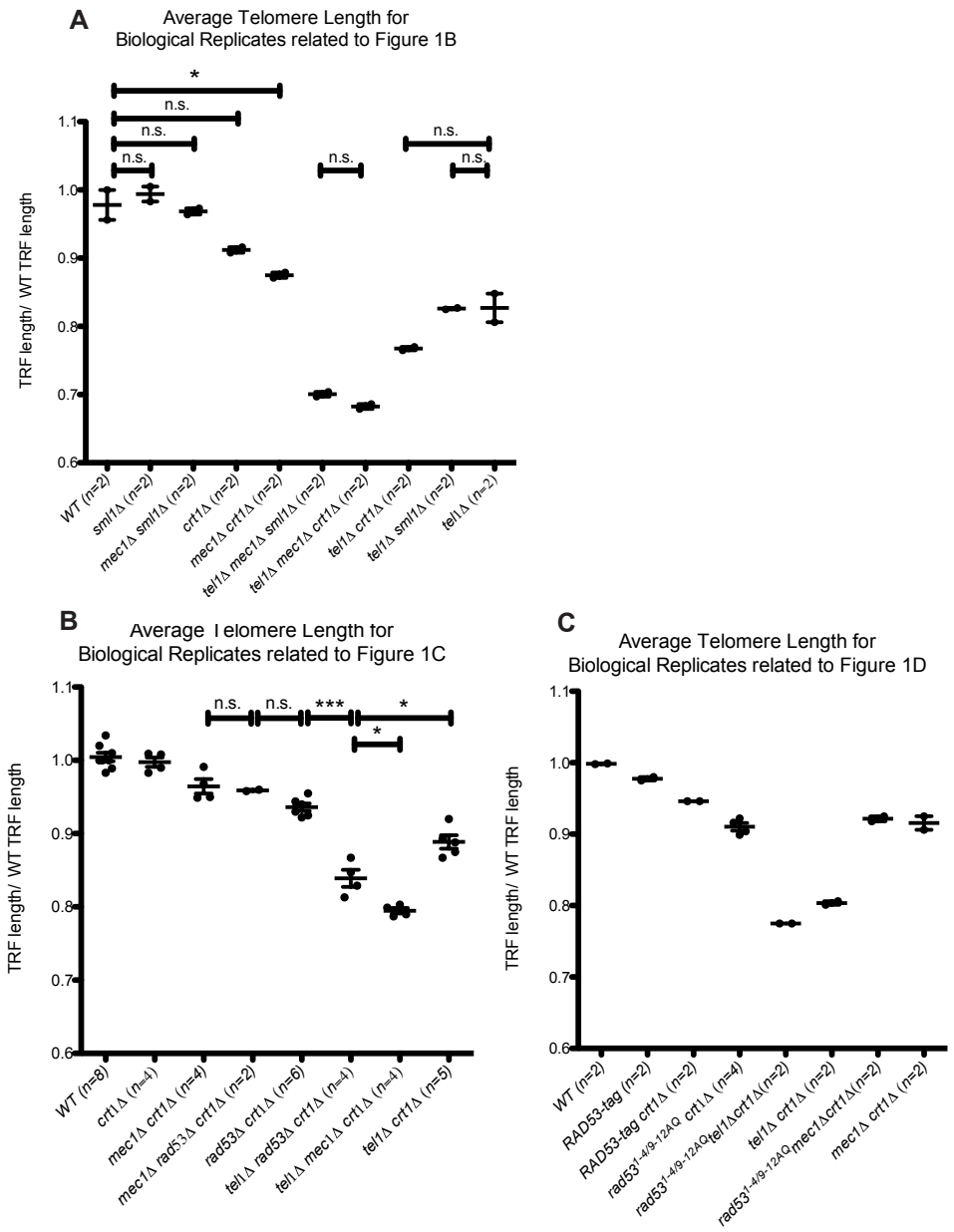
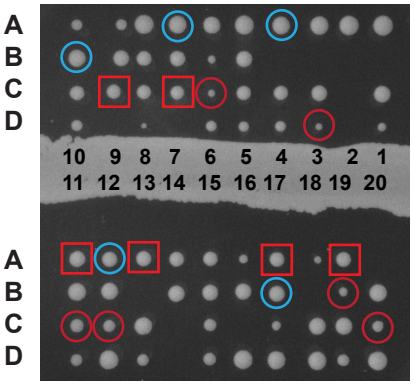


Figure S1 Quantitation of Figure 1 Southerns

(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 ahd exactly the same values, making it impossible to estimate variance.

