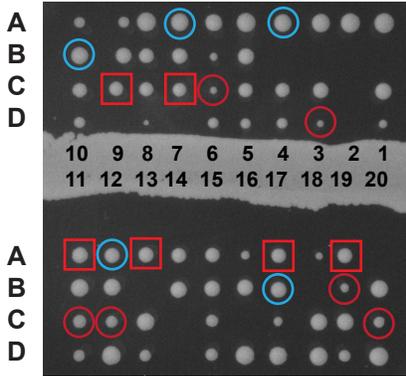


Figure S1 Quantitation of Figure 1 Southern

(A-C) The median length of the telomere restriction fragment (TRF) was quantified in ImageQuant TL. To compare samples run on different Southern blots, each sample is normalized to the first *WT* sample on that Southern (see Methods). The mean and standard error of the mean are indicated. The number of biological replicates used for the quantitative analysis is given in parentheses next to each genotype. The number of replicates quantitated is smaller than the total replicates for a given genotype because we only used samples that were passaged the same number of times before running the Southern blot. An unpaired two-tailed *t*-test was used to determine statistical significance. n.s. = not significant. * *p*-value < 0.05 *** *p*-value < 0.0001 (A) Samples were passaged for approximately 120 population doublings. (B) Samples were passaged minimally. (C) Samples were passaged for approximately 100 population doublings. Statistical analysis could not be applied because several data points had exactly the same values, making it impossible to estimate variance.

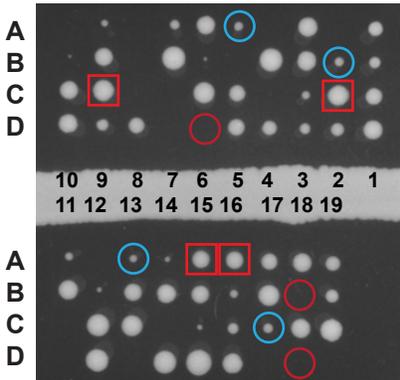
A



Diploid Genotype: *TEL1-hy909/tel1Δ MEC1/mec1Δ SML1/sml1Δ*

Haploid Genotype	Viable Segregants
□ <i>TEL1-hy909</i>	7C, 9C, 11A, 13C, 16D, 17A, 19A
○ <i>TEL1-hy909 mec1Δ</i>	3D, 6C, 10A, 11C, 12C, 15C, 15D, 18A, 19B, 20C
□ <i>TEL1-hy909 sml1Δ</i>	3A, 4D, 6D, 9B, 13A, 14A, 14B, 16B, 18D, 20D
○ <i>TEL1-hy909 mec1Δ sml1Δ</i>	4A, 7A, 10B, 12A, 17B
○ <i>telΔ</i>	4C, 6A, 10C, 12D, 17D
○ <i>tel1Δ mec1Δ</i>	none
○ <i>tel1Δ sml1Δ</i>	3C, 7B, 10D, 11B, 12B, 15A, 15B, 18C, 19C, 20B
○ <i>tel1Δ mec1Δ sml1Δ</i>	6B, 9A, 11D, 13D, 16A, 17C, 19D

B

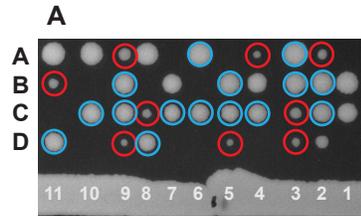


Diploid Genotype: *TEL1/TEL1-hy909 MEC1/mec1Δ RAD53/rad53Δ CRT1/crt1Δ XRS2/xrs2Δ*

Haploid Genotype	Viable Segregants
□ <i>TEL1-hy909</i>	1D, 2C, 9C, 12D, 15A, 16A
○ <i>TEL1-hy909 rad53Δ</i>	none
□ <i>TEL1-hy909 crt1Δ</i>	13C, 14D, 17B
○ <i>TEL1-hy909 rad53Δ crt1Δ</i>	2B, 5A, 13A, 17C
□ <i>TEL1-hy909 xrs2Δ</i>	1C, 3A, 5C, 6A, 9B
□ <i>TEL1-hy909 rad53Δ xrs2Δ</i>	3C
□ <i>TEL1-hy909 crt1Δ xrs2Δ</i>	10C, 19A
□ <i>TEL1-hy909 rad53Δ crt1Δ xrs2Δ</i>	10B, 15D, 16B
□ <i>WT</i>	10D, 14B, 19C
○ <i>rad53Δ</i>	none
○ <i>crt1Δ</i>	6C, 12C, 18A
○ <i>rad53Δ crt1Δ</i>	1A, 3D, 9D, 15C, 16C, 19B
○ <i>xrs2Δ</i>	3B, 13B, 15B, 16D, 17A, 18D
○ <i>rad53Δ xrs2Δ</i>	none
○ <i>crt1Δ xrs2Δ</i>	2D, 5D, 18C
○ <i>rad53Δ crt1Δ xrs2Δ</i>	1B, 6B, 9A, 12B, 14A

