δi* triple mutant. Each data point represents the frequency of chromosome loss in a cohort of ~50 cells. Unpaired T-test (*<0.05, **<0.005, ***<0.0005). **C)** Frequency of anaphase mis-segregation of chromosome II at indicated aging time (top) in cells of indicated genotype. *3xΔi stands for GLC7-Δi MCM21-Δi NBL1-Δi* triple mutant. Each data point represents mis-segregation frequency in a cohort of ~50 anaphases. Loss frequencies in the same age category are compared to wt. Unpaired T-test (*<0.05, **<0.005, ***<0.0005). **D)** Replicative lifespan upon intron removal (n>200 cells, Log-rank (Mentel Cox) test, *<0.05, **<0.005, ***<0.0005).

chromosome segregation in young cells (**Figure 4B**), cell growth and mitotic timing (**Sup. Figure 3A-C**), Interestingly, whereas the removal of *GLC7*'s intron decreases the expression of the gene by about 50% and affects cell proliferation [39–41], we note that the *GLC7*-Δ*i* allele is duplicated in our strain, probably owing to recombination between transposon LTRs surrounding it (**Sup Figure 3E-F**). We suspect that this duplication explains why our *GLC7*-Δ*i* mutant strain grew as well as wild type cells. Simultaneously removing all three introns nearly completely suppressed chromosome loss in old cells (**Figure 4B-C**). Removing them individually or in pair did so as well, albeit the effects were milder. Importantly, the simultaneous removal of *NBL1*, *MCM21* and *GLC7* introns did not affect the duration of the last budding event (**Sup Figure 3D**), indicating that it suppressed chromosome loss without alteration cell cycle progression. Removing the introns of the two α-tubulin genes *TUB1* and *TUB3* simultaneously had no effect, indicating that the observed effects were specifically related to the introns of *NBL1*, *MCM21* and *GLC7* and not to introns in general (**Figure 4B**). The effects observed were similar for chromosome II and chromosome IV, indicating that the role of these introns was not chromosome specific (**Sup Figure 2B**). Furthermore, removing these three introns also suppressed the occurrence of asymmetric anaphases in old cells (**Figure 4C**), supporting the view that asymmetric chromosome segregation and chromosome loss go hand-in-hand and are under the control of these three introns. Strengthening this conclusion, removal of these introns also suppressed chromosome loss in the *ipl1-321* mutant cells (**Sup Figure 1D**).

Remarkably, simultaneous removal of *MCM21*, *NBL1* and *GLC7* introns had also a substantial effect on the longevity of the cells, extending their replicative lifespan up to ~20% (**Figure 4D**). A similar effect was observed in the *ipl1-321* mutant cells (Sup Fig1C). Thus, chromosome loss may indeed be the first direct cause of death for many yeast cells upon aging, although other mechanisms eventually take over when chromosome loss is abrogated.

Basket destabilization causes intron-dependent chromosome loss in young cells

Our data were consistent with the hypothesis that basket dissociation from NPCs promotes chromosome loss through an intron-dependent dampening of the error correction pathway. To test this hypothesis in greater depth we asked whether mutations affecting the nuclear basket also promote chromosome loss outside of the aging context. Thus, we characterized chromosome segregation in young *mlp1*Δ cells (**Figure 5A-C**). Analysis of chromosome II segregation indicated that the *mlp1*Δ mutant cells not only exhibited a four-fold increase in their chromosome mis-segregation frequency compared to the young wild type cells, but ~80% of these mis-segregation events followed the old SPB, as in old cells (**Figure 5C**). Furthermore, this chromosome loss was suppressed upon removal of the introns of *MCM21*, *NBL1* and *GLC7* (**Figure 5B**). Therefore, although to a lower extent than in old cells, defects in the nuclear basket result in asymmetric chromosome partitioning in an intron-dependent manner in young cells as well. Interestingly, removal of these three introns also rescued the age dependent chromosome loss and partially restored the longevity of the *mlp1*Δ mutant cells (**Figure 5D-E**). We concluded that intron-dependent dampening of the error correction pathway is one, even if not the only progeric effects of the *mlp1*Δ mutation. Possibly, other introns promote additional aging phenotypes. Thus, we conclude that basket displacement promotes chromosome loss in young mutant cells and old wild type mother cells.

Intron deletion suppresses the effect of DNA circle accumulation on chromosome loss

As seen above, chromosome loss correlates well with conditions that promote ERC accumulation and their anchorage to NPCs. This idea gained in traction as we progressed, because ERC attachment, and the attachment of any DNA circle, to NPCs is known to mediate nuclear basket

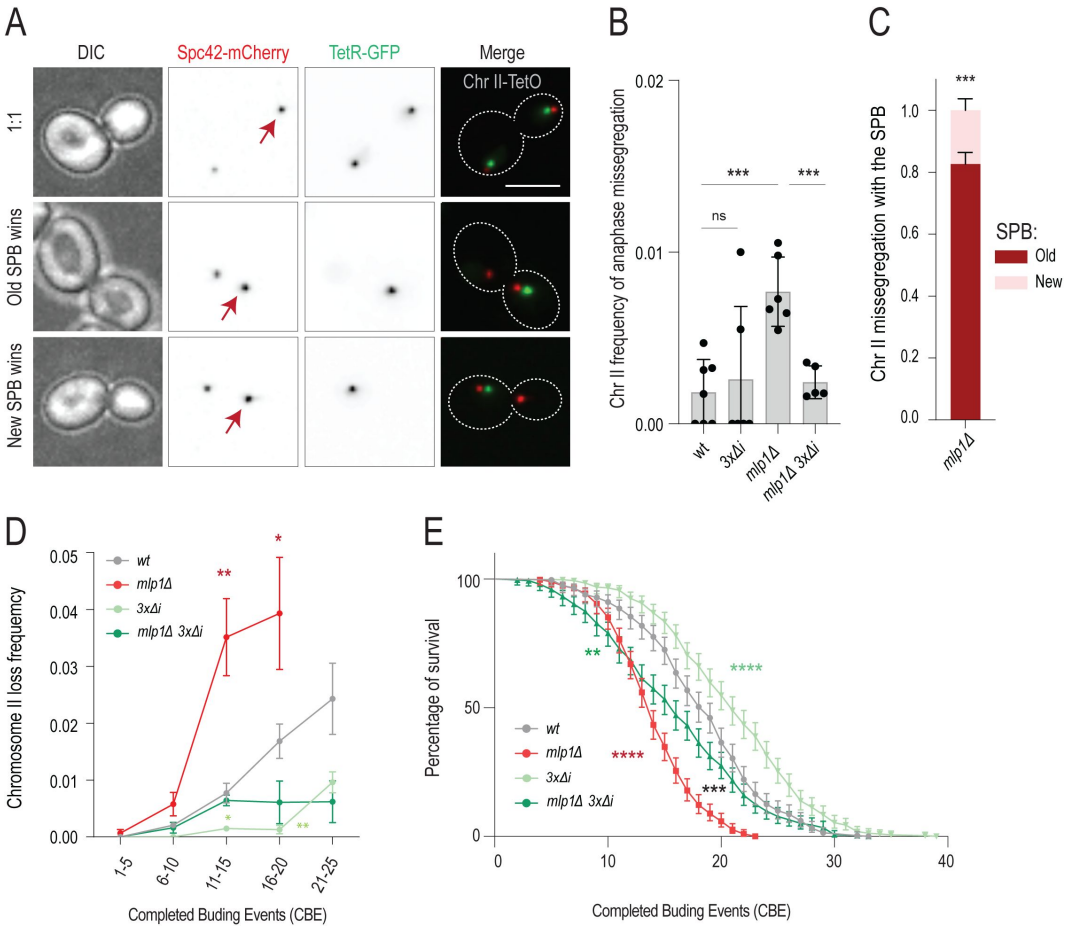

Figure 5

Basket displacement causes intron-dependent chromosome loss in young and old cells

A) Stills of symmetric and asymmetric anaphases in young cells. Arrow marks the old SPB. Scale bar (upper right panel) is 5µm **B)** Frequency of anaphase mis-segregation of chromosome II in cells of indicated genotypes *(n>2500 cells).* Each data point represents anaphase mis-segregation frequency in a cohort of >300 cells. **C)** Fraction of chromosome II mis-segregation with old (red) or new SPB (pink) in anaphase cells of indicated genotype (n=30 cohorts of ~10 mis-segregation events) Unpaired T-test (*<0.05, **<0.005, ***<0.0005) **D)** Chromosome II loss frequency in indicated genotype as a function of CBE (categories of 5xCBE) *(N, n(cells/divisions) wt=458/8600, mlp1Δ=302/4270,3xΔi=469/9974 mlp1Δ 3xΔi=295/4757*) **E)** Replicative lifespan of listed genotypes. Red and green stars represent *p-values* between the wt and the corresponding genotype. Black stars represent the p-value between *mlp1Δ* and *mlp1Δ 3xΔi* (n>300 cells, Log-rank (Mentel Cox) test, *<0.05, **<0.005, ***<0.0005).

displacement[5].Thus, we further investigated the relationships between DNA circle accumulation and chromosome loss by investigating how the removal of *NBL1*, *MCM21* and *GLC7* introns affected chromosome loss observed upon DNA circle accumulation. For this, we followed two parallel approaches. First, we tested whether introducing a replicative DNA circle into the cells is sufficient to drive chromosome loss during aging, and if so, whether this event required the presence of the introns of *NBL1*, *MCM21* and *GLC7*. Thus, we transformed the wild type and the *3xΔi* mutant strains carrying the labelled chromosome II as reporter with the yeast replicative plasmid YRp17[42]. We then monitored the frequency at which these transformed cells lost the reporter chromosome as they underwent aging. YRp17 contains a replication origin (*ARS1*) but no centromere, such that it accumulates in the mother cells with each replicative cycle (**Figure 6A**). As expected[7], the cells carrying YRp17 showed a much shorter lifespan (10 CBE median RLS, **Figure 6B**). Remarkably however, the effect of YRp17 on longevity was substantially suppressed by the 3xΔi mutations (**Figure 6B**). Analysis of chromosome loss indicated that introducing this DNA circle promoted chromosome loss, starting early in life and increasing rapidly with age (**Figure 6C**). Furthermore, removal of the introns of *NBL1*, *MCM21* and *GLC7* (*3xΔi* cells) largely suppressed the effect of YRp17 on chromosome loss. However, note that the suppressive effect of the *3xΔi* mutations on longevity and chromosome loss was only partial, indicating that YRp17 promotes chromosome loss through both a mechanism that involves these three introns, and at least one additional mechanism independent of these introns.

As a second approach, we investigated whether removing the introns of *NBL1*, *MCM21* and *GLC7* mitigates the chromosome loss phenotypes of the *sir2*Δ mutant cells. Supporting this hypothesis, the *3xΔi* triple mutation significantly, though modestly, improved the longevity phenotype of these cells (**Figure 6D**). As in cells carrying YRp17, the triple intron removal indeed ameliorated the premature chromosome loss phenotype of the *sir2*Δ mutant cells (**Figure 6E**). As for YRp17, the effect of the 3xΔi was substantial and significant, but partial. Thus, we concluded that the accumulation of DNA circles, such as ERCs and others, which promote the dissociation of the basket from NPCs and thereby drive chromosome loss at least in part through the introns of *NBL1*, *MCM21* and *GLC7*. While this effect appeared to be the dominant one in physiologically aged cells, additional effects seem to further promote chromosome loss in the *sir2*Δ mutant cells and cells carrying an exogenous DNA circle, such as YRp17.

Old cells leak pre-mRNA to the cytoplasm

Putting our different observations together, our data suggested that removal of the nuclear basket from NPCs in response to the accumulation of DNA circles with age causes old yeast mother cells to lose chromosomes to their last bud. Furthermore, the effect of nuclear basket removal from NPCs was primarily mediated by three introns. This suggested that the nuclear basket of NPCs enforces proper chromosome segregation through its function in pre-mRNA processing or quality control. To test this possibility, we next investigated whether old mother cells show defects in pre-mRNA quality control. Since, basket mutant cells fail to retain unspliced pre-mRNAs in the nucleus [32,33], we reasoned that old yeast mother cells also might leak pre-mRNAs to the cytoplasm.