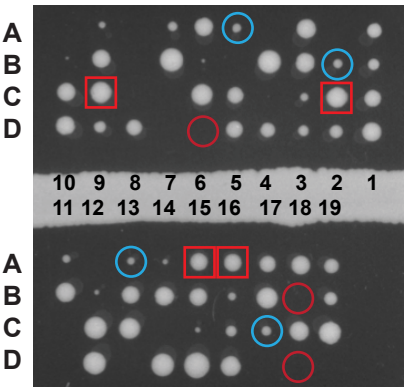
A



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

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

B

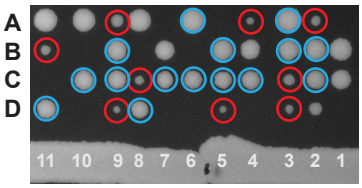


Diploid Genotype: *TEL1/TEL1-hy909 MEC1/mec1Δ RAD53/rad53Δ CRT1/crt1Δ XRS2/xrs2Δ*

Haploid Genotype	Viable Segregants
TEL1-hy909	1D, 2C, 9C, 12D, 15A, 16A
TEL1-hy909 rad53Δ	none
TEL1-hy909 crt1Δ	13C, 14D, 17B
TEL1-hy909 rad53Δ crt1Δ	2B, 5A, 13A, 17C
TEL1-hy909 xrs2Δ	1C, 3A, 5C, 6A, 9B
TEL1-hy909 rad53Δ xrs2Δ	3C
TEL1-hy909 crt1Δ xrs2Δ	10C, 19A
TEL1-hy909 rad53Δ crt1Δ xrs2Δ	10B, 15D, 16B
WT	10D, 14B, 19C
rad53Δ	none
crt1Δ	6C, 12C, 18A
rad53Δ crt1Δ	1A, 3D, 9D, 15C, 16C, 19B
xrs2Δ	3B, 13B, 15B, 16D, 17A, 18D
rad53Δ xrs2Δ	none
crt1Δ xrs2Δ	2D, 5D, 18C
rad53Δ crt1Δ xrs2Δ	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*Δ *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*Δ *crt1*Δ segregants. The strain in this tetrad dissection is yRK5028.

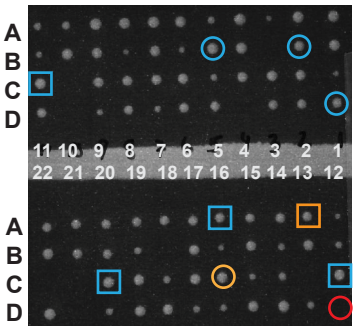
A



Diploid Genotype: *prad53$^{1\text{-}4/9\text{-}12AQ}$ RAD53/rad53Δ*

Haploid Genotype	Viable Segregants
rad53Δ	none
◯ *rad53Δ prad53$^{1\text{-}4/9\text{-}12AQ}$*	2A, 2D, 3C, 3D, 4A, 5D, 8C, 9A, 9D, 11B
RAD53	1B, 1C, 4B, 7A, 8A, 10A, 11A
◯ *RAD53 prad53$^{1\text{-}4/9\text{-}12AQ}$*	2B, 2C, 3A, 3B, 4C, 5B, 5C 6A, 6C, 7C, 8D, 9B, 9C 10C, 1D

B



Diploid Genotype: *RAD53/rad53$^{1\text{-}4/9\text{-}12AQ}$ MEC1/mec1Δ TEL1/tel1Δ SML1/sml1Δ CRT1/crt1Δ*

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

Figure S3 Integrated rad53$^{1\text{-}4/9\text{-}12AQ}$ is lethal while plasmid-expressed rad53$^{1\text{-}4/9\text{-}12AQ}$ 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*$^{1\text{-}4/9\text{-}12AQ}$ plasmid. The blue circle identifies *RAD53* segregants that retained the *rad53*$^{1\text{-}4/9\text{-}12AQ}$ 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*$^{1\text{-}4/9\text{-}12AQ}$ segregants. The blue circle identifies *rad53*$^{1\text{-}4/9\text{-}12AQ}$ *crt1*Δ segregants. The orange circle represents *rad53*$^{1\text{-}4/9\text{-}12AQ}$ *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.