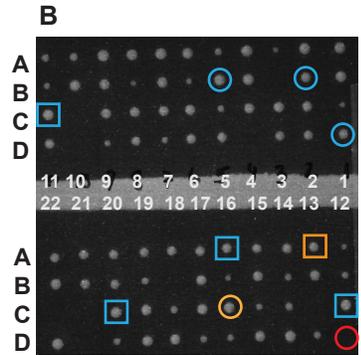
Figure S2 *TEL1-hy909* can rescue *mec1* Δ but not *rad53* Δ lethality

Pictures of tetrad dissection plates where each haploid from a tetrad was placed in a vertical column designated A, B, C, or D (indicated to the left of the picture). Tetrads are assigned numbers from 1-20, as indicated in the center of the plate. One diploid strain was dissected on each plate; the diploid genotype is written at the top of the table. Every possible haploid genotype is listed in the table under haploid genotype. Each haploid with that genotype is listed in the adjacent row under Viable Segregants. In cases where three out of four segregants are viable, it is possible to deduce the genotype of the inviable segregants. (A) The red square indicates *TEL1-hy909* segregants, the red circle indicates *TEL1-hy909 mec1* Δ segregants, and the blue circle indicates *TEL1-hy909 mec1* $\Delta sml1$ Δ segregants. The strain in this tetrad dissection is yRK5055. (B) The red square indicates *TEL1-hy909* segregants, the red circle indicates *TEL1-hy909 rad53* Δ segregants, and the blue circle indicates *TEL1-hy909 rad53* $\Delta crt1$ Δ segregants. The strain in this tetrad dissection is yRK5028.



Diploid Genotype: *rad53^{1-4/9-12AQ} RAD53/rad53 Δ*

Haploid Genotype	Viable Segregants
<i>rad53Δ</i>	none
○ <i>rad53Δ rad53^{1-4/9-12AQ}</i>	2A, 2D, 3C, 3D, 4A, 5D, 8C, 9A, 9D, 11B
<i>RAD53</i>	1B, 1C, 4B, 7A, 8A, 10A, 11A
○ <i>RAD53 rad53^{1-4/9-12AQ}</i>	2B, 2C, 3A, 3B, 4C, 5B, 5C, 6A, 6C, 7C, 8D, 9B, 9C, 10C, 1D



Diploid Genotype: *RAD53/rad53^{1-4/9-12AQ} MEC1/mec1 Δ TEL1/tel1 Δ SML1/sml1 Δ CRT1/crt1 Δ*

Haploid Genotype	Viable Segregants
○ <i>rad53^{1-4/9-12AQ}</i>	none
○ <i>rad53^{1-4/9-12AQ} crt1Δ</i>	1D, 2B, 5B
○ <i>rad53^{1-4/9-12AQ} sml1Δ</i>	16C
<i>rad53^{1-4/9-12AQ} sml1Δ crt1Δ</i>	10B
<i>rad53^{1-4/9-12AQ} tel1Δ</i>	none
<i>rad53^{1-4/9-12AQ} mec1Δ</i>	none
<i>rad53^{1-4/9-12AQ} mec1Δ sml1Δ</i>	20B
<i>rad53^{1-4/9-12AQ} mec1Δ sml1Δ crt1Δ</i>	4C, 22D
<i>rad53^{1-4/9-12AQ} tel1Δ crt1Δ</i>	13B
<i>rad53^{1-4/9-12AQ} tel1Δ sml1Δ crt1Δ</i>	18A
<i>rad53^{1-4/9-12AQ} tel1Δ mec1Δ sml1Δ</i>	1C, 11B, 12A, 16B
<i>rad53^{1-4/9-12AQ} tel1Δ mec1Δ sml1Δ crt1Δ</i>	5A, 11A, 12B, 13D, 20D
<i>tel1Δ</i>	20A
<i>tel1Δ mec1Δ</i>	none
<i>tel1Δ sml1Δ</i>	1A, 4A
<i>tel1Δ crt1Δ</i>	22B
<i>tel1Δ sml1Δ crt1Δ</i>	21A
<i>tel1Δ mec1Δ sml1Δ</i>	5C
<i>tel1Δ mec1Δ crt1Δ</i>	16D
<i>tel1Δ mec1Δ sml1Δ crt1Δ</i>	10A, 18C
<i>mec1Δ</i>	none
<i>mec1Δ sml1Δ</i>	22A
<i>mec1Δ crt1Δ</i>	1B
□ <i>sml1Δ</i>	13A
□ <i>crt1Δ</i>	11C, 12C, 16A, 20C
<i>sml1Δ crt1Δ</i>	21B
<i>wildtype</i>	5D, 11D, 18D

Figure S3 Integrated *rad53*^{1-4/9-12AQ} is lethal while plasmid-expressed *rad53*^{1-4/9-12AQ} is viable