As a first proof of principle, we characterized the localization of intronic RNA sequences in young and old cells, using single molecule RNA fluorescence in situ hybridization (smRNA FISH). Most yeast introns are short (50-150 nucleotides) and well below the minimal size detectable by smRNA FISH (>500 nts, ideally >1 kb). In the yeast genome, only 20 introns are longer than 500 nucleotides. Thus, we created probes for the longest introns, namely those of *DBP2* (766 nts) and *YRA1* (1002 nts), and of *GLC7* because of its role in chromosome segregation (525 nts). The introns of *NBL1* (66 nts) and *MCM21* (88 nts) are too short for smRNA FISH. Cohorts of old cells were collected using the mother enrichment program (MEP)[43] followed by FACS sorting to reach high homogeneity (see methods). By inducing cell death in the daughter cells specifically, the MEP ensures that aging mother cells are diluted linearly instead of exponentially in the population, greatly facilitating the isolation of very old cells. FACS sorting ensured that the cohorts were free of young cells and young cell debris. With each of these three series of smRNA FISH probes, we

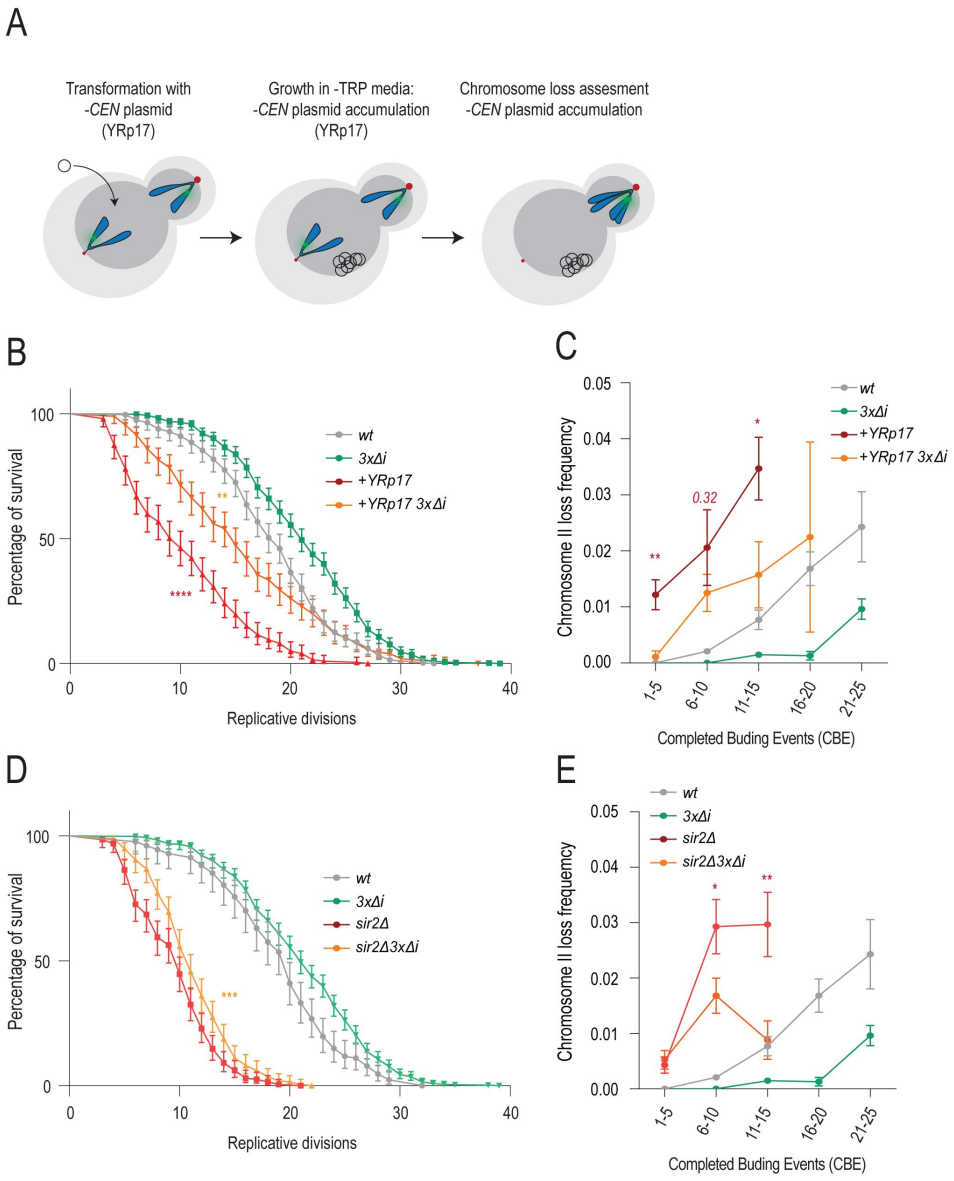

Figure 6

Non-centromeric DNA is sufficient to drive aging and chromosome loss

A) Schematic of transformation of chromosome II reporter strains with YRp17 (-*CEN* plasmid) and the accumulation of non-centromeric plasmids. **B)** Replicative lifespan of strains with and without YRp17 plasmid. Red stars represent *p-value* between the YRp17 and YRp17 *3xΔi* strain. Orange stars represent the *p-value* between YRp17 *3xΔi* and wt (n>200 cells, Log-rank (Mentel Cox) test, *<0.05, **<0.005, ***<0.0005) **C)** Chromosome II loss frequency in indicated genotypes as a function of CBE (divided into categories of 5xCBE) *(N, n(cells/divisions) wt=458/8600,3xΔi=469/9974,*+YRp17 *=199,2097,* + YRp17 *3xΔi=200,3134).* **D)** Replicative lifespan of *sir2Δ and sir2Δ3xΔi* strain. Stars represent *p-value* between *sir2Δ and sir2Δ3xΔi* (n>200 cells, Log-rank (Mentel Cox) test, *<0.05, **<0.005, ***<0.0005). **E)** Chromosome II loss frequency in indicated genotype as a function of CBE (divided into categories of 5xCBE) *(N, n(cells/divisions) wt=458/8600,3xΔi=469/9974, sir2Δ=351,3500, sir2Δ3xΔi=350,3840*).

detected one or two nuclear foci in young cells (**Figure 7A**). Only very few cells ((<3%) showed a focus in the cytoplasm. No foci were detected upon deleting the corresponding introns (Δi), demonstrating probe specificity (**Sup Figure 4A-B**). In contrast, for each of the three introns tested, smRNA FISH detected intronic RNA sequences in the cytoplasm of most old cells. This was not due to an increase in overall smRNA FISH foci number during aging (See **Sup Figure 4C**), but due to the change in foci distribution between nucleus and the cytoplasm. For example, in the case of *GLC7*, ~70% of smRNA FISH foci were found in the cytoplasm of old mother cells, compared to ~3% in young cells (**Figure 7A-B**). Thus, 3 out of 3 tested introns showed a strong tendency to leak to the cytoplasm in old mother cells. However, this first test did not clarify whether the full pre-mRNA or only the intron escaped from the nucleus.

To directly address this question, we turned to a functional assay and monitored the translation of an unspliced pre-mRNA reporter in the cytoplasm. This fluorescent reporter contains the coding sequence for mCherry in the intron and expresses GFP upon proper splicing of the nascent transcript. Therefore, mCherry is only translated when the unspliced pre-mRNA leaks out of the nucleus (**Figure 7C**)[44]. Cells carrying this construct integrated at the *TRP1* locus were trapped and imaged in our microfluidics platform. As cells aged, we measured the number of completed budding events and the fluorescence levels of the two reporter fluorophores. Fluorescence signal analysis established that the mCherry/GFP ratio increased in old compared to young cells, demonstrating elevated pre-mRNA translation in the cytoplasm of a large majority of aged cells (**Figures 7D-E**).

Consistent with ERC-dependent leakage and translation of this reporter, the mCherry/GFP ratio increased prematurely and to a higher level in the *sir2*Δ mutant, compared to the wild type cells (**Figures 7D-F**). The fact that signal was already observable in young *sir2*Δ mutant cells suggests that the pleiotropic functions of Sir2 cause a fraction of these effects. Conversely, the *fob1*Δ mutant cells showed a lowered mCherry/GFP ratio, which did not increase much during their lifespan (**Figures 7D-F**). Thus, we conclude that ERC formation and accumulation promotes pre-mRNA leakage during yeast aging, as expected from their effect on the nuclear basket.

Pre-mRNA leakage causes intron-dependent chromosome loss in young cells

Given the results above, we wondered whether chromosome loss in old cells is linked to their failure to retain pre-mRNAs in the nucleus. Thus, we asked whether inducing pre-mRNA leakage in young cells would trigger chromosome loss. The two yeast SR-protein Gbp2 and Hrb1 have been implicated in the retention of pre-mRNAs in the nucleus by binding intron-containing transcripts and delaying their export until they are properly spliced or degraded[45,46]. Accordingly, the *hrb1*Δ *gbp2*Δ double mutant cells leak pre-mRNA to the cytoplasm at a higher rate than wild type cells[45,47]. Thus, we asked whether these double mutant cells lost the labelled chromosome II at an increased frequency. As a control, we asked whether the *snu66*Δ mutation, which affects the spliceosome itself directly[48,49], lead to similar phenotypes.

The young spliceosome-defective *snu66*Δ mutant cells did exhibit a five-fold elevated level of chromosome mis-segregation compared to young wild type cells. However, this mis-segregation was not biased towards any specific SPB (**Figures 8A-B**), suggesting that it involved a distinct mechanism than that observed in old cells and in young cells lacking the basket protein Mlp1. In contrast to the *snu66*Δ mutant, the *hrb1*Δ *gbp2*Δ double mutant cells, showed an even further increased frequency of chromosome mis-segregation, which was more than ten-fold above that of young wild type cells (**Figure 8A**). Furthermore, sister chromatid mis-segregation was strongly biased towards the old SPB (**Figure 8B**). Removing the introns of *GLC7*, *MCM21* and *NBL1* suppressed this chromosome segregation defect (**Figure 8A**). Therefore, pre-mRNA leakage is sufficient to induce asymmetric chromosome partitioning and is contingent on the presence of

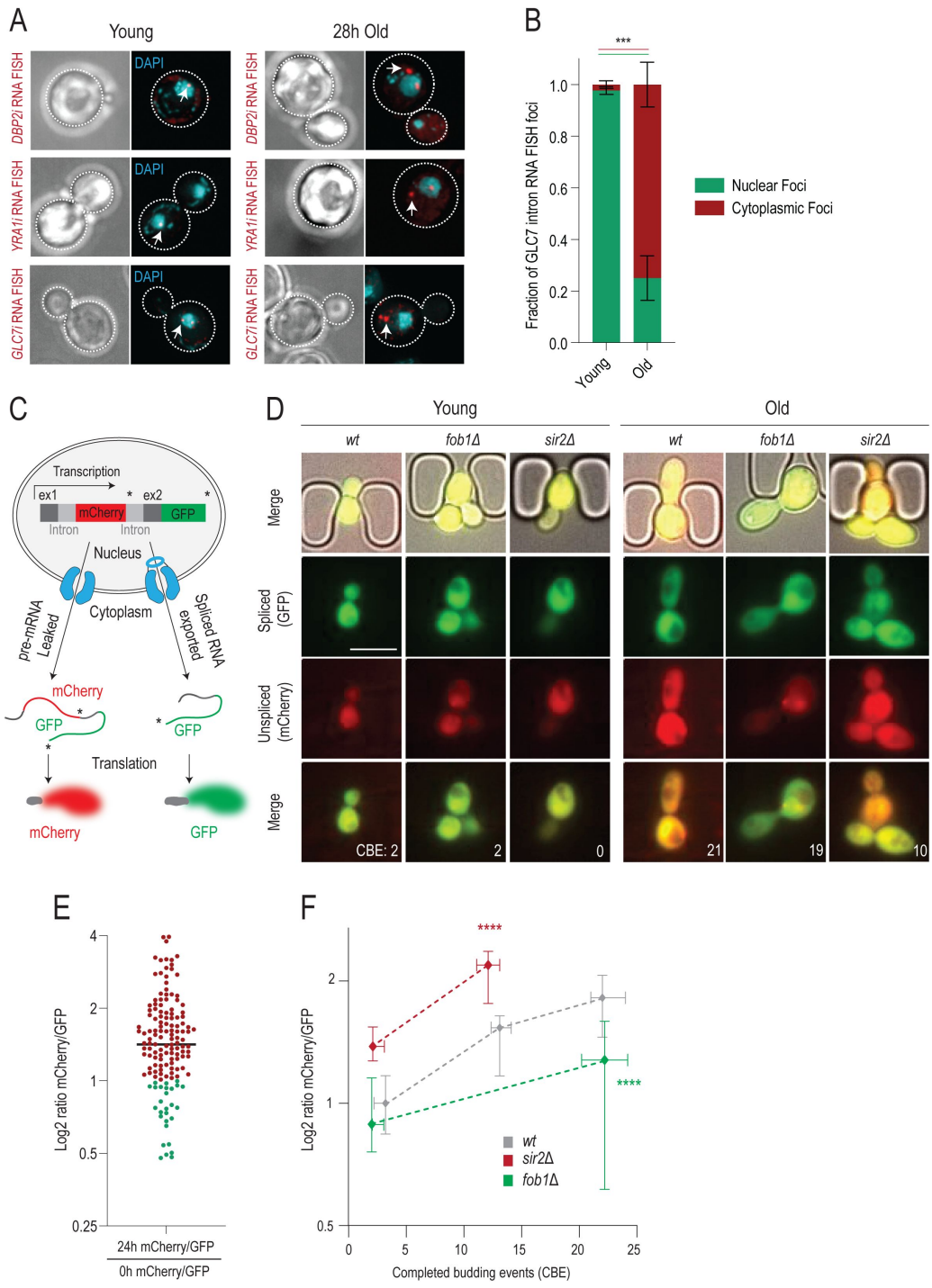

Figure 7

Pre-mRNA leaks to the cytoplasm of old cells

A) Images of RNA FISH targeting three long introns (*GLC7, YRA1, DBP2*) in young and old cells. **B)** Quantification of *GLC7* intron RNA FISH foci localization in young and old cells (n=295, cohorts of ~100 cells) **C)** Principle of the pre-mRNA translation reporter (left panel) adapted from (*Sorenson et al. 2014*). **D)** Images of the pre-mRNA translation reporter in cells (right panel) of indicated genotype (top) at indicated ages (bottom). **E)** Ratio of mCherry/GFP signal in the same cells at 0h and after 24 hours of aging. 119/146 cells have a ratio greater than "1" **F)** Log2 ratio of mCherry/GFP in cells of indicated genotype as a function of age, normalized to wt median at 0h (n=60, 35, 32). Unpaired T-test comparing mCherry/GFP intensity of cells at 12 or 24h (*<0.05, **<0.005, ***<0.0005).