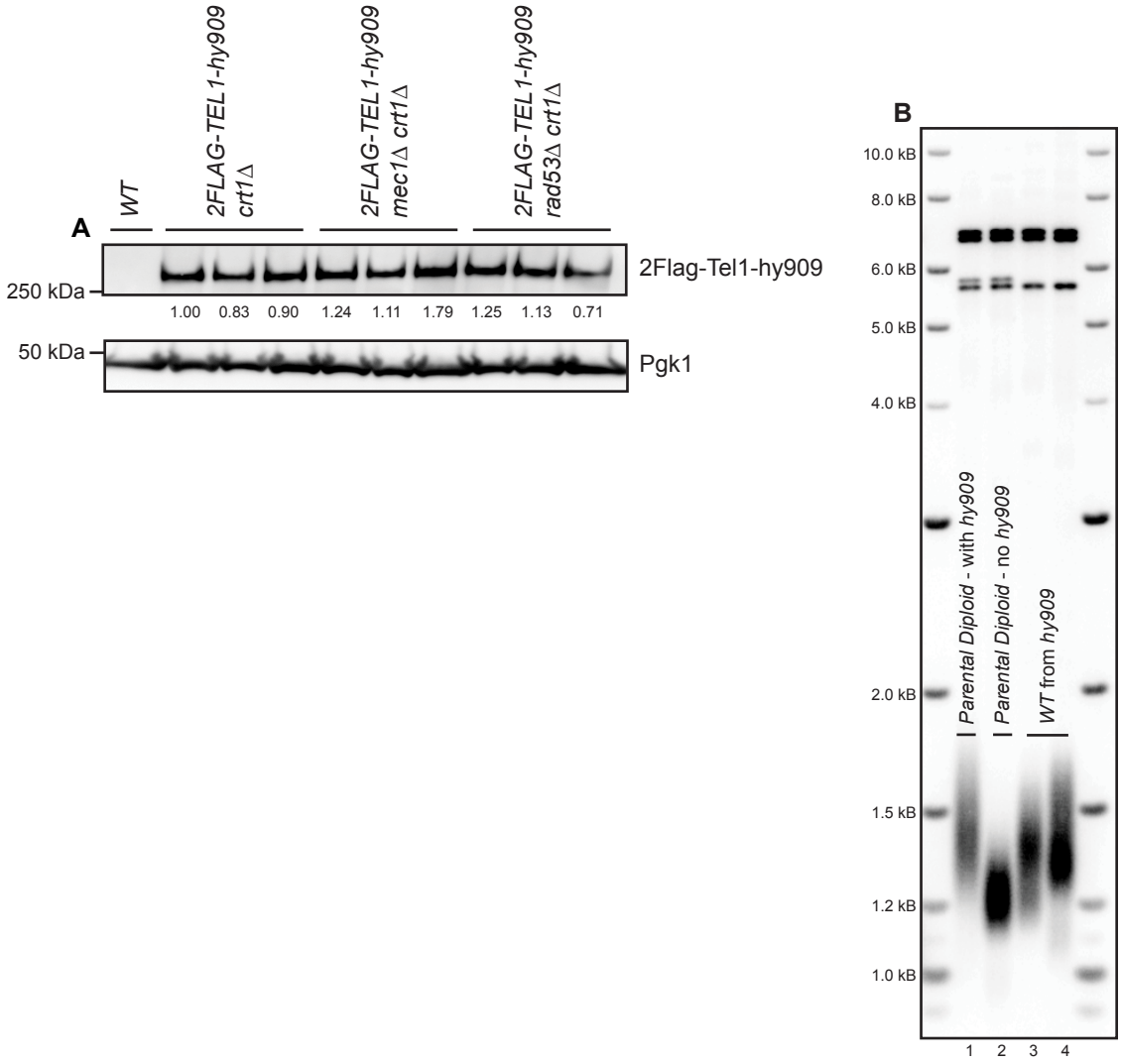


Figure S4 Tel1-hy909 increases telomere length heterogeneity and is stably expressed in the absence of Mec1 and Rad53

(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.