Pictures of tetrad dissection plates where each haploid from a tetrad was placed in a vertical column designated A, B, C, or D (indicated to the left of the picture). Tetrads are assigned numbers from 1-22, as indicated in the center of the plate. One diploid strain was dissected on each plate; the diploid genotype is indicated at the top of the table. Each haploid with that genotype is listed in the adjacent row under Viable Segregants. In cases where three out of four segregants are viable, it is possible to deduce the genotype of the inviable segregant. (A) The listed haploid genotypes reflect those that had viable segregants. There are no inferred genotypes in this experiment because the plasmid does not segregate in a predictable pattern. The red circle identifies the inferred location of segregants that are *rad53* Δ and retained the *rad53*^{1-4/9-12AQ} plasmid. The blue circle identifies *RAD53* segregants that retained the *rad53*^{1-4/9-12AQ} plasmid. The strain in this tetrad dissection is yRK6017-2. (B) The listed haploid genotypes reflect those that had viable segregants or inferred inviable segregants. Additional possible genotypes are not listed because no segregants with that genotype were present in this tetrad dissection. The red circle identifies the inferred location of *rad53*^{1-4/9-12AQ} segregants. The blue circle identifies *rad53*^{1-4/9-12AQ} *crt1* Δ segregants. The orange circle represents *rad53*^{1-4/9-12AQ} *sml1* Δ segregants. The blue square represents *crt1* Δ segregants. The orange square represents *sml1* Δ segregants. The strain in this tetrad dissection is yRK6007.

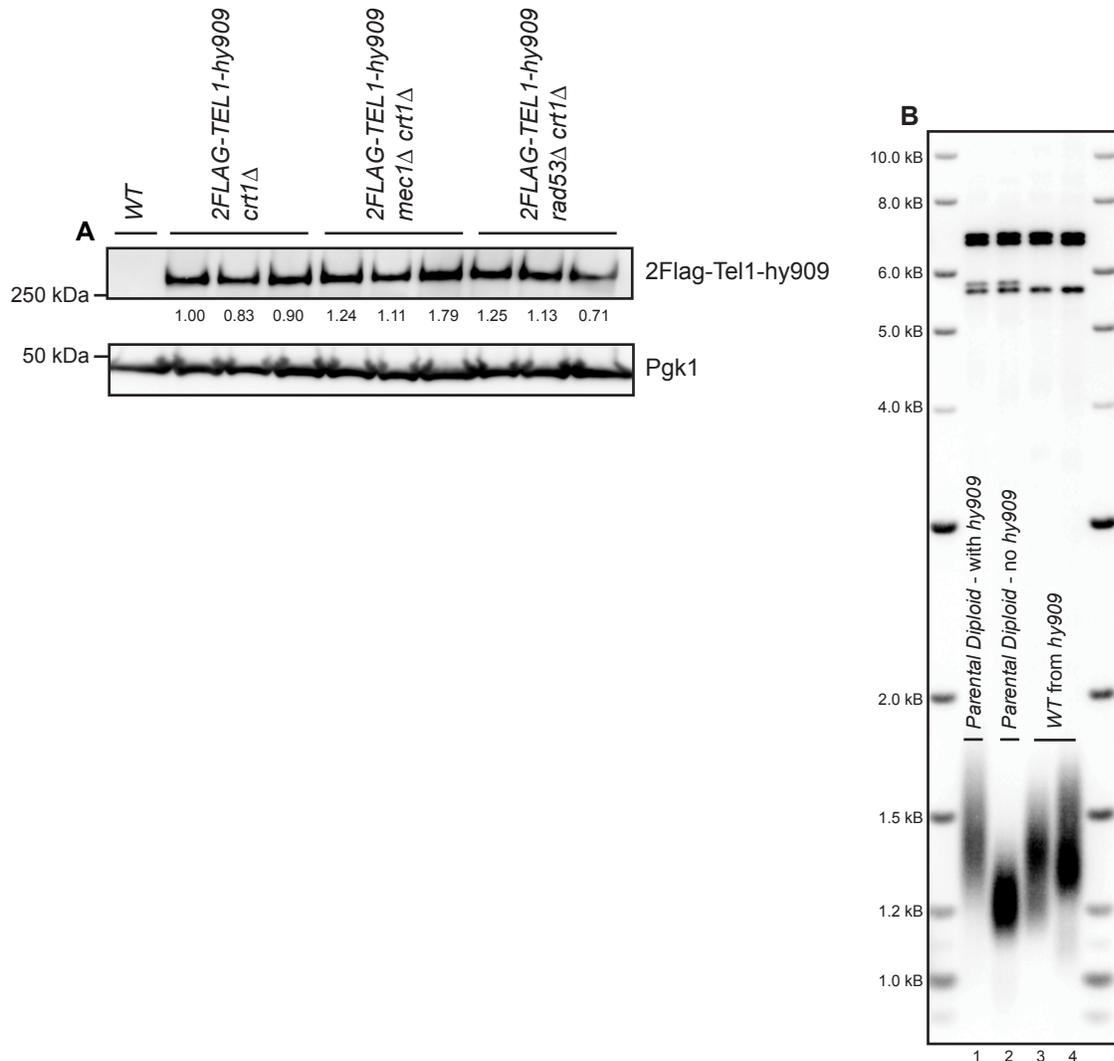


Figure S4 Tel1-hy909 increases telomere length heterogeneity and is stably expressed in the absence of Mec1 and Rad53