introns in the error correction genes *NBL1*, *MCM21* and *GLC7*. The fact that mutations affecting the spliceosome did not promote asymmetric chromosome segregation to any specific SPB with age indicates that the chromosome loss observed in old cells is not due to splicing defects.

We conclude that accumulation of extrachromosomal DNA causes the displacement of the NPC basket and subsequent leakage of pre-mRNAs. Leakage of intron-containing transcripts into the cytoplasm impairs correction of syntelic attachments by the Ipl1/aurora B pathway, likely because three genes involved in this pathway contain introns. This results in asymmetric partitioning of sister chromatids, and age-associated chromosome loss. In summary, basket displacement and pre-mRNA leakage impair error correction via an as-yet unknown mechanism involving the introns of *NBL1*, *MCM21* and *GLC7*.

Discussion

Aging manifests itself through a broad diversity of conserved cellular phenotypes, but in most cases, we know little about how these are causally linked to each other[1–3]. In this study we investigated whether NPC remodelling in old yeast cells contributed to the emergence of other aging phenotypes, using chromosome loss as a case study[5]. We showed that NPC remodelling is both necessary and sufficient to drive the age-associated increase in chromosome loss and we identified the mechanisms involved. Specifically, interventions that promoted displacement of the NPC basket, or basket defects more broadly, enhanced non-disjunction and mis-segregation of sister chromatids to the bud, thereby promoting chromosome loss in old yeast cells. These interventions included mutations that stimulated ERC formation and removed the main yeast TPR isoform, Mlp1. Conversely, interventions that prevented NPC remodelling, such as delaying ERC accumulation or preventing their anchorage and the recruitment of SAGA to NPCs, restored proper chromosome segregation in old cells and supressed chromosome loss. Thus, NPC remodelling appeared to be the pivotal point for triggering chromosome loss in old cells.

Two potential caveats should be considered. First, the *sir2*Δ and *fob1*Δ mutations each have effects beyond influencing ERC formation rates. The *sir2*Δ mutant cells lose silencing of the hidden mating type loci, genetically behaving as diploids. They fail to silence subtelomeric regions and act through some as-yet unknown mechanism on the maintenance of proteostasis[50–53]. Likewise, the *fob1*Δ mutation renders the rDNA locus unstable, which might interfere with rRNA transcription and ribosome biosynthesis[54]. However, their only clear common denominator is that they both affect ERC formation, and hence NPC remodelling even if in opposite manners[27,28]. Moreover, the *sgf73*Δ mutation phenocopies the effects of the *fob1*Δ mutation. Here again the only known functional overlap between Fob1 and Sgf73 is that they promote ERC accumulation in the mother cell indirectly (Fob1) or directly (Sgf73), ERC anchorage to NPCs and the consequent displacement of NPC basket[5,12]. Finally, introducing another DNA circle in the form of a non-centromeric replicative plasmid, a process that also causes basket displacement[5], promoted chromosome loss as well. Thus, our data establish that DNA circle accumulation and attachment to NPCs trigger chromosome loss. Destabilizing the basket independently of DNA circle accumulation also promotes the premature onset of chromosome loss, indicating that it is the basket displacement from NPCs that triggers chromosome loss upon DNA circle accumulation.

A second possible caveat is that chromosome loss could be linked to aging in some other manner. To address this possibility, we investigated the molecular mechanisms that link basket displacement and chromosome loss. Our data reveal that chromosome loss is due to the non-correction of syntelic chromosome attachments in old cells. In young cells, the kinase aurora B and its accessory factors enforce such corrections[24]. Our data also show that the inhibition of error correction in old cells requires the presence of three introns, those of the genes *NBL1*, *MCM21* and *GLC7*. These three genes are the only chromosome segregation machinery genes that contain an intron. Strikingly, the proteins Nbl1, Mcm21 and Glc7 are all involved in the error correction

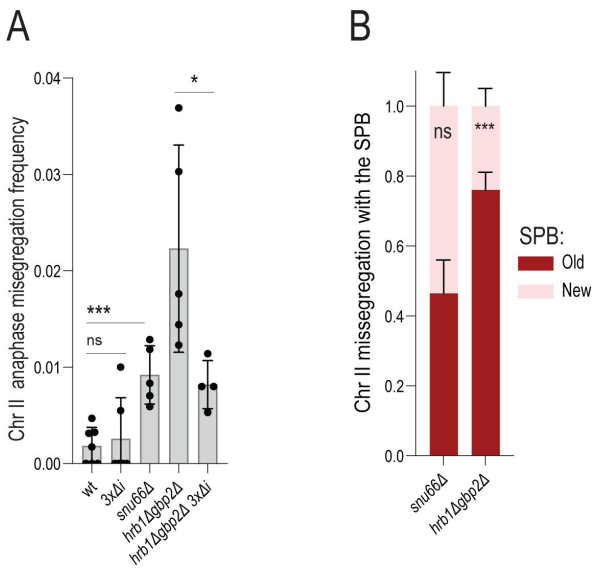

Figure 8

Pre-mRNA leakage is sufficient to drive asymmetric chromatid partitioning

A) Chromosome II mis-segregation in cells of indicated genotypes *(n=2774,3000,5269,2650,1607).* Each data point represents anaphase mis-segregation frequency in the cohort of >300 cells **B)** Fraction of chromosome II mis-segregation with old (red) or new SPB (pink) in anaphase cells of indicated genotype (n=50,30 cohorts of ~10 mis-segregation events). Unpaired T-test (*<0.05, **<0.005, ***<0.0005).

pathway, together with aurora B/Ipl1[36–38]. Removing their introns restored proper attachment correction and chromosome segregation in old cells and supressed the premature chromosome loss phenotype of the *ipl1-321* mutant cells grown at semi-permissive temperature. Intron removal delays cell death but does not abrogate aging. Furthermore, removing these introns not only supresses chromosome mis-segregation in old wild type cells, but also in young *mlp1*Δ mutant cells. Thus, intron removal separates aging and chromosome loss, indicating that these introns are part of the proximal cause of chromosome loss and act independently of age. Therefore, our data indicate that there is a direct, intron-dependent link between basket displacement from NPCs and chromosome loss. Our data indicate that this link involves the leakage of unspliced pre-mRNAs out of the nucleus upon basket removal and affects the basket's function in mRNA quality control. The fact that another, independent set of mutations that also interfere with pre-mRNA retention in the nucleus, such as the *gbp2*Δ *hrb1*Δ double mutant cells, also increase the frequency of chromosome loss through the same three introns strengthens this conclusion. Thus, together our data establish that displacement of the nuclear basket from NPCs in old mother cell directly triggers chromosome non-disjunction and loss through causing the leakage of the pre-mRNAs of the intron containing genes *NBL1*, *MCM21* and *GLC7*.

This conclusion has several consequences and opens new questions. The key question stems from our observation that releasing pre-mRNA from the nucleus relaxes the control that cells impose on chromosome attachment. The question is, how does this work? Does it involve the translation of the pre-mRNAs in the cytoplasm? Does it come about through the correlated loss of properly mature mRNAs and hence, the reduced expression of their products? Two arguments speak against these interpretations. First, if any of these two options were to account for the effect of pre-mRNA leakage, mutations affecting splicing efficiency would lead to the same effects. However, tempering with the spliceosome does not affect the correction of chromosome attachment errors to any extent and specificity close to the effects of pre-mRNA leakage. Second, since Mcm21 and Glc7 have opposite effects on chromosome correction it is unclear how impairing the expression of both would lead to the synergistic effects that we observe between them. Finally, analysis of RNA-seq data from replicatively aged cells indicate that if aging indeed impairs the splicing of some transcripts, such as those encoding ribosomal proteins, it stimulates that of others[55]. This suggests that aging has a non-linear effect on intron-containing transcripts. It will be important to determine whether basket displacement contributes to these effects, how it does so and whether this could explain the effect it has on the error correction pathway.

Our observation that basket displacement has such profound and highly specific impacts on processes functionally very distant from that of the NPC, such as chromosome segregation, has several consequences as well. First, it means that NPC remodelling could affect many more cellular functions than only chromosome segregation. Interestingly, beyond mRNA quality control the basket of the NPC is also involved in protein quality control and the activation of environmentally regulated genes, two processes that are impaired in old cells[1,2]. Thus, basket displacement might contribute to these phenotypes in aging cells. It is also very likely that the role of the basket in mRNA quality control could affect more than only chromosome segregation. Curiously, while budding yeasts have kept only a subset of the introns of their ancestors, these introns are not distributed randomly and seem to be enriched in very specific processes, such as genes coding for ubiquitin-conjugating enzymes and proteasome subunits, components of the mitochondrial respiratory chain and ribosomal proteins[56]. Interestingly, decay of the ubiquitin-mediated protein degradation, mitochondrial membrane potential and of ribosome assembly are classical and ubiquitous hallmarks of aging[1–3]. Thus, it is possible that basket displacement in old cells contributes to the emergence of these aging phenotypes as well.

A second type of consequences emerging from our observations stems from the fact that displacement of the NPCs' basket is also observed in stressed cells and not solely in the context of aging. For example, heat shock also causes basket displacement in yeast[57]. Therefore, it is possible that the downstream effects we observe in old cells also take place during stress response.

In this respect, it is interesting to note that heat shock has been associated with a strong rise in aneuploidy in yeast[58–60]. Furthermore, aneuploidy is a common response to environmental stress and a primary mechanism for the emergence of stress resistant clones in fungi[61]. Therefore, it is tempting to speculate that the chromosome mis-segregation resulting from basket displacement might be a selected response to stress. It might be selectively advantageous for cells to actively promote karyotype variations when their survival is in question. It is therefore worth considering that a similar process, perhaps using similar mechanisms, underlies the aneuploidy of cancer cells.

Third, the role of basket displacement in the emergence of aging phenotypes is likely not a peculiarity of yeast. Using proteomics, basket displacement has been inferred to take place in aging cells such as the hepatocytes of old mice and humans[62,63]. Little is known about whether the nuclear basket of metazoans plays a similar role in pre-mRNA retention in the nucleus as that of yeast, but it is striking that intron-retention is one of the hallmarks of aging on the metazoan transcriptome, and that pre-mRNA processing genes are important for mitotic stability[64–67]. Intron retention would be expected if some transcripts escaped the nucleus prematurely. More strikingly, perhaps the most ubiquitous effect of the expression of the progeric variant of lamin A, progerin, across cell types is the displacement of the nuclear basket protein TPR from NPCs[68]. We know still very little about the impact of basket displacement in the aetiology of the Gilford-Hutchinson Progeria Syndrome, but our study suggest that it might be relevant. If so, investigating the potential effects of progerin on pre-mRNA leakage might become an essential step towards understanding how progerin expression causes premature aging.

