Supplementary Material

The fission yeast MRN complex tethers dysfunctional telomeres for NHEJ repair

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Supplementary figure 1. The reduced amount of telomere fusions detected in nbs1Δ rap1Δ cells is not due to shorter telomeres.
NotI-digested genomic DNA was analyzed by pulsed-field gel electrophoresis followed by Southern blot analysis using a non-telomere probe for the terminal fragments of chromosomes I and II (LMIC probe). End chromosomal fusions are also detected using this probe in ctp1Δ rap1Δ, but not nbs1Δ rap1Δ, G1-arrested cells. Non-specific, non-telomere bands are indicated by an asterisk. Please note that lanes come from the same Southern blot and that only irrelevant lanes have been removed in the interest of clarity. Full-sized source images of the original scans can be found as an online supplement to this paper.

Supplementary figure 2. Rad3 and Tel1 PI3-kinases are dispensable for unprotected taz1Δ and rap1Δ NHEJ telomere fusions.
Southern blot analysis using a non-telomere probe for the terminal fragments of chromosomes I and II (LMIC bands) shows that the reduced detection of telomere fusions of rad3Δ tel1Δ taz1Δ or rad3Δ tel1Δ rap1Δ triple mutants in PFGE (shown in fig. 3c) is due to the loss of telomere sequences in these strains. Asterisks depict non-specific, non-telomere bands. Please note that lanes come from the same Southern blot and that only irrelevant lanes have been removed in the interest of clarity. Full-sized source images of the original scans can be found as an online supplement to this paper.
Supplementary figure 3. PCR confirmation of colonies transformed with of two unlinked DNA fragments.

A- PCR reactions performed with Leu2F and KanF. B- PCR reactions performed with Leu2R and KanF. Lanes: 1- HyperLadder I; 2 to 6- cells resulting form co-transformation of digested pKan1 plasmid and LEU2 fragment; 7- untransformed cells; 8- cells transformed with digested pKan1 plasmid alone. Please note that lanes come from the same Southern blot and that only irrelevant lanes have been removed in the interest of clarity. Full-sized source images of the original scans can be found as an online supplement to this paper.

Supplementary figure 4. The ability of rad32 mutants to join two independent DNA fragments in vivo is not correlated with their DNA damage sensitivity.

The nuclease-dead rad32-D65N and the dimerization-impaired rad32-L77K and rad32-L154D alleles are mildly sensitive to DNA damage and partially retain the DNA repair ability of the MRN complex. Ten-fold serial spots were made, and cells were subjected to UV irradiation, hydroxyurea (HU), and camptothecin (CPT) at the indicated doses. Plates were photographed after a 4-day incubation at 32°C.

Supplementary figure 5. MRN is dispensable to process incompatible plasmid DNA ends.

NHEJ plasmid repair assays were performed in strains from the indicated genotypes as described in Figure 5 except that the cut vector was simultaneously digested with Kpnl and BglII thus generating non-cohesive ends. The results are plotted as the ratio of the number of transformants obtained with linearized plasmid over those transformed with uncut plasmid. A minimum of 3 independent experiments was perfomed. Error bars represent 2xSEM.
Supplementary Figure 1

(LMIC probe)
Supplementary Figure 2

A

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(LMIC probe)

B

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(Telomere probe)
Supplementary Figure 3
Supplementary Figure 4

- wt
- rad32Δ
- rad50Δ
- rad32-L77K
- rad32-L154D
- rad32-H68S
- rad32-D65N
- rad32-H134S

Untreated, 2mM HU, 4mM HU

Dimerization domain
Nuclease domain

- wt
- rad32Δ
- rad50Δ
- rad32-L77K
- rad32-L154D
- rad32-H68S
- rad32-D65N
- rad32-H134S

200J/m² UV, 400J/m² UV, 0.2μM CPT, 1.0μM CPT
Supplementary Figure 5

![Graph showing the cut/uncut ratio for different genotypes and domains.](image-url)