(A) Western blot analysis of 2Flag-Tel1-hy909 protein levels. Samples were quantified in ImageQuant TL (see Methods) and normalized to the Pgk1 loading control, then to the Flag signal in lane 2. An unpaired two-tailed student *t*-test was performed pair-wise between samples and no significant effect was observed. Each sample is an independent segregant from yRK5177 except for the untagged WT sample which is a yRK6003 segregant. (B) Southern blot analysis of telomeres from strains with the indicated genotype. The Parental Diploid without *TEL1-hy909* is JHUy957-1 (*RAD53/rad53Δ::kanMX4 CRT1/crt1Δ::URA3 XRS2/xrs2Δ::LEU2*). The Parental Diploid with *TEL1-hy909* is yRK5028 (*TEL1/tel1Δ::TEL1-hy909-HIS3 RAD53/rad53Δ::kanMX4 CRT1/crt1Δ::URA3 XRS2/xrs2Δ::LEU2*). The haploids are segregants derived from yRK5028. 25 independent biological replicates were assayed for WT segregants from a parental diploid with the *TEL1-hy909* allele. Two independent parental diploids were assayed for telomere length.

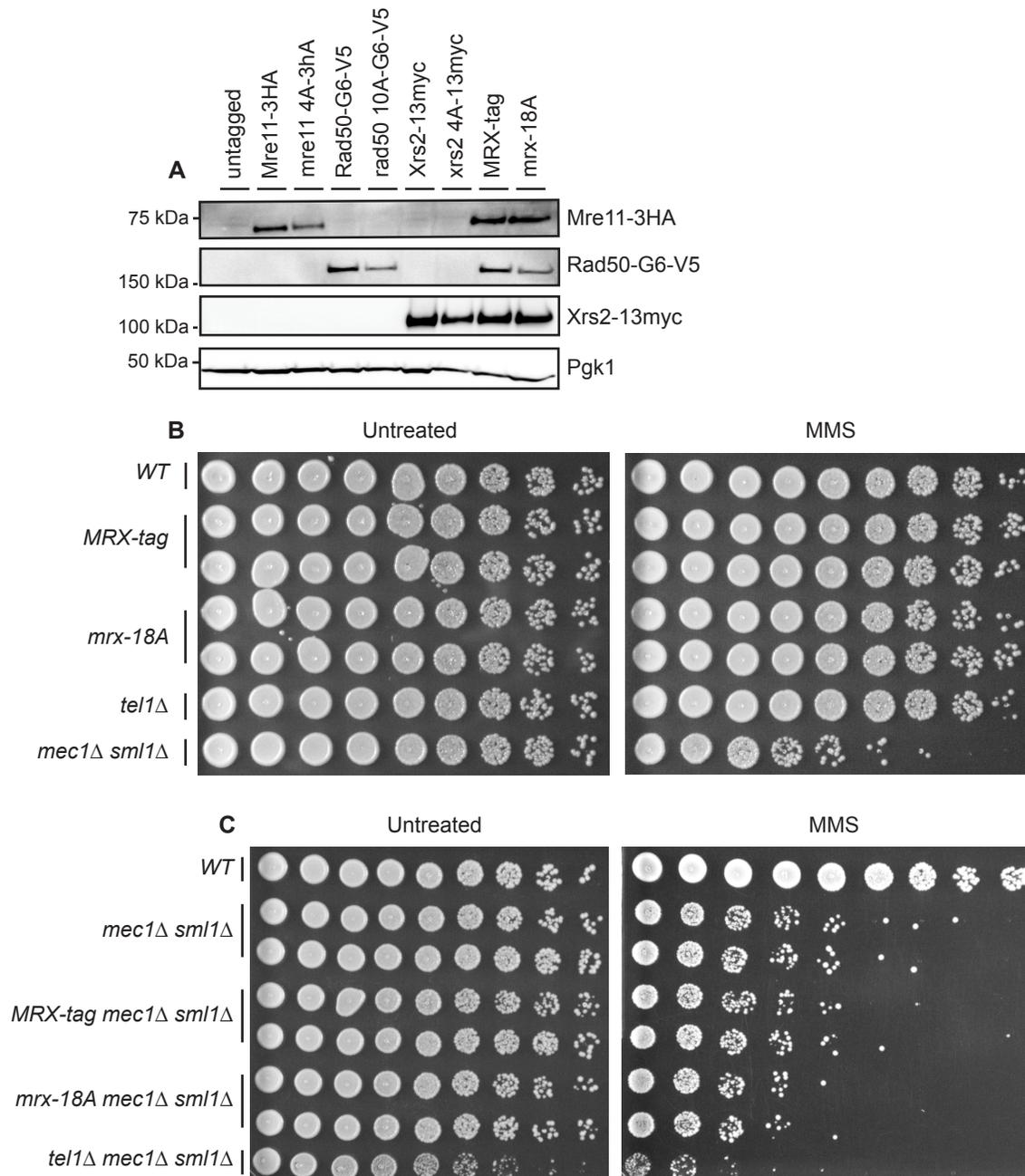


