File S1

SUPPORTING MATERIAL AND METHODS

DNA isolation and denaturing in-gel hybridization: Yeast genomic DNA was isolated using a Yeast DNA Extraction Kit (Thermo Scientific). The DNA was digested with XhoI restriction endonuclease before running on a 0.8% agarose gel. Denaturing in-gel hybridization using a telomeric CA oligonucleotide radio- labeled probe was performed as described (DIONNE and WELLINGER 1996)

Telospot: Telospot assays were performed as previously described (CRISTOFARI *et al.* 2007), except that the membrane was not denatured with NaOH. For Figure 6C, Telospot reactions were performed with 35 nM 5′-biotinylated $(TTAGGG)_3$ primer, which were then purified using 10 µl of streptavidin-coated M-280 Dynabeads (Invitrogen) according to the manufacturer's protocol. Samples were heated to 98°C for 5 min in 98% formamide-10 mM EDTA, resolved on a 12% polyacrylamide-urea gel, and transferred onto a positively charged Nylon membrane (GE Healthcare) using a semi-dry electrophoretic transfer cell (Transblot SD, BIO-RAD). After UV-crosslinking, the membrane was probed as in a standard Telospot assay.

SUPPORTING INFORMATION REGARDING THE VIABILITY OF *rnr1Δ*

To further examine the viability of an *rnr1Δ* deletion mutation in the BY4741 background, we backcrossed MCY602 (*rnr1Δ::kanMX* in the BY4741 background) to W9100-12C (a wild type strain of the W303 background). Half of the *rnr1Δ* progeny were dead (22 of 42 possible spores from 21 tetrad dissections), suggesting that one genetic locus was responsible for the difference in viability in the two genetic backgrounds.

Since Rnr3 protein levels are increased in *rnr1Δ* mutants (Figure 5), we wanted to determine if this single genetic locus was linked to *RNR3*. To do so, we crossed MCY605 (*rnr3Δ::natMX*; BY4742 background) and MCY606 (*yil067cΔ::natMX*; BY4742 background) to MCY602. *YIL067C* is adjacent to *RNR3* on chromosome IX. We were able to obtain viable *rnr1Δ yil067cΔ* double mutants, but not *rnr1Δ rnr3Δ* double mutants, indicating that Rnr3 is required for viability in the absence of Rnr1. We then crossed MCY607 (*rnr1Δ::kanMX yil067cΔ::natMX*; BY4741 background) to W9100-12C. As expected, half of the *rnr1Δ* progeny were dead (13 of 24 possible spores from 12 tetrad dissections). If the locus in question is linked to *RNR3*, then all the viable *rnr1Δ* strains should also contain *yil067cΔ::natMX* (of the 11 viable *rnr1Δ* colonies, only 8 were big enough to be genotyped by replica plating). This was not the case: only half did (5 of the 8). Thus, while a single genetic locus is responsible for the viability of *rnr1Δ* in the BY4741 background, the locus is not linked to *RNR3*.