In a broader sense, our findings might bring fundamental insights towards our understanding of aging. For decades, the role of ERCs in aging has been perceived as a yeast peculiarity. However, the effect it has on NPCs may close the gap. On one hand, it seems to illuminate what might be the commonality between ERC-driven aging in yeast and other aging processes in other eukaryotes. Indeed, the basket-centric view of aging that we propose here would explain how ERCs actually cause aging in yeast and indicate that while ERC accumulation might be a yeast idiosyncrasy, its downstream effects are not. On the other hand, this idea open two corollary questions. 1) What is the biological significance of eccDNAs promoting basket displacement in yeast? 2) What causes basket displacement in other aging systems? We are intrigued by the idea that ERCs and any replicating eccDNA for that matter are the closest mimics that laboratory yeast strain may encounter of pathogenic DNA of exogenous origin. In that perspective, basket displacement and the ensuing effects might have first appeared as a response to infections. In other words, yeast aging might reflect the activity of a death pathway that normally functions in pathogen control. It might represent a fungal version the inflammatory or auto-immune response in metazoans. Therefore, it will be interesting to investigate whether exogenous or non-chromosomal DNA, such as that of viruses, also triggers the displacement of the nuclear basket from NPCs in yeast and other systems, and whether this contributes to either or both of anti-viral defence and aging. If so, aging might have emerged as an anti-viral defence.

Materials and methods

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Yves Barral (yves.barral@bc.biol.ethz.ch).

Materials availability

This study generated ~50 novel yeast strains, derived either by crossing or transformation. All strains, primers and RNA FISH probes are listed in **Supplementary table 1**. All resources are available to the public upon request. All the raw data used in this manuscript is available in

Supplementary file 1.

Experimental Model Details

Strains

All yeast strains and plasmids used in this study are listed in the **Supplementary table 1**. Strains are derived from S288C background. For every strain derived from a cross, an appropriate control strain was always selected from the same tetrad. Protein C-terminal yeGFP-tag and knock-out strains were generated using methods described in *Janke et al 2004* [69]. All cultures were grown using standard conditions at 160 rpm, in YPD or synthetic drop-out medium supplemented with 0.1% BSA for aging chips (SD-medium; ForMedium, Norfolk, UK) at 25°C or 30°C.

Intron-less strains

Intron deletions were conducted in two independent colonies of the strain JPY10I, as outlined previously in Parenteau *et al* 2008 [40]. Briefly, each intron was individually deleted using a modified two-step process (pop-in / pop-out), ensuring the absence of any additional sequence or markers post-deletion. The deletions were done in diploid strains, confirmed via PCR, and three distinct haploid strains bearing the confirmed deletions were chosen for subsequent analyses. Combinations of different Δ-intron strains have been derived by crossing and validated by PCR for the absence of the intron.

Chromosome and Plasmid reporter strains

Multiple chromosomes and minichromosome visualization strains have been used in this study: **1)** Chromosome II reporter strain, with the centromere proximal TetO array (*POA1* locus) strain was kindly provided by Elisa Dultz from the Weis Lab, ETH, institute of Biochemistry (internal reference KWY3759). **2)** Chromosome IV TetO (Centromere proximal) and LacO (centromere distal) were acquired from strains used in *Neurohr et al 2011* [17], originally derived from *Vas AC et al 2006* [70]. **3)** Ubi-GFP minichromosome (PYB 2665) was derived by cloning UBIYdkGFP* from *Houser et al 2012* (PNC 1136) [71] into a PYB 2640 backbone by homologous recombination in yeast. **4)** A minichromosome reporter containing the TetO array was previously described in *Denoth-Lippuner et al 2014* [12].

Plasmids

For the assessment of centromeric plasmid loss using the TeO-TetR-GFP system, we used the plasmid already published in *Dennoth-Lipptuner et al 2014*[12]. For construction of centromeric plasmid encoding GFP as a reporter, we used a short-lived GFP (UBIYΔkGFP*–SpHis5–TIM9 in pUC118) available from Addgene(pNC1136). Ubi-GFP was amplified from PNC1136 with primers MM 311/312 (listed in Supplementary table1). The resulting PCR product contained 50 bp homologous overhangs to another plasmid containing a centromere, pADH1 promoter and a pUG23 backbone (PYB2640). The template plasmid (PYB2640) was digested with MscI and Sac II and co-transformed with amplified UBIYΔkGFP* PCR product to yeast to assemble the plasmid with *CEN* and pADH1-UBIYΔkGFP* via homologous recombination. Successful recombinants were then selected on *-LEU* media after transformation. Recombinant plasmid was extracted from yeast and transformed into bacteria. The correct plasmid assembly was validated by sequencing. A full sequence of the assembled plasmid is available in supplementary data. For transformation of yeast with a non-centromeric plasmid, we have utilized Yeast Replicative Plasmid 17(YRp17) from *Mann et al 1983*. The full sequence of YRp17 is available online and in the supplementary data.

Materials and methods

Microscopy

For confocal fluorescent microscopy, yeast cells were precultured in YPD and then washed in synthetic drop-out medium. One ml of cells from exponential growing cultures with OD <1 was concentrated by centrifugation at 1500 rcf, washed in SD complete, and resuspended in ~10 µl of low fluorescent SD-medium, spotted on a round coverslip and immobilized with a SD/agar patch. The cells were imaged in >15 z-stacks slices with 0.3 µm spacing, with a 100×/1.4 NA objective on a DeltaVision microscope (Applied Precision) equipped with a CCD HQ2 camera (Roper), 250 W Xenon lamps, Softworx software (Applied Precision) and a temperature chamber set to 30°C.

Aging microfluidic platform

Chromosome segregation and protein intensity during aging were assessed using the high-throughput yeast aging analysis (HYAA) microfluidics dissection platform [19]. The PDMS (polydimethylsiloxane) microchannel is made by soft-lithography and bonded on the 30 mm micro-well cover glass in the 55 mm glass bottom dish (Cellvis, CA, USA). For lifespan analyses, a chip with a new cell trapping design was used [5], to ensure excellent retention of old cells.

To start the aging experiment, yeast cells were pre-cultured for 24 hr in SD-full media supplemented with 0.1% Albumin Bovine Serum (protease free BSA; Acros Organics, Geel, Belgium). Young cells from an exponentially growing culture were captured in the traps of the microfluidic chip; the chip was continuously flushed with fresh medium at a constant flow of 10 µl/min, using a Harvard PHD Ultra syringe pump (Harvard Apparatus, Holiston, MA, USA) with 60 ml syringe per lane, with inner diameter 26.7 mm (Becton Dickinson, Franklin Lakes, NJ, USA). Bright field images in a single z focal plane were recorded every 15 min throughout the duration of the entire experiment to measure replicative age of cells. To record fluorescent signals, images with a florescent lamp and at least 13x z-stacks (0.5 µm step) were acquired in 12h intervals. For imaging we used an epi-fluorescent microscope (TiE, Nikon Instruments, Tokyo, Japan) controlled by Micro-Manager 1.4.23 software [72], with a Plan Apo 60 × 1.4 NA objective. For fluorescence illumination of the GFP and mCherry labelled proteins, a Lumencor Spectra-X LED Light Engine was used. Z stacks of >13 slices with 0.5 µm spacing were recorded for fluorescent imaging every 12 hours to cover the entire volume of the nucleus during the aging process. Transmitted light imaging was done in a single Z plane to minimize the light exposure of the cells. The age of the cell was defined by the number of daughter cells that emerged during the budding cycles (CBE).

Chromosome loss assessment

For the assessment of chromosome and mini-chromosome loss and mis-segregation in aging, trapped cells in the chip that had the labeled chromosomes at the initial time point of 0h (~virtually all cells) were followed in transmitted light channel and fluorescent images were acquired every 12 hours. Only cells where the entire volume of the nucleus was in focus and imaged, and where SPB was clearly visible were considered in the analysis. The background fluorescence was subtracted in FIJI using a subtract background function. The aged nuclei were examined by scrolling through the z stack (>13 stacks, 0,5um step) in both channels, to validate the presence of the SPBs and TetR foci in the mother or the daughter cell. “Chromosome loss” refers to two quantified events: 1) The absence of the chromosome from a mother or a daughter cell compartment during anaphase 2) A G1 mother cell that just underwent its final division and is clearly viable, with a present SPB dot, but no chromosome dot. When “Chromosome loss” is listed, it pertains to the sum of these two events. When “chromosome loss in anaphase” is listed, it only pertains to the loss frequency observed during anaphase. Cells which lost chromosomes due to abnormal mitotic events in the last replicative division, such as SPB overamplification or full

nuclei migration into the bud (Combined ~10% of all cells in the final replicative division) were discarded from the quantifications. All cells were examined in the DIC channel for signs of disrupted morphology indicating cell death and followed up in DIC to confirm cell death after chromosome loss. For the assessment of minichromosome loss in aging (TetO array or ubi-GFP minichromosome) the yeast cultures were grown in SD-LEU or SD-URA selective media enabling minichromosome maintenance in all starting young cells. When loaded onto an aging chip cell were transferred to SD complete media, allowing them to lose the minichromosome during aging without inducing cell death. As the cells were grown in selective media, the minichromosome loss rate at 0h is N/A. In general, aging chips are not suitable for measurement of chromosome loss frequency in young cells, as cells within the young population that are missegregating chromosomes are usually stressed and enlarged, and thus cannot be loaded into the microfluidic traps intended for normal young cells. For chromosome mis-segregation frequency in young cells, we have used classical confocal imaging on a patch of SD agar, as described in the Microscopy section.

Measurements of chromosome loss per CBE

To measure chromosome loss probability per Completed Budding Event (CBE), we utilized at least 150 cells per genotype and quantified the number of cells that lost the reporter chromosome during a specific replicative division. The number of losses at a specific CBE was then divided by total number of cells which passed through mitosis at that CBE number to give the probability of chromosome loss per CBE. Categories of 5x CBE and its mean value of chromosome loss were plotted along with its SEM.

Mother Enrichment Program (MEP) with FACS and RNA FISH

Mother Enrichment Program was performed according to the standard procedure, using the strains acquired from the Gotchling Lab [43]. Cells were labeled with Biotin and aged for 28h, after which they were fixed in 4% PFA for 30 minutes. The cells were then stained with streptavidin conjugated with a FACS compatible fluorophore (647nm) and sorted using FACSAria III at the Flow Cytometry Core Facility at ETH Zurich to enrich for old mother cells. After FACS sorting, single molecule RNA FISH with Stellaris® probes designed for introns of *GLC7, YRA1, DBP2* was performed as described in *Rahman et al 2013* [73].

Quantification and Statistical analysis

Statistical analyses were performed using GraphPad Prism 10 software

Resources table

REAGENT or RESOURCE	SOURCE	Reference
Critical commercial assays		
GEL extraction	QIAGEN	Ref:28604
Plasmid extraction	MACHEREY-NAGEL	Ref:740588.250
Deposited data		
Raw data from this study	Mihailo Mirkovic	Supp table 2
Experimental models: Organisms/strains		
Full strain list	Mihailo Mirkovic	Supp table 1
Primers	Mihailo Mirkovic	See Sup. Table 1
smRNA FISH probes	Biosearch Technologies	See Sup. Table 1
Recombinant DNA		Internal reference
CEN TetO-plasmid	*Dennoth-L et al 2014*	YYB 16094
CEN Ubi-GFP plasmid	This study	PYB 2665
YRp17 plasmid	*Mann C et al 1983*	PYB 546
Software and algorithms		
Graphpad Prism	Dotmatics	
ImageJ-FIJI		
Microsoft Excel	Microsoft	
Microsoft Word	Microsoft	
Adobe Illustrator	Adobe	