Figure S5 *mrx-18A* MMS sensitivity is similar to *MRX-tag*

(A) Western blots examining stability of the MRX complex in *MRX-tag* and *mrx-18A* haploids. Samples were run and transferred on duplicate gels simultaneously. The membranes were cut such that each protein could be independently probed. Strains used in western blot are yRK114, yRK106, yRK94, yRK138, yRK139, yRK133, yRK135, yRK102, and yRK90. (B) Yeast dilution series on untreated cells or cells cultured in 0.02% MMS for two hours. Genotypes are indicated to the left of the panels. Segregants are from JHUy816, yRK79, yRK80, yRK81, and yRK83. (C) Yeast dilution series on untreated cells or cells treated with 0.04% MMS for two hours. The genotype is indicated to the left of the panels. This defect was quantified in a separate colony forming assay shown in Figure 3C. Segregants are derived from JHUy816, yRK79, yRK80, yRK81, and yRK83.

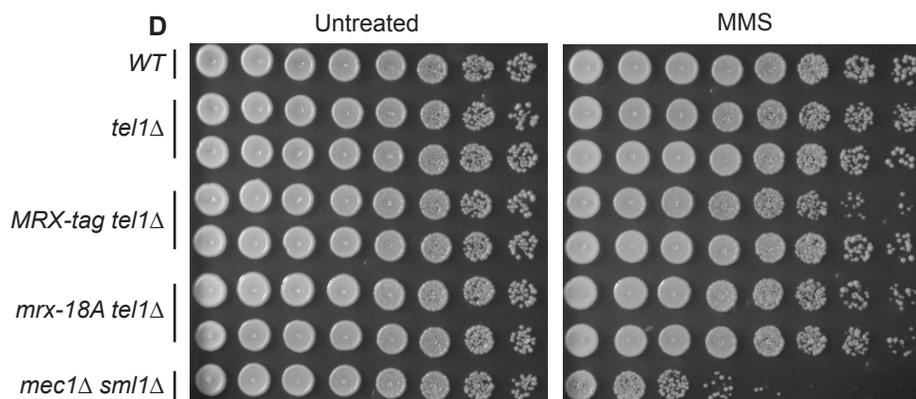
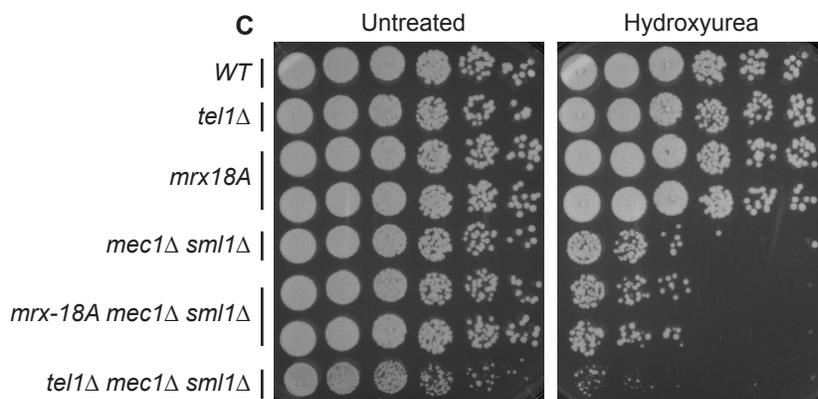
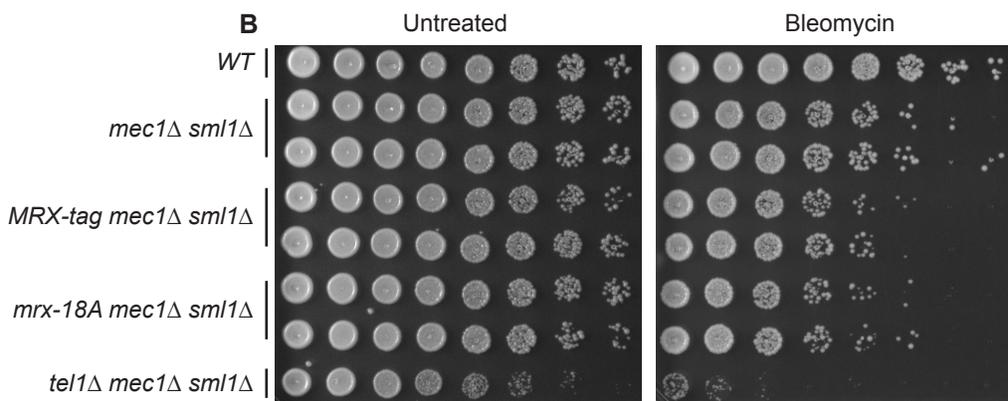
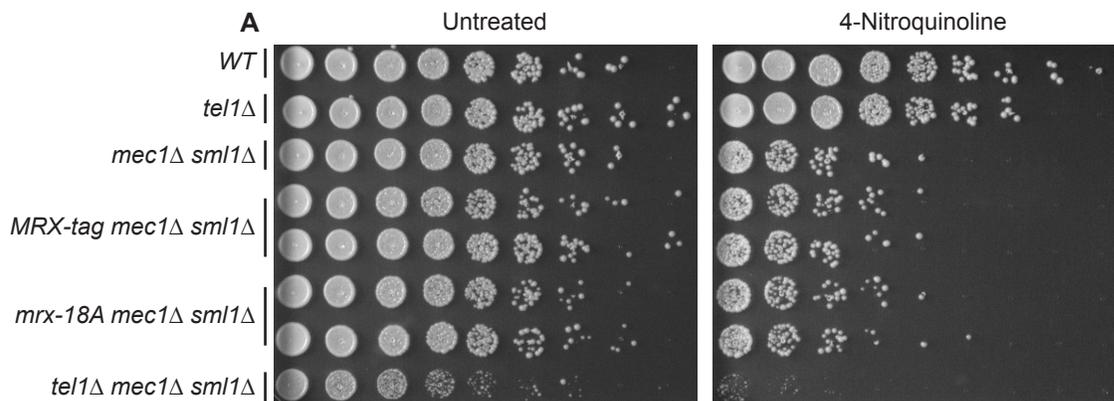