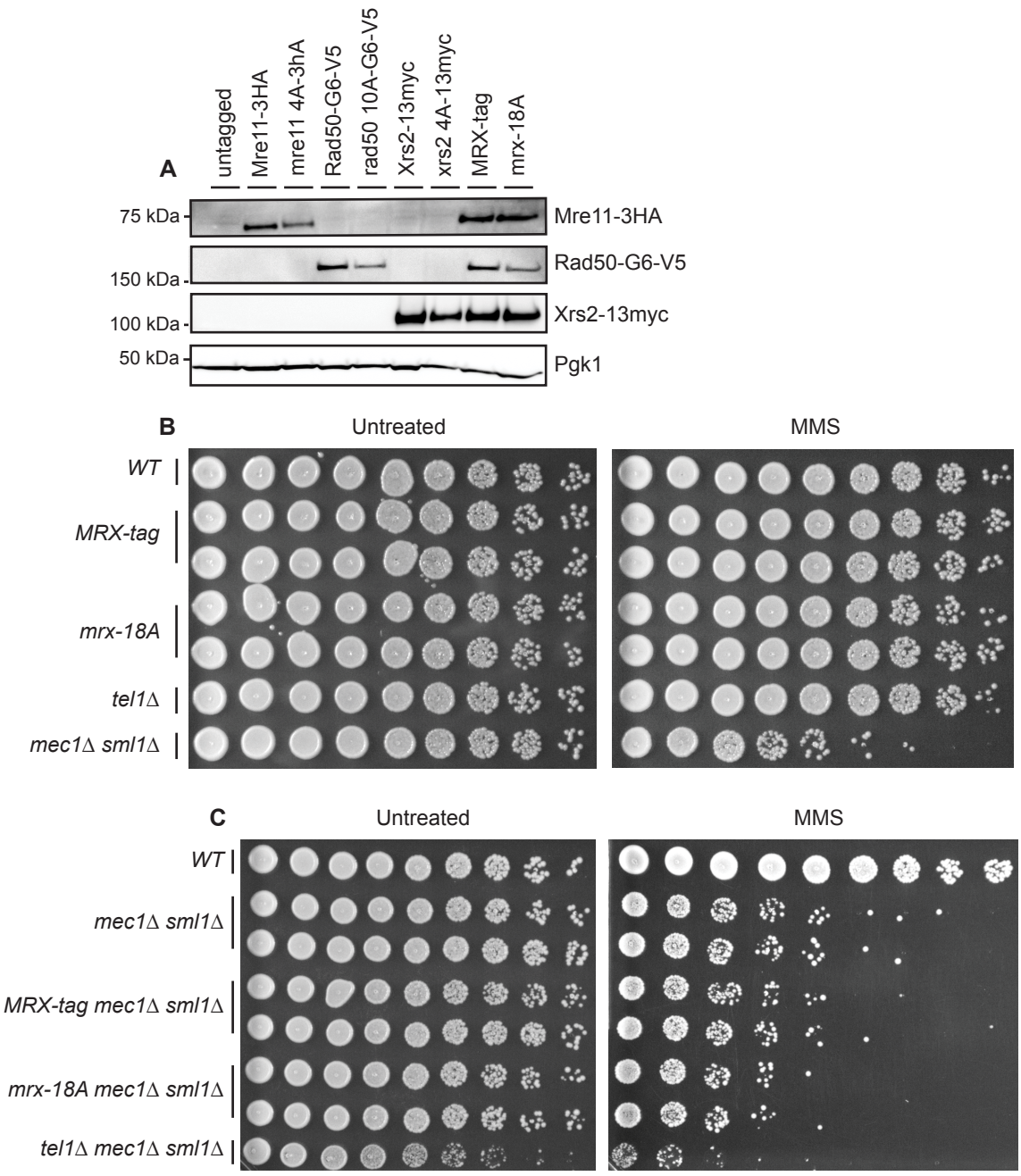


Figure S5 *mrx-18A* MMS sensitivity is similar to *MRX-tag*
(A) Western blots examining stability of the MRX complex in *MRX-tag* and *mrx-18A* haploids. Samples were run and transferred on duplicate gels simultaneously. The membranes were cut such that each protein could be independently probed. Strains 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.