YYB number	Genotype	Mating type	Plasmid	Strain made by
962	wt deletion collection	A		Deletion collection
3416	hoD::SCW11pr-Cre-EBD78-NatMX loxP-UBC9-loxP-LEU2 loxP-CDC20-Intron-loxP-HPHMX	α		Dan Gottschling lab
3476	trp1::TetO:TRP1, lys4::LacO:LEU2 ,his3::LacR-GFP:HIS3 ,TetR-RFP	A		Gabriel Neurohr
14507	LEU2::TetR-3mCherry POA1::TetO112:URA3	A		Karsten Weis lab(KWY3759)
16094	Spc42-mCherry:NAT leu2::LEU2 TetR-GFP	A	rec-URA3-CEN-rec-tetO-LEU2	Anna Marzelliusadottir
16128	lys4::LacO:LEU2 his3::LacR-GFP:HIS3 Spc42-mCherry:NAT	α		Anna Marzelliusadottir
16157	trp1::pRS405_Trp1_SplicingReporter	A		Anne Meinema
16161	fob1::KAN, trp1::pRS405_Trp1_SplicingReporter	A		Anne Meinema
16301	sir2::kanMX, trp1::pRS405_Trp1_SplicingReporter	A		Anne Meinema
16648	mad1::kanMX	A		Deletion collection
16934	lys4::LacO:LEU2 his3::LacR-GFP:HIS3 Spc42-mCherry:NAT	A		Mihailo Mirkovic
16935	lys4::LacO:LEU2 his3::LacR-GFP:HIS3 Spc42-mCherry:NAT *lpl1-321*	α		Mihailo Mirkovic
16936	lys4::LacO:LEU2 his3::LacR-GFP:HIS3 Spc42-mCherry:NAT *GLC7-Δi*	A		Mihailo Mirkovic
16937	lys4::LacO:LEU2 his3::LacR-GFP:HIS3 Spc42-mCherry:NAT lpl1-321 *GLC7-Δi*	α		Mihailo Mirkovic
17198	trp1::TetO:TRP1, lys4::LacO:LEU2 ,his3::LacR-GFP:HIS3 ,TetR-mRFP fob1::hphNT1	A		Mihailo Mirkovic
17213	mad2::KanMX	A		Deletion collection
17590	wt elela intronless collection	A	PYB 2665 Ubi-GFP	Mihailo Mirkovic
17591	*GLC7Δi*	A	PYB 2665 Ubi-GFP	Mihailo Mirkovic
17592	*lpl1-321*	α	PYB 2665 Ubi-GFP	Mihailo Mirkovic
17593	*lpl1-321 GLC7Δi*	α	PYB 2665 Ubi-GFP	Mihailo Mirkovic
17677	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2,Spc42-mCherry-hphNT1	α		Mihailo Mirkovic
17678	POA1::TetO112:URA3,leu2::TetR-GFP:LEU2,Spc42-mCherry-hphNT1 *GLC7Δi*	α		Mihailo Mirkovic
17679	POA1::TetO112:URA3,leu2::TetR-GFP:LEU2,Spc42-mCherry-hphNT1 *GLC7/MCM21/NBL1 ΔiΔiΔi*	A		Mihailo Mirkovic
17689	*lpl1-321*	A		Mihailo Mirkovic
17690	*lpl1-321 GLC7Δi*	α		Mihailo Mirkovic
17691	*lpl1-321 MCM21/NBL1ΔiΔi*	A		Mihailo Mirkovic
17692	*lpl1-321 GLC7/MCM21/NBL1-ΔiΔiΔi*	A		Mihailo Mirkovic
17693	wt lpl1 crosses	α		Mihailo Mirkovic
17694	*GLC7Δi*	A		Mihailo Mirkovic
17695	*GLC7/MCM21/NBL1 ΔiΔiΔi*	A		Mihailo Mirkovic
17834	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2,Spc42-mCherry-hphNT1 fob1::Nat	α		Mihailo Mirkovic
17838	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2,Spc42-mCherry-hphNT1 sir2::Nat	α		Mihailo Mirkovic
17924	lpl1-321 Glc7Δi POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42:mCherry:hphNT1	α		Mihailo Mirkovic
18019	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42-mCherry-hphNT	α		Mihailo Mirkovic
18028	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42:mCherry:hphNT1	α		Mihailo Mirkovic
18029	lpl1-321 POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42:mCherry:hphNT	A		Mihailo Mirkovic
18069	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42-mCherry-hphNT1	α		Jordan Mccarthy
18070	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42-mCherry-hphNT1 hrb1::kan gbp2::kan	α		Jordan Mccarthy
18071	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42-mCherry-hphNT1 hrb1::kan gbp2::kan *GLC7/MCM21/NBL1-ΔiΔiΔi*	A		Jordan Mccarthy
18100	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42-mCherry-hphNT1 *TUB1/TUB3-ΔiΔi*	A		Mihailo Mirkovic
18100	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42-mCherry-hphNT1 *GLC7/MCM21/NBL1-ΔiΔiΔi*	A		Mihailo Mirkovic
18213	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42-mCherry-hphNT1 snu66del::Nat	α		Mihailo Mirkovic
18214	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42-mCherry-hphNT1 *TUB1/TUB3-ΔiΔi* snu66::Nat	α		Mihailo Mirkovic
18493	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42-mCherry-hphNT1 sgf73::Nat	α		Mihailo Mirkovic
18661	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42-mCherry-hphNT1 mlp1::nat	α		Mihailo Mirkovic
18663	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42-mCherry-hphNT1 mlp1::nat , *GLC7/MCM21/NBL1-ΔiΔiΔi*	A		Mihailo Mirkovic
18683	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42:mCherry:hphNT1 *NBL1Δi MCM21Δi*	A		Mihailo Mirkovic
19347	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42-mCherry-hphNT1 *GLC7/MCM21/NBL1-ΔiΔiΔi* sir2::Nat	A		Mihailo Mirkovic
19348	POA1::TetO112:URA3 leu2::TetR-GFP:LEU2 Spc42-mCherry-hphNT1 trp1::TetO:TRP1	A		Mihailo Mirkovic
19453	POA1::TetO112:URA3,leu2::TetR-GFP:LEU2,Spc42-mCherry-hphNT1	α	YRp17(PYB546)	Mihailo Mirkovic
19454	POA1::TetO112:URA3,leu2::TetR-GFP:LEU2,Spc42-mCherry-hphNT1 *GLC7/MCM21/NBL1 ΔiΔiΔi*	A	YRp17(PYB546)	Mihailo Mirkovic

Supplementary Figure Legends

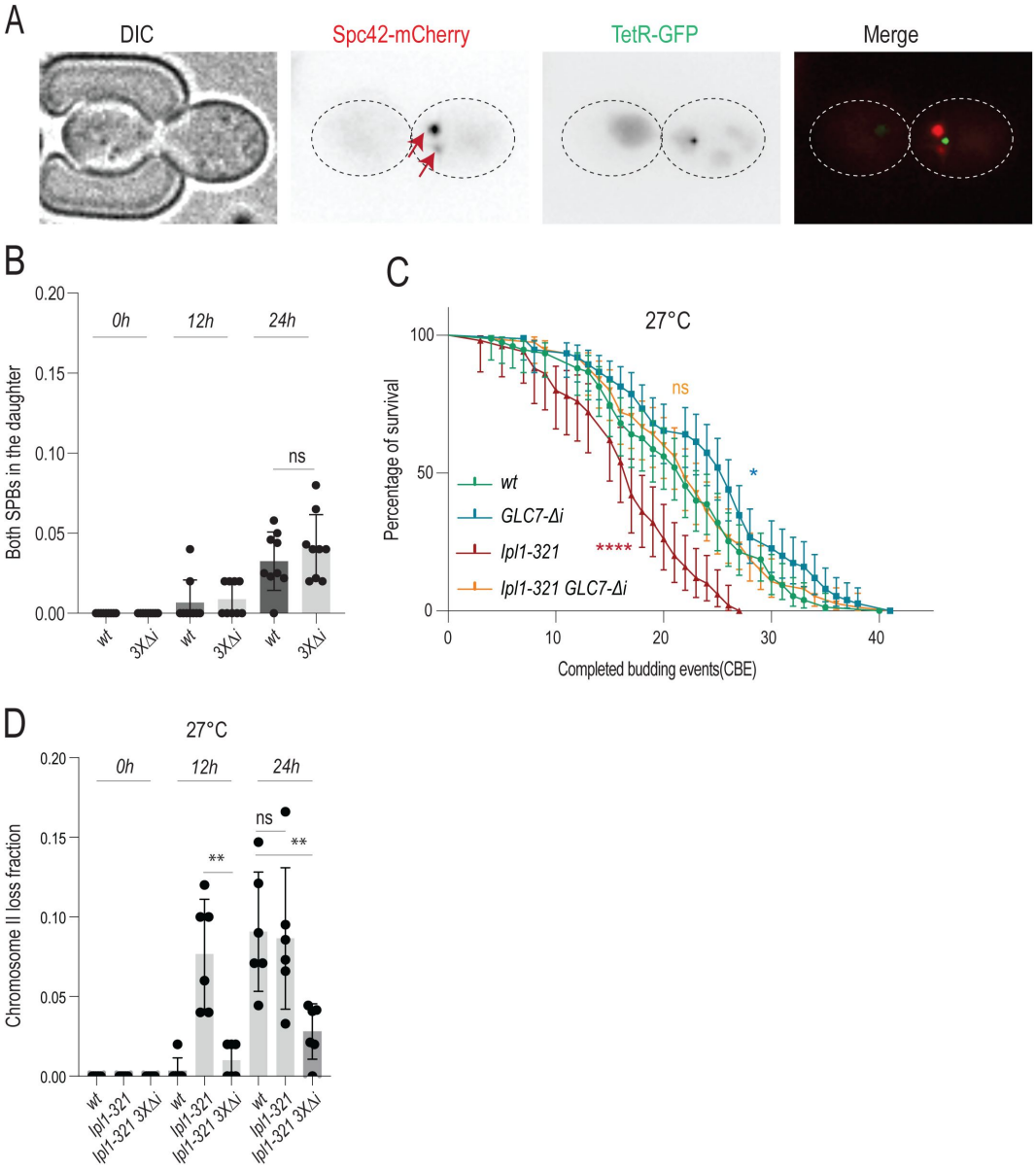

Figure S1

Introns affect chromosome loss, but not whole nuclei mis-segregation in aging

A) Stills of a whole nuclear mis-segregation event, with both SPBs ending up in the daughter cell. **B)** Quantification of the frequency of whole nuclear mis-segregation events in wt and 3xΔ*i* (n=450 cells, cohorts of 50 cells, Unpaired T-test. **C)** Replicative lifespan of wt, *GLC7Δi, Ipl1-321* and *Ipl1-321 GLC7Δi* cells grown at 27 Celsius (n=300), Log-rank (Mentel Cox) test * <0.05,**<0.005,***<0.0005.**D)** Frequency of chromosome II loss in wt, *Ipl1-321*, *Ipl1-321 3xΔi (GLC7-Δi MCM21-Δi NBL1Δi*) at 0h (~0-3 CBE), 12h (~8-12 CBE) and 24h (~18-22 CBE) at 27°C. Each data point represents loss frequency in a cohort of ~50 cells. Unpaired T-test (*<0.05, **<0.005).

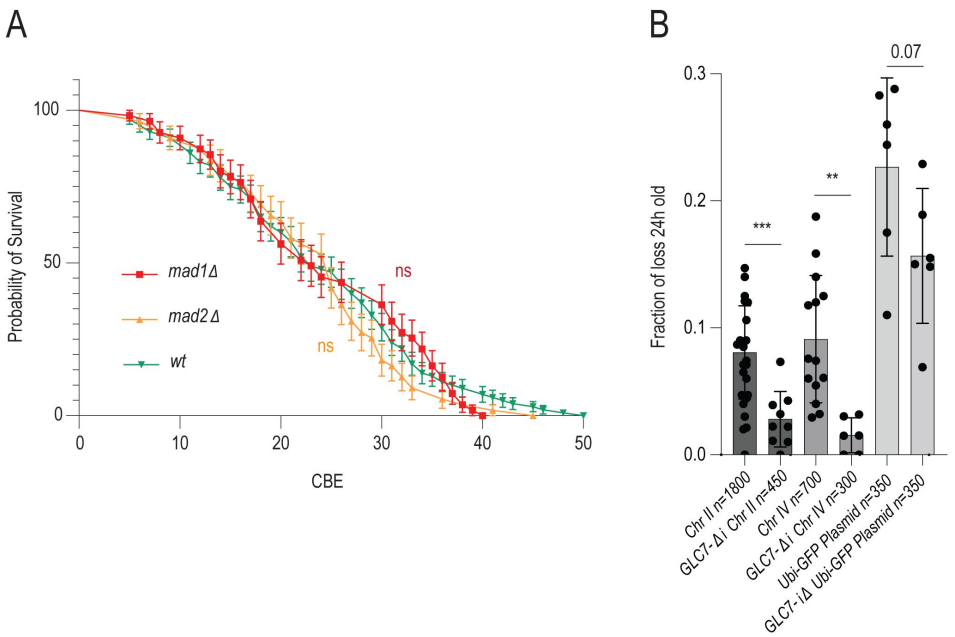

Figure S2

The effect of introns on chromosome loss is not chromosome-specific

A) Replicative lifespan of *wt, mad1*Δ, *mad2*Δ cells. n>100 cells, Log-rank (Mentel Cox) test. **B)** Frequency of chromosome II, IV and GFP minichromosome loss (See **Fig 1B**) in wt and upon removal of intron of *GLC7* at 24h (~18-22 CBE). Each data point represents a fraction of loss in a cohort of ~50 cells. Unpaired T-test (*<0.05, **<0.005, ***<0.0005).