Figure S6 *mrx-18A mec1* Δ *sml1* Δ and *mrx-18A tel1* Δ do not show increased sensitivity to various mutagens

(A) Yeast dilution series on untreated cells or cells treated with 5 μ M 4-nitroquinoline for one hour. Segregants were yRK114, yRK126, yRK128, yRK104, yRK141, yRK92, yRK93, and yRK122. (B) Yeast dilution series on untreated cells or cells treated with 300 μ g/mL bleomycin for one hour. Segregants were derived from JHUy816, yRK79, yRK80, yRK81, and yRK83. (C) Yeast dilution series on untreated cells or cells treated with 200 mM hydroxyurea for one hour. Segregants were derived from JHUy816, yRK72, and yRK73. (D) Yeast dilution series on untreated cells or cells treated with 0.04% MMS for two hours. Segregants were derived from yRK86, yRK87, yRK88, and yRK89.

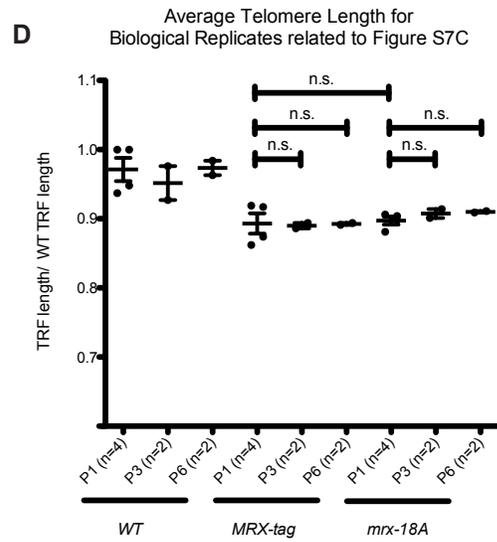
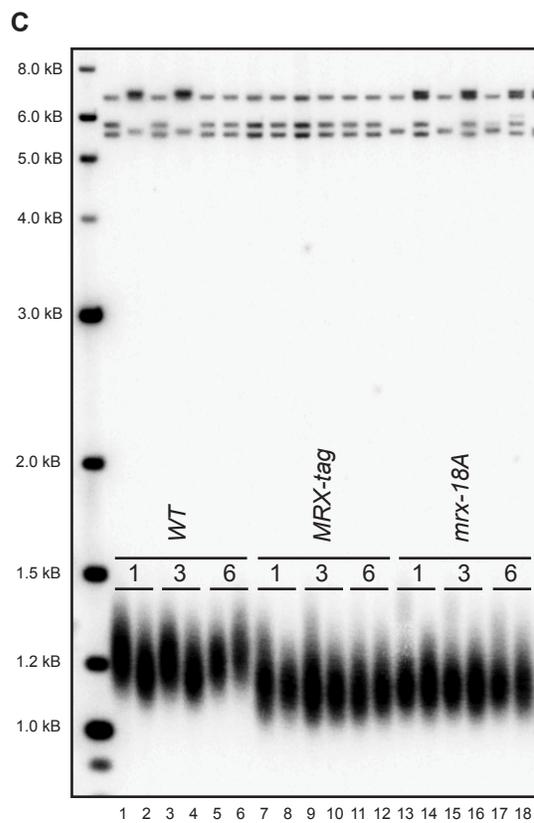
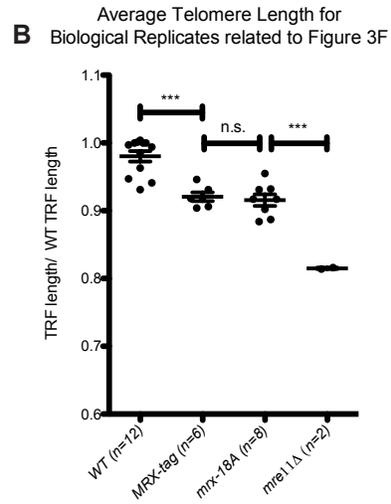
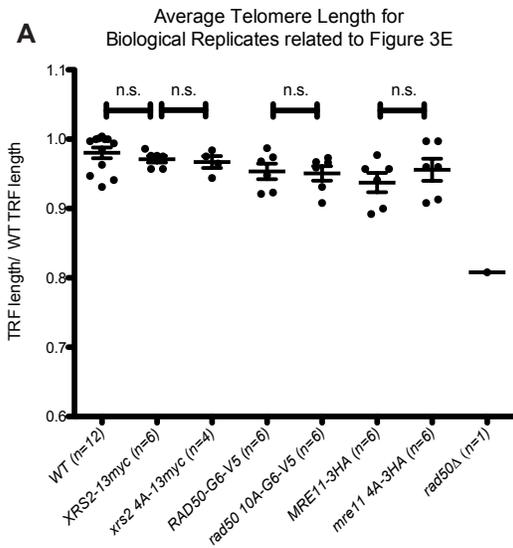
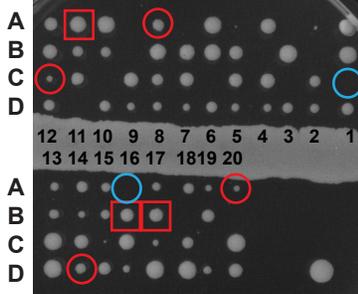