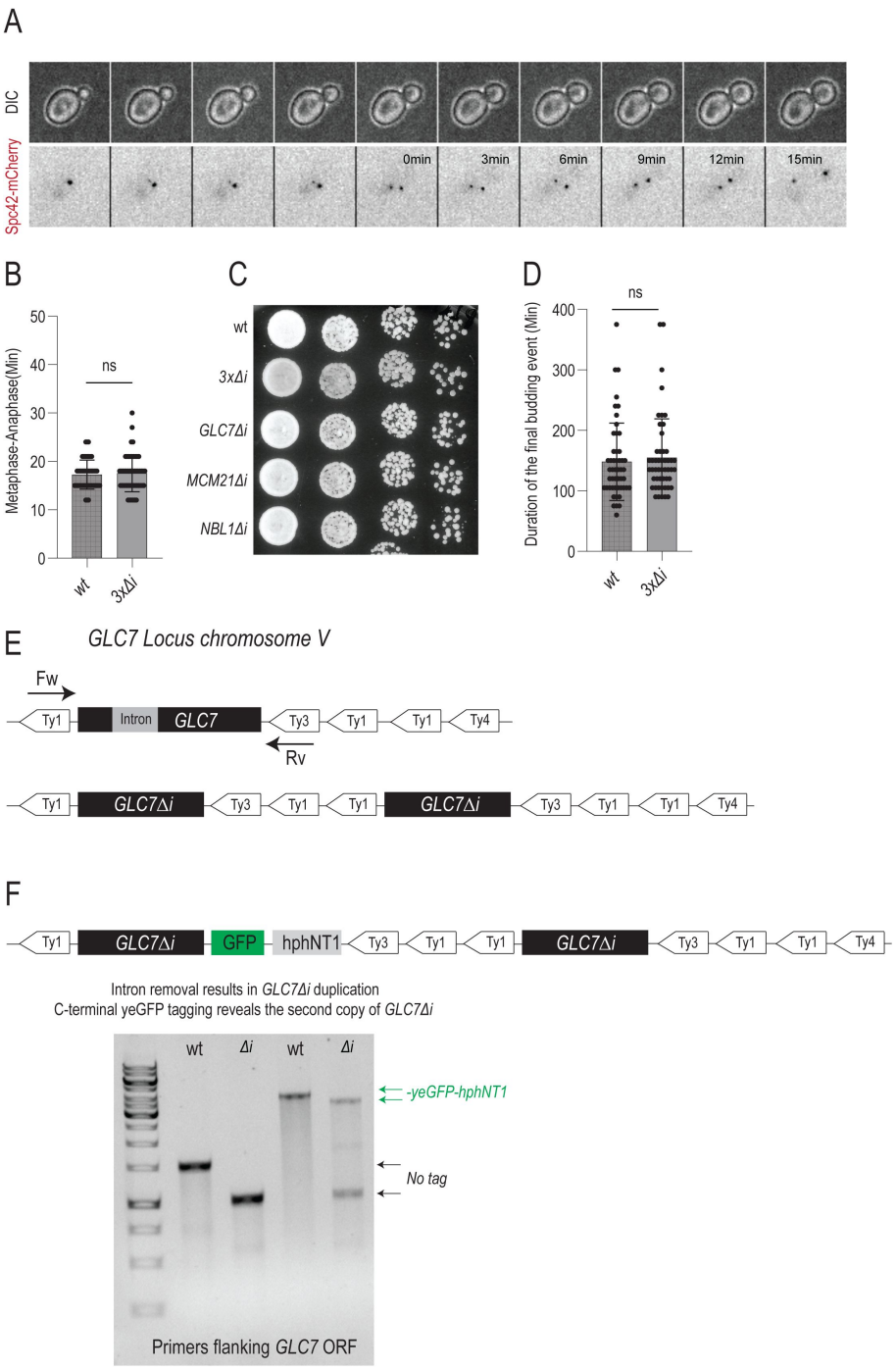

Figure S3

The 3x*Δi* removal does not alter mitotic timing, growth or budding in old age due to *GLC7Δi* duplication.

A) Stills depicting the method of mitotic timing quantification in young cells, measuring the time between SPB alignment and SPB separation, marking anaphase onset **B)** Mitotic timing in wt and *3xΔi* cells (n>50, unpaired T-test). **C)** 10-fold serial dilution spot assay for different intron removal strains. YPD, two days of growth, 30°C. **D)** Measuring the timing between the emergence of the bud and cytokinesis in the final completed division of the replicative lifespan in wt and *3xΔi* cells(n>50, unpaired T-test). **E)** Map of the *GLC7* locus on chromosome V, the flanking Ty1 elements, and the result of Ty1 transposition. **F)** The result of tagging the duplicated locus with C-terminal ye-GFP tag. Upon tagging the *GLC7-Δi* but not the wt *GLC7* locus, the locus duplication becomes evident.

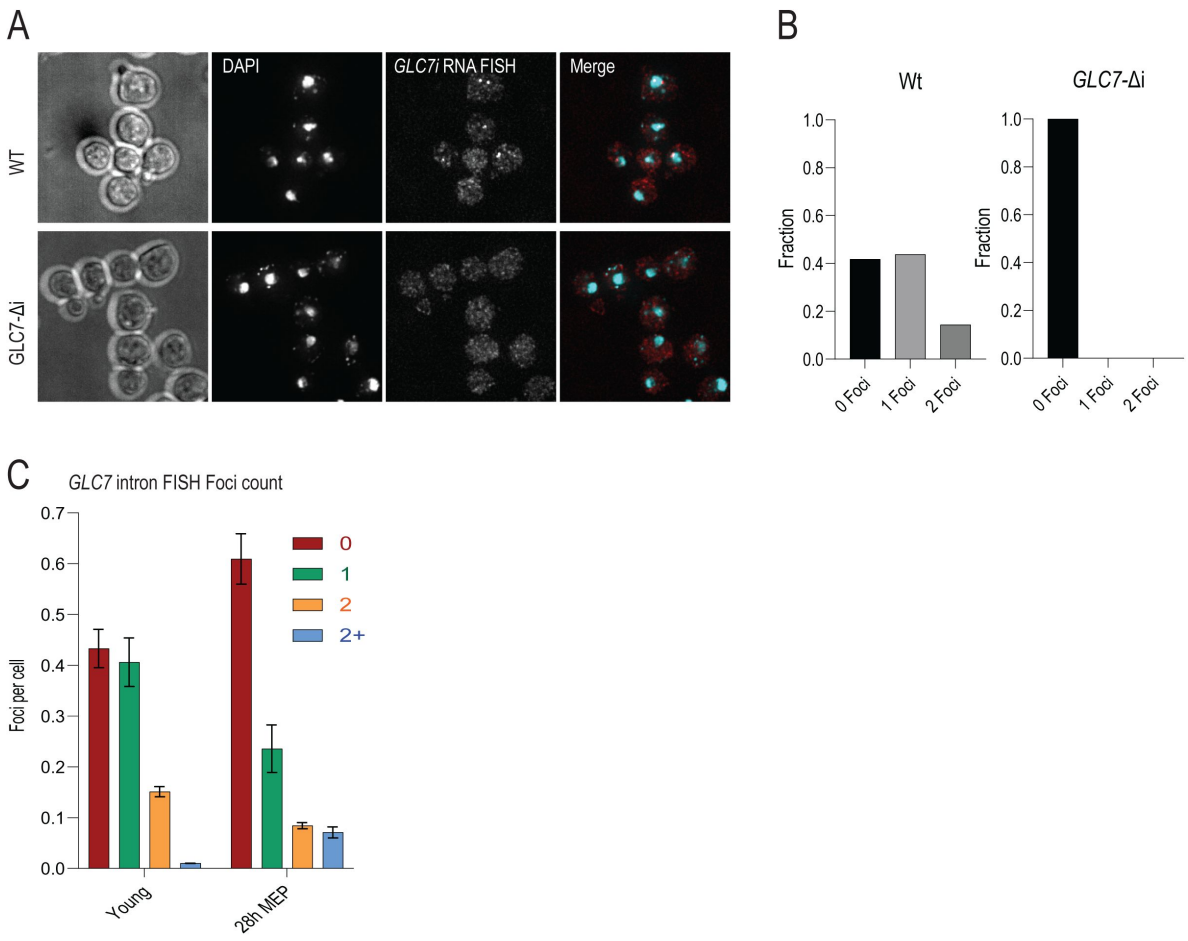

Figure S4

smRNA FISH probes are intron specific

A) Images of smRNA FISH experiments in wt and *GLC7*-Δ*i* cells. **B)** Quantification of smRNA FISH foci per cell in wt and *GLC7*-Δ*i* cells. **C)** Quantification of smRNA FISH foci per cell in wt cells at 0 and 28h of aging (n>300, cohorts of 100+ cells).

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Additional information

Data and materials availability

All the raw data generated in the manuscript will be deposited in the Mendelay online repository.

Author Contributions

Conceptualization: MM, ACM, YB Investigation: MM, JM, ACM Formal Analysis: MM,JM, ACM,CD Writing-original draft: YB,MM Writing – review & editing: MM,JM,JP,SAE,YB Resources: JP,SSL Supervision: YB Funding acquisition: YB

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Reviewer #1 (Public review):

Summary:

In this study, the authors explore a novel mechanism linking aging to chromosome mis-segregation and aneuploidy in yeast cells. They reveal that, in old yeast mother cells, chromosome loss occurs through asymmetric partitioning of chromosomes to daughter cells, a process coupled with the inheritance of an old Spindle Pole Body. Remarkably, the authors identify that remodeling of the nuclear pore complex (NPC), specifically the displacement of its nuclear basket, triggers these asymmetric segregation events. This disruption also leads to the leakage of unspliced pre-mRNAs into the cytoplasm, highlighting a breakdown in RNA quality control. Through genetic manipulation, the study demonstrates that removing introns from key chromosome segregation genes is sufficient to prevent chromosome loss in aged cells. Moreover, promoting pre-mRNA leakage in young cells mimics the chromosome mis-segregation observed in old cells, providing further evidence for the critical role of nuclear envelope integrity and RNA processing in aging-related genome instability.

Strengths:

The findings presented are not only intriguing but also well-supported by robust experimental data, highlighting a previously unrecognized connection between nuclear envelope integrity, RNA processing, and genome stability in aging cells, deepening our understanding of the molecular basis of chromosome loss in aging.

Weaknesses:

The authors have satisfactorily addressed my concerns.

https://doi.org/10.7554/eLife.104530.2.sa3

Reviewer #2 (Public review):

Summary:

The authors make the interesting discovery of increased chromosome non-dysjunction in aging yeast mother cells. The phenotype is quite striking and well supported with solid experimental evidence. This is quite significant to a haploid cell (as used here) - loss of an essential chromosome leads to death soon thereafter. The authors then work to tie this phenotype to other age-associated phenotypes that have been previously characterized: accumulation of extrachromosomal rDNA circles that then correlate with compromised nuclear pore export functions, which correlates with "leaky" pores that permit unspliced mRNA messages to be inappropriately exported to the cytoplasm. They then infer that three intron containing mRNAs that encode portions in resolving sister chromatid separation during mitosis, are unspliced in this age-associated defect and thus lead to the non-dysjunction problem.

Strengths:

The discovery of age-associated chromosome non-dysjunction is an interesting discovery, and it is demonstrated in a convincing fashion with "classic" microscopy-based single cell fluorescent chromosome assays that are appropriate and seem robust. The correlation of this phenotype with other age-associated phenotypes - specifically extrachromosomal rDNA circles and nuclear pore dysfunction - is supported by in vivo genetic manipulations that have been well-characterized in the past.

In addition, the application of the single cell mRNA splicing defect reporter showed very convincingly that general mRNA splicing is compromised in aged cells. Such a pleiotropic event certainly has big implications.

Weaknesses:

The authors have addressed my major concerns with experimentation or clarification.

https://doi.org/10.7554/eLife.104530.2.sa2

Reviewer #3 (Public review):

Summary:

Mirkovic et al explore the cause underlying development of aneuploidy during aging. This paper provides a compelling insight into the basis of chromosome missegregation in aged cells, tying this phenomenon to the established Nuclear Pore Complex architecture remodeling that occurs with aging across a large span of diverse organisms. The authors first establish that aged mother cells exhibit aberrant error correction during mitosis. As

extrachromosomal rDNA circles (ERCs) are known to increase with age and lead to NPC dysfunction that can result in leakage of unspliced pre-mRNAs, Mirkovic et al search for intron-containing genes in yeast that may be underlying chromosome missegregation, identifying three genes in the aurora B-dependent error correction pathway: MCM21, NBL1, and GLC7. Interestingly, intron-less mutants in these genes suppress chromosome loss in aged cells, with a significant impact observed when all three introns were deleted (3xΔi). The 3xΔi mutant also suppresses the increased chromosome loss resulting from nuclear basket destabilization in a mlp1Δ mutant. The authors then directly test if aged cells do exhibit aberrant mRNA export, using RNA FISH to identify that old cells indeed leak intron-containing pre-mRNA into the cytoplasm, as well as a reporter assay to demonstrate translation of leaked pre-mRNA, and that this is suppressed in cells producing less ERCs. Mutants causing increased pre-mRNA leakage are sufficient to induce chromosome missegregation, which is suppressed by the 3xΔi.