Figure S7 *MRX-tag* and *mrx-18A* telomere length is stable over 170 population doublings

(A-B) The median length of the telomere restriction fragment (TRF) was quantified in ImageQuant TL. In order to compare samples run on different Southern blots, each sample is normalized to the first *WT* sample on that Southern (see Methods). The mean and standard error of the mean are indicated. An unpaired student *t*-test was used to assay statistical significance. n.s. = not significant *** p-value < 0.0001. Samples are considered biological replicates only if they were passaged the same number of times before running the Southern blot. The number of biological replicates is given in parentheses next to each genotype. (C) Southern blot analysis of telomeres from strains with the indicated genotype. Two independent, haploid segregants were assayed for each genotype. Biological replicates are reported in (D). The *rad50Δ* haploid was yRK2024 and was passaged for approximately 170 generations. All other genotypes were segregants from yRK3018, yRK35, or yRK36 and were not passaged. (D) Telomere length was quantified as described for (A-B).

Diploid Genotype: *TEL1-hy909/tel1Δ MEC1/mec1Δ SML1/sml1Δ MRE11/mre11Δ*



Haploid Genotype	Viable Segregants
□ <i>TEL1-hy909</i>	11A, 16B, 17B
○ <i>TEL1-hy909 mec1Δ</i>	8A, 12C, 14D, 20A
<i>TEL1-hy909 sml1Δ</i>	6B, 9C, 14A
<i>TEL1-hy909 mec1Δ sml1Δ</i>	1B, 6D, 7C, 10D, 17D, 18A
<i>TEL1-hy909 mre11Δ</i>	4D
○ <i>TEL1-hy909 mec1Δ mre11Δ</i>	none
<i>TEL1-hy909 sml1Δ mre11Δ</i>	4A, 7D, 9D, 10B, 11B, 18C, 20D
<i>TEL1-hy909 mec1Δ sml1Δ mre11Δ</i>	8D, 12A
<i>WT</i>	7B, 8B, 10A, 18D
<i>mec1Δ</i>	none
<i>sml1Δ</i>	1A, 16C, 20C
<i>mec1Δ sml1Δ</i>	4C, 11C
<i>mre11Δ</i>	1D, 6A, 12B, 14C, 17A
<i>mec1Δ mre11Δ</i>	none
<i>sml1Δ mre11Δ</i>	8C
<i>mec1Δ sml1Δ mre11Δ</i>	14B, 16D, 17C

Figure S8 *TEL1-hy909* rescue of *mec1Δ* lethality depends on the MRX complex

Pictures of tetrad dissection plates where each haploid from a tetrad was placed in a vertical column designated A, B, C, or D (indicated to the left of the picture). Tetrads are assigned numbers from 1-20, as indicated in the center of the plate. One diploid strain was dissected on each plate; the diploid genotype is written at the top of the table. Every possible haploid genotype is listed in the table under Haploid Genotype. Each haploid with that genotype is listed in the adjacent row under Viable Segregants. In cases where three out of four segregants are viable, it is possible to deduce the genotype of the inviable segregants. The red square indicates *TEL1-hy909* segregants, the red circle indicates *TEL1-hy909 mec1Δ* segregants, and the blue circle indicates *TEL1-hy909 mec1Δ mre11Δ* segregants. The strain in this tetrad dissection is yRK5063.

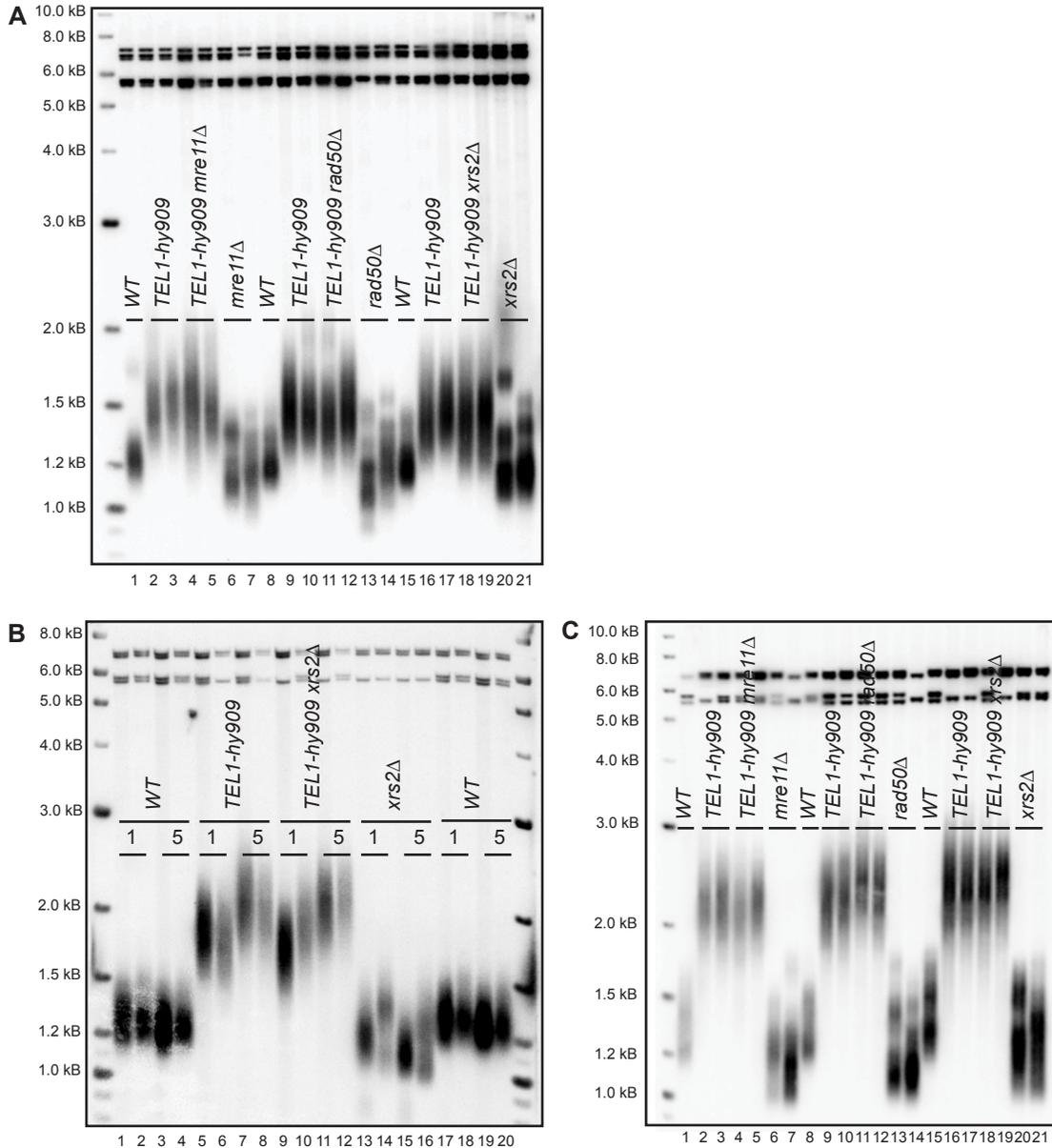


Figure S9 *TEL1-hy909* lengthens telomeres in the absence of the MRX complex in the W303 background

(A)-(C) Southern blot analysis of telomeres from strains with the indicated genotype. Two independent haploids segregants were assayed for each genotype. (A) Cells underwent minimal propagation before genomic DNA was prepared. Segregants are all W303 background and derived from yRK5069, yRK5070, yRK5071, yRK5072, yRK5073, and yRK5074. Biological replicates assayed in this background were *WT* n=3, *TEL1-hy909* n=6, *TEL1-hy909 mre11Δ* n=2, *mre11Δ* n=2, *TEL1-hy909 rad50Δ* n=2, *rad50Δ* n=2, *TEL1-hy909 xrs2Δ* n=2, *xrs2Δ* n=2.

(B) Haploid cells were passaged on solid media for approximately 120 population doublings. *xrs2Δ* had a mild growth defect that was constant over time (not shown). Segregants are all BY background and derived from yRK5028 and yRK5059. For passage 1 (1) samples *WT* n= 35, *TEL1-hy909* n=42, *TEL1-hy909 xrs2Δ* n=4, *xrs2Δ* n=4. For passage 5 (5) samples there were two biological replicates for each genotype. (C) Haploid cells were passaged on solid media as in (B) and only passage 5 is shown. Biological replicates assayed at the fifth passage were *WT* n=5, *TEL1-hy909* n=8, *TEL1-hy909 mre11Δ* n=2, *TEL1-hy909 rad50Δ* n=2, *TEL1-hy909 xrs2Δ* n=4, *mre11Δ* n=2, *rad50Δ* n=2, *xrs2Δ* n=4.