Strengths:

The finding that deleting the introns of 3 genes in the Aurora B pathway can suppress age-related chromosome missegregation is highly compelling. Additionally, the rationale behind the various experiments in this paper is well-reasoned and clearly explained.

Weaknesses:

My main concerns have been thoroughly addressed by the authors.

https://doi.org/10.7554/eLife.104530.2.sa1

Author response:

The following is the authors' response to the original reviews.

> ***Reviewer #1 (Public review):***
>
> *Summary:*
>
> *In this study, the authors explore a novel mechanism linking aging to chromosome mis-segregation and aneuploidy in yeast cells. They reveal that, in old yeast mother cells, chromosome loss occurs through asymmetric partitioning of chromosomes to daughter cells, a process coupled with the inheritance of an old Spindle Pole Body. Remarkably, the authors identify that remodelling of the nuclear pore complex (NPC), specifically the displacement of its nuclear basket, triggers these asymmetric segregation events. This disruption also leads to the leakage of unspliced pre-mRNAs into the cytoplasm, highlighting a breakdown in RNA quality control. Through genetic manipulation, the study demonstrates that removing introns from key chromosome segregation genes is sufficient to prevent chromosome loss in aged cells. Moreover, promoting pre-mRNA leakage in young cells mimics the chromosome mis-segregation observed in old cells, providing further evidence for the critical role of nuclear envelope integrity and RNA processing in aging-related genome instability.*
>
> *Strengths:*
>
> *The findings presented are not only intriguing but also well-supported by robust experimental data, highlighting a previously unrecognized connection between nuclear envelope integrity, RNA processing, and genome stability in aging cells, deepening our understanding of the molecular basis of chromosome loss in aging.*

We thank the reviewer for this very positive assessment of our work

> *Weaknesses:*
>
> *Further analysis of yeast aging data from microfluidic experiments will provide important information about the dynamic features and prevalence of the key aging phenotypes, e.g. pre-mRNA leakage and chromosome loss, reported in this work.*

We thank the reviewer for bringing this point, which we have addressed in the revised version of the manuscript. In short, chromosome loss is an abrupt, late event in the lifespan of the cells. To examine its prevalence, we have quantified the combined loss frequency of two chromosomes when both are labelled in the same cell. Whereas single chromosomes are lost at a frequency of 10-15% per cell, less than 5% of the cells lose both at the same time. Thus, the different chromosomes are lost largely but not fully independently from each other. Based on these data, and on the fact that yeast cells have 16 chromosomes, we evaluate that about half of the cells lose at least one chromosome in their final cell cycle.

We also tried to estimate the prevalence of the pre-mRNA leakage phenotype, based on the increased mCherry to GFP ratio observed between 0h and 24 hours of aging for 146 individual cells. For this analysis, we compared the mCherry/GFP ratio at 0 and 24h for the same individual cell. This analysis indicates that 81% of the cells show a fold change strictly above 1 as they age. Furthermore, the data appears to be unimodal. Thus, we can conservatively conclude that a majority of the cells show premRNA leakage at 24 hours. Since not all cells are at the end of their life at that time, this is possibly an underestimate.

> *In addition, a discussion would be needed to clarify the relationship between "chromosome loss" in this study and "genomic missegregation" reported previously in yeast aging.*

Genomic mis-segregation is characterized by the entry of both SPBs and all the chromosomes into the daughter cell compartment (PMID: 31714209). We have observed these events in our movies as well. However, the chromosome loss phenotype that we are focusing on affects only some chromosomes (as discussed above) and takes place under proper elongation of the spindle, with one SPB remaining in the mother cell whereas the other one goes to the bud, as shown in the manuscript's Figure 2. In our movies, chromosome loss is at least three-fold more frequent (for a single chromosome) than full genome mis-segregation (Sup Fig 1A-B). Furthermore, whereas chromosome loss is alleviated by the removal of the introns of MCM21, NBL1 and GLC7, genomic mis-segregation is not (Sup Fig 1B). Thus, genomic mis-segregation mentioned by the reviewer is a process distinct from the chromosome loss that we report. This discussion and the relevant data have been added to the manuscript.

We thank the reviewer for bringing up the possible confusion between these two phenotypes, allowing us to clarify this point.

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *The authors make the interesting discovery of increased chromosome non-dysjunction in aging yeast mother cells. The phenotype is quite striking and well supported with solid experimental evidence. This is quite significant to a haploid cell (as used here) - loss of an essential chromosome leads to death soon thereafter. The authors then work to tie this phenotype to other age-associated phenotypes that have been previously characterized: accumulation of extrachromosomal rDNA circles that then correlate with compromised nuclear pore export functions, which correlates with "leaky" pores that permit unspliced mRNA messages to be inappropriately exported to the cytoplasm. They then infer that three intron containing mRNAs that encode portions in resolving sister chromatid*

> *separation during mitosis, are unspliced in this age-associated defect and thus lead to the non-dysjunction problem.*
>
> *Strengths: The discovery of age-associated chromosome non-dysjunction is an interesting discovery, and it is demonstrated in a convincing fashion with "classic" microscopy-based single cell fluorescent chromosome assays that are appropriate and seem robust. The correlation of this phenotype with other age-associated phenotypes - specifically extrachromosomal rDNA circles and nuclear pore dysfunction - is supported by in vivo genetic manipulations that have been well-characterized in the past.*
>
> *In addition, the application of the single cell mRNA splicing defect reporter showed very convincingly that general mRNA splicing is compromised in aged cells. Such a pleiotropic event certainly has big implications.*

We thank the reviewer for this assessment of our work. To avoid confusion, we would like to stress out, however, that our data do not show that splicing per se is defective in old cells. Actually, we specifically show that the cells are unlikely to show splicing defect (last figure of the original and the revised version of the manuscript). Our data specifically show that unspliced mRNAs tend to leak out of the nucleus of old cells.

> *Weaknesses:*
>
> *The biggest weakness is "connecting all the dots" of causality and linking the splicing defect to chromosome disjunction. I commend the authors for making a valiant effort in this regard, but there are many caveats to this interpretation. While the "triple intron" removal suppressed the non-dysjunction defect in aged cells, this could simply be a kinetic fix, where a slowdown in the relevant aspects of mitosis, could give the cell time to resolve the syntelic attachment of the chromatids.*

The possibility that intron-removal leads to a kinetic fix is an interesting idea that we have now considered. In the revised manuscript, we now provide measurements of mitotic duration in the “triple intron” mutant compared to wild type cells and the duration of their last cell cycle (See supplementary figure 3A-D). There is no evidence that removing these introns slows down mitosis. Thus, the kinetic fix hypothesis is unlikely to explain our observation about the effect of intron removal.

> *To this point, I note that the intron-less version of GLC7, which affects the most dramatic suppression of the three genes, is reported by one of the authors to have a slow growth rate (Parenteau et al, 2008 - https://doi.org/10.1091/mbc.e07-12-1254)*

The reviewer is right, removing the intron of GLC7 reduces the expression levels of the gene product (PMID: 16816425) to about 50% of the original value and causes a slow growth phenotype. However, the cells revert fairly rapidly through duplication of the GLC7-Δi gene (see supplementary Figure 3EF). As a consequence, neither the GLC7-Δi nor the 3xΔi mutant strains show noticeable growth phenotypes by spot assays. We now document these findings in supplementary figure 3.

> *Lastly, the Herculean effort to perform FISH of the introns in the cytoplasm is quite literally at the statistical limit of this assay. The data were not as robust as the other assays employed through this study. The data show either "no" signal for the young cells or a signal of 0, 1, or 2 FISH foci in the aged cells. In a Poisson distribution, which this follows, it is improbable to distinguish between these differences.*

This is correct, this experiment was not the easiest of the manuscript... However, despite the limitations of the assay, the data presented in figure 7B are very clear. 300 cells aged by MEP

were analysed, divided in the cohorts of 100 each, and the distribution of foci (nuclear vs cytoplasmic) in these aged cells were compared to the distribution in three cohorts of young cells. For all 3 aged cohorts, over 70% of the visible foci were cytoplasmic, while in the young cells, this figure was around 3%. A t-test was conducted to compare these frequencies between young and old cells (Figure 7B). The difference is highly significant. Therefore, we are clearly not at the statistical limit.

What the reviewer refers to is the supplementary Figure 4, where we were simply asking i) is the signal lost in cells lacking the intron of GLC7 (the response is unambiguously yes) and ii) what is the general number of dots per cell between young and old wild type cells (without distinguishing between nuclear and cytoplasmic) and the information to be taken from this last quantification is indeed that there is no clearly distinguishable difference between these two population of cells, as the reviewer rightly concludes. In other word, the reason why there are more dots in the cytoplasm of the old cells in the Figure 7B is not because the old cells have much more dots in general (see supplementary Figure 4C). We hope that these clarifications help understand the data better. We have edited the manuscript to avoid confusion.

> ***Reviewer #3 (Public review):***
>
> *Summary:*
>
> *Mirkovic et al explore the cause underlying development of aneuploidy during aging. This paper provides a compelling insight into the basis of chromosome missegregation in aged cells, tying this phenomenon to the established Nuclear Pore Complex architecture remodelling that occurs with aging across a large span of diverse organisms. The authors first establish that aged mother cells exhibit aberrant error correction during mitosis. As extrachromosomal rDNA circles (ERCs) are known to increase with age and lead to NPC dysfunction that can result in leakage of unspliced pre-mRNAs, Mirkovic et al search for intron-containing genes in yeast that may be underlying chromosome missegregation, identifying three genes in the aurora B-dependent error correction pathway: MCM21, NBL1, and GLC7. Interestingly, intron-less mutants in these genes suppress chromosome loss in aged cells, with a significant impact observed when all three introns were deleted (3xΔi). The 3xΔi mutant also suppresses the increased chromosome loss resulting from nuclear basket destabilization in a mlp1Δ mutant. The authors then directly test if aged cells do exhibit aberrant mRNA export, using RNA FISH to identify that old cells indeed leak intron-containing pre-mRNA into the cytoplasm, as well as a reporter assay to demonstrate translation of leaked pre-mRNA, and that this is suppressed in cells producing less ERCs. Mutants causing increased pre-mRNA leakage are sufficient to induce chromosome missegregation, which is suppressed by the 3xΔi.*
>
> *Strengths:*
>
> *The finding that deleting the introns of 3 genes in the Aurora B pathway can suppress age-related chromosome missegregation is highly compelling. Additionally, the rationale behind the various experiments in this paper is well-reasoned and clearly explained.*

We thank the reviewer for their very positive assessment of our work

> *Weaknesses:*
>
> *In some cases, controls for experiments were not presented or were depicted in other figures.*

We are sorry about this confusion. We have improved our presentation of the controls, bringing them back each time they are relevant. We have also added those that were missing (such as those mentioned by reviewer 2, see above). Note that the frequencies of centromeric plasmid loss at 0h in Figure 1C is not meaningful and therefore not presented. Since the cells were grown on selective medium before loading on to the ageing chip, we cannot report a plasmid loss frequency here. The ageing experiments themselves were subsequently conducted in full medium, to allow for centromeric plasmid loss without killing the cell. We explain this in the materials and methods section.

> *High variability was seen in chromosome loss data, leading to large error bars.*

We thank the reviewer for this comment. The variance in those two figures (3A and 5D) comes from the suboptimal plotting of this data. This is now corrected as follows. We divided the available data into 4 cohorts and then plotted the average loss frequency across these cohorts for the indicated age groups. This filters out much of the noise and improves the statistical resolution.

> *The text could have been more polished.*

Thank you for this comment. We have gone through the manuscript again in detail.

> ***Reviewer #1 (Recommendations for the authors)**:*
>
> *(1) A previous study (PMID: 31714209). showed that aging yeast cells undergo genomic missegregation in which material was abnormally segregated to the daughter cells, leading to cell cycle arrest. After that, the missegregation is either corrected by returning aberrantly segregated genetic material to the mother cells so that they can resume cell cycles, or if not corrected, the mother cells will terminally exist the cell cycle and eventually die. That paper also showed that this agedependent genomic missegregation is related to rDNA instability. Is the chromosome loss in this work related to the genomic missegregation reported before? Is it partially reversible like genomic missegregation? Are all the chromosomes lost in one cell division, like in the case of genomic missegregation? Some additional characterization and a discussion would be helpful.*

As mentioned above, indeed the phenotype of full genome mis-segregation described by Crane et al. (2019) is observable in our data as well. At 24h ~3% of the cells segregate both SPBs to the bud, as they previously described (Supp Figure 1A and B). This phenomenon is clearly distinct from asymmetric chromosome partition, where cells undergo anaphase, separate the SPBs and segregate one to the mother cell and one to the bud (Figure 2A). Also, asymmetric chromosome partitioning affects only a subset of the chromosomes (see below), not the entire genome. Finally, unlike asymmetric chromosome partitioning, the frequency of genome mis-segregation in ageing was not alleviated by intron removal (Supp Figure 1B). Thus, these two processes are clearly distinct and driven by different mechanisms. Note that asymmetric chromosome partitioning appears 3 to 5 times more frequently than genomic mis-segregation.

Supporting further the notion that these two processes are distinct, chromosome loss seals the end of the life of the cell, as we reported, indicating that this is not a reversible event. Also, it does not involve all chromosomes at once. Cells that contain the labelled versions of both chromosome II and IV at the same time, the loss frequency of both chromosomes is less than 5%, whereas each chromosome is lost in 10-15% of the cells (Figure 1C). Thus, most cells lose one and keep the other. Furthermore, this indicates that there are many more cells losing at least one chromosome than the 15% that lose chromosome IV for example, probably 50%

or more. Thus, chromosome loss by asymmetric segregation is much more frequent than the partly transient transfer of the entire nucleus to the bud.

> *(2) What percentage of aging WT cells undergo pre-mRNA leakage (using the GFP/mCherry reporter) during their entire lifespan? Is it a sporadic, reversible process or an accumulative, one-way deterioration? Previous studies (PMID: 32675375; PMID: 24332850; PMID: 36194205; PMID: 31291577) showed that only a fraction of yeast cells age with rDNA instability and ERC accumulation, as indicated by excessive rRNA transcription and nucleolar enlargement. Are they the same fraction of aging cells that undergo pre-mRNA leakage and chromosome loss? This information will indicate the prevalence of the key aging phenotypes reported in this work and should be readily obtainable from microfluidic experiments. In addition, a careful discussion would be helpful.*

Pre-mRNA leakage is relatively widespread in the population, but it is difficult to put a precise number on it. Analysis of how the mCherry/GFP ratio changes in 146 individual cells between 0 and 24 hours and imaging in our microfluidics platform indicates that ~80% show an increase and 50% of the cells show an increase above 1.5-fold. Therefore, the frequencies of pre-mRNA leakage and chromosome loss are probably similar. We have modified the discussion to account for these considerations. This would be in the same range as the frequency of aging by ERC accumulation (mode 1) estimated by PMID: 32675375.

> ***Reviewer #2 (Recommendations for the authors)***
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> *The manuscript could use a bit of editing in places - please go through it once more.*
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> *Editing suggestions:*
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> *Line 80 – irrespective*

Corrected.

> *Line 97 - these are not "rates" but frequencies. Please correct this error throughout.*
>
> *Replaced “rate” with “frequency throughout the manuscript and the figures, when pertaining to chromosome loss*
>
> *Line 328 - increase in chromosome…*

Corrected.

> *Line 379 - tampering*
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> ***Reviewer #3 (Recommendations for the authors)**:*
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> *Specific Feedback to Authors*
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> *(a) Major Points*
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> *(i) While the proposed connection between ERC-mediated nuclear basket removal and erroneous error correction was clearly stated, this connection is correlative and was not directly tested. Specifically, although mutants impacting ERC levels were tested for missegregation, it was not directly tested if increased missegregation levels occurred due to ERC tethering to the NPC and subsequent nuclear basket removal. It is possible that the increased ERCs may be driving missegregation via a different pathway. Authors should consider experiments to strengthen this idea, such as looking at chromosome loss frequency in a sir2Δ 3xΔi double mutant, or a sir2Δ sgf73Δ double mutant.*

This connection is addressed in the original version of the manuscript, where we show that preventing attachment of ERCs to the NPC, by removing the linker protein Sgf73, alleviates chromosome loss. The link is further substantiated by the fact that removing the basket on its own promote chromosome loss and that in both cases, namely during normal aging, i.e., upon ERC accumulation, and upon basket removal the mechanism of chromosome loss is the same. In both cases, it depends on the introns of the GLC7, MCM21 and NBL1 genes.

However, we acknowledge that the mutants tested have pleiotropic effects, making interpretation somewhat difficult, even when examining chromosome loss in multiple mutants that affect ERC formation and NPC remodelling, as we have done. As recommended by the reviewer, we have characterized the phenotype of the sir2Δ 3xΔi mutant strain. Intron removal in the sir2Δ mutant cells largely rescued the elevated chromosome loss frequency of these cells and slightly extended their replicative lifespan (Figure 6D-E). We conclude that intron removal can remedy the chromosome loss phenotype of the sir2Δ. Although clearly significant, the effect on the replicative lifespan was not very strong, likely due to the sir2Δ affecting other ageing processes.

Touching on this question, we added a new set of experiments asking whether any accumulating DNA circle causes chromosome loss in an intron-dependent manner. Thus, we have introduced a noncentromeric replicative plasmid in wild type and 3xΔi mutant strains carrying the labelled version of chromosome II (Figure 6A-C). These studies show that these cells age much faster than wild type cells, as expected, and lose chromosomes at a higher frequency than non-transformed cells. Finally, the effect is at least in part alleviated by removing the introns of NBL1, MCM21 and GLC7.

Therefore, after adding this new and more direct test of the role of DNA circles in chromosome loss, we are confidently concluding that ERC-mediated basket removal is the trigger of chromosome loss in old cells.

> *(b) Minor Points*
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> *(i) In Figure 1C, the text (lines 91-92) argues that chromosome loss happens abruptly as cells age; however the data only show loss at young and old time points, not an intermediate, which leaves open the possibility that chromosome loss is occurring gradually. While cells that lost chromosomes should fail to divide further, we don't know if these events happened and were simply excluded.*

We agree with the reviewer that formally the conclusion drawn in the lines 91-92 (of the original manuscript), namely that chromosome loss takes place abruptly as cells age, cannot be drawn from the Figure 1C alone but only from subsequent observations. However, since chromosome loss is lethal in haploid, as we mention in the text and the reviewer notes as well, it is difficult to envision how cells could lose chromosomes before the end of their lifespan and must therefore increase abruptly as the cells reach that point. This is now underlined in the revised version of the manuscript. Accordingly, the frequency of chromosome loss per age group, which is depicted in Figure 3A, shows that the wild type cells that have budded less than 10 times show no chromosome loss. The chromosome loss frequency starts to ramp up only pass that point. Therefore, chromosome loss does not increase linearly with age.

> *Additionally, cells that lost minichromosome should not arrest. We suggest that the interpretation of these data should be softened in the text, or that chromosome loss fraction could be more effectively portrayed as a Kaplan-Meier survival curve depicting cells that have not lost chromosomes, if these data are easily available. Or, chromosome loss at an intermediate time point could be depicted.*

Since we cannot visualize more than 2 chromosomes at a time, it is not possible to plot the KaplanMeier curve of cells that have not lost chromosomes. However, as mentioned above, the chromosome loss frequencies at intermediate time points are depicted in Figure 3A and Figure 4B and shows that it increases with age.

> *(ii) Also regarding Figure 1, it would be helpful to expound on the purpose of the minichromosomes, as well as how the Ubi-GFP minichromosome is constructed.*

We now explained why we tested the loss of minichromosome, namely, as a mean to test whether the centromere is necessary and sufficient to drive the loss of the genetic material linked to it, i.e., chromosomes, in old cells. Concerning the Ubi-GFP minichromosome, the Materials and methods section is now updated and reports plasmid construction, backbone used, primers as well as the plasmid sequence being available in the supplementary data.

> *The purpose of the minichromosome initially appears to be the engineering of an eccDNA (ERC) with a CEN to demonstrate distinct behaviour, but it is unclear whether this was actually conducted or if the minichromosome are simply CEN plasmids and/or if this was the intended goal. Furthermore, lines 102-103 state that the presence of a centromere was necessary and sufficient for minichromosome loss. However, since no constructs lacking a centromere were tested, necessity cannot be concluded. Please clarify this in the text and include experimental details to help readers understand what was tested.*

We apologize for having been too short here. The behaviour of the CEN-less version of this plasmid has been characterized in detail in previous studies (Shcheprova et al., 2008; Denoth-Lippuner 2014, Meinema et al 2022). Here we focused on the behaviour of the CEN+ version of an otherwise Identical plasmid. We now clarify in the text that this plasmid is retained in the mother cell when CEN-less and cite the relevant literature.

> *(iii) It is unclear how cells at 0-3 budding events were identified in assays using the microfluidics platform. Can the authors clarify the known "age" of the cells once captured, i.e. how do the authors know how many divisions a cell has undergone prior to capture?*

The reviewer is right; we do not know the exact age of these cells. However, in any asynchronous population of yeast cells, which is what we start from, 50% of the cells are newborn daughters, 25% have budded once, 12.5 have budded twice, 6.25 % have budded three times… Therefore, at the time of loading, 93% of the cells have budded between 0 and 3 times. For this reason, we report to this population as cells age 0-3 CBE. We acknowledge that this is an approximation, but it remains a relatively safe one.

> *(iv) While the schematic in Figure 2D is generally helpful, a different depiction of the old and new SPBs would be beneficial in cases where the new SPB and TetR-GFP are depicted as colocalized, it is difficult to see that the red is fainter for the new SPB.*

We have corrected this issue by completely separating the SPB and the Chromosome signals in the Figure 2D.

> *(v) In Figure 2F, the grey colour of the 12h Ipl1-321 data bar did not have high enough contrast when the manuscript was printed-would recommend changing this to a darker shade.*

We have corrected this issue by using a darker shade of grey.

> *(vi) In Figure 3A, 'Budding' is misspelled on X-axis label*

We have corrected this error.

> *(vii) In Figure 4, the authors should clarify the differences between the analyses in panels B and C. The distinction is not immediately clear and may be difficult to grasp upon initial reading.*

We have corrected this issue in the main text as well as figure legend.

> *(viii) In Figure 5, It would aid comparisons to depict the 3xΔi only as well on panels B, D, and E.*

We have added 3xΔi data to Figure 5,6 and 8.

> *(ix) In Figure 6D, it is unclear why there was an appreciable level of unspliced RNA in the wild-type and sir2Δ young cells. Additionally, it is unclear why there is so much signal observed in the Merge image for the old wild-type cell, especially regarding the apparent bright spot. Is that nuclear signal? Please clarify.*

The pre-mRNA processing reporter is not very efficiently spliced. It was selected as such during design (Sorenson et al 2014; DOI: 10.1261/rna.042663.113) to provide sensitivity. As for the bright spot occurring, translation of the unspliced reporter produces the N-terminal part of a ribosomal protein, a fraction of which forms some sort of nuclear aggregate in a fraction of the population.

> *(x) In Figure 6E, why does the sir2Δ exhibit higher mCherry/GFP than the wild-type and fob1Δ at "young age"? Is this due to disrupted proteostasis in the sir2Δ, or a different pleiotropic effect of sir2Δ? Please comment on this observation in the text.*

Indeed, as we have stated in the text the sir2Δ mutation already perturbs pre-mRNA processing in young cells. We do not know the reason of this but indeed it is most probably reflective of its pleiotropic function. Following the reviewer's request, we now state this in the text. For example, Sir2 may regulate the acetylation state of the basket itself. The genetic interactions observed between sir2Δ and quite a few nucleoporin mutations seem to support this possibility.

> *(xi) Throughout, the authors switch between depicting aging in Completed Budding Events versus hours, which made it difficult to compare data across figures*

Ideally, all the data in this manuscript should be plotted according to the CBE age of the cell. To ensure that the major findings are plotted in such a way, we have done so for over ~3000 combined cells and thousands of replicative divisions in Figures 3,5-7. All the measurements of chromosome loss at a specific CBE had to be done manually, due to the absence of algorithms that would be able to accurately detect chromosome loss and replicative age. Therefore, doing this for the entirety of our dataset, encompassing well over 50 ageing chips and tens of thousands of cells is not easily doable at this stage.

> *(xii) Typo on line 12 (Sindle Pole Body)*

We have corrected this error.

> *(xiii) The phrase should be 'chromosome partitioning' rather than 'chromosome partition', throughoutfor example, line 17*

Replaced “chromosome partition” with “chromosome partitioning” throughout the text.

> *(xiv) There are inconsistencies between plural and singular references throughout sentences-example, lines 35-37, and lines 44-45.*

We carefully combed through the manuscript again and hope that we caught all inconsistencies.

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