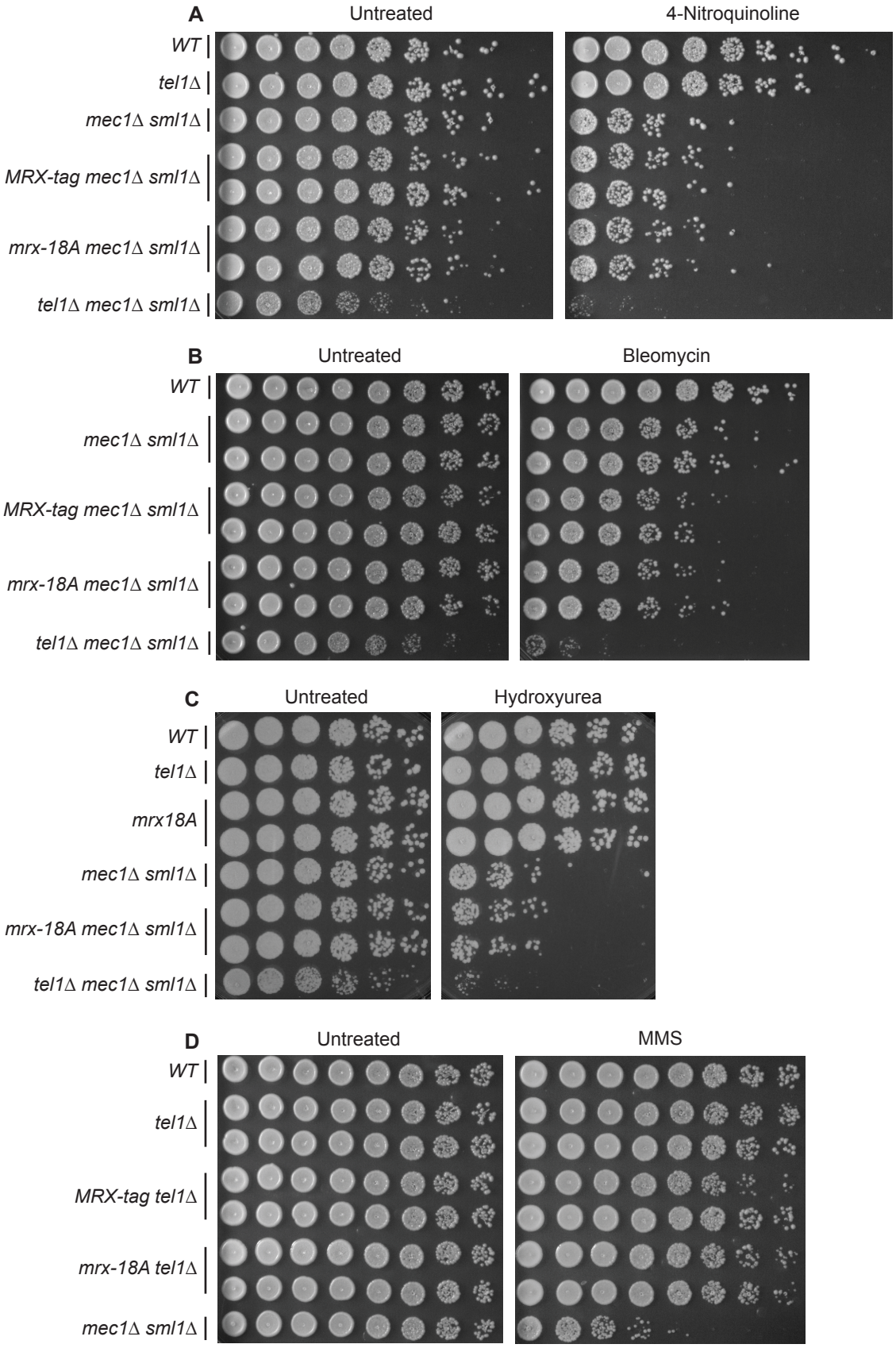

A
Untreated
4-Nitroquinoline
WT
tel1Δ
mec1Δ sml1Δ
MRX-tag mec1Δ sml1Δ
mrx-18A mec1Δ sml1Δ
tel1Δ mec1Δ sml1Δ
B
Untreated
Bleomycin
WT
mec1Δ sml1Δ
MRX-tag mec1Δ sml1Δ
mrx-18A mec1Δ sml1Δ
tel1Δ mec1Δ sml1Δ
C
Untreated
Hydroxyurea
WT
tel1Δ
mrx18A
mec1Δ sml1Δ
mrx-18A mec1Δ sml1Δ
tel1Δ mec1Δ sml1Δ
D
Untreated
MMS
WT
tel1Δ
MRX-tag tel1Δ
mrx-18A tel1Δ
mec1Δ sml1Δ

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

(A) Yeast dilution series on untreated cells or cells treated with 5 uM 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 ug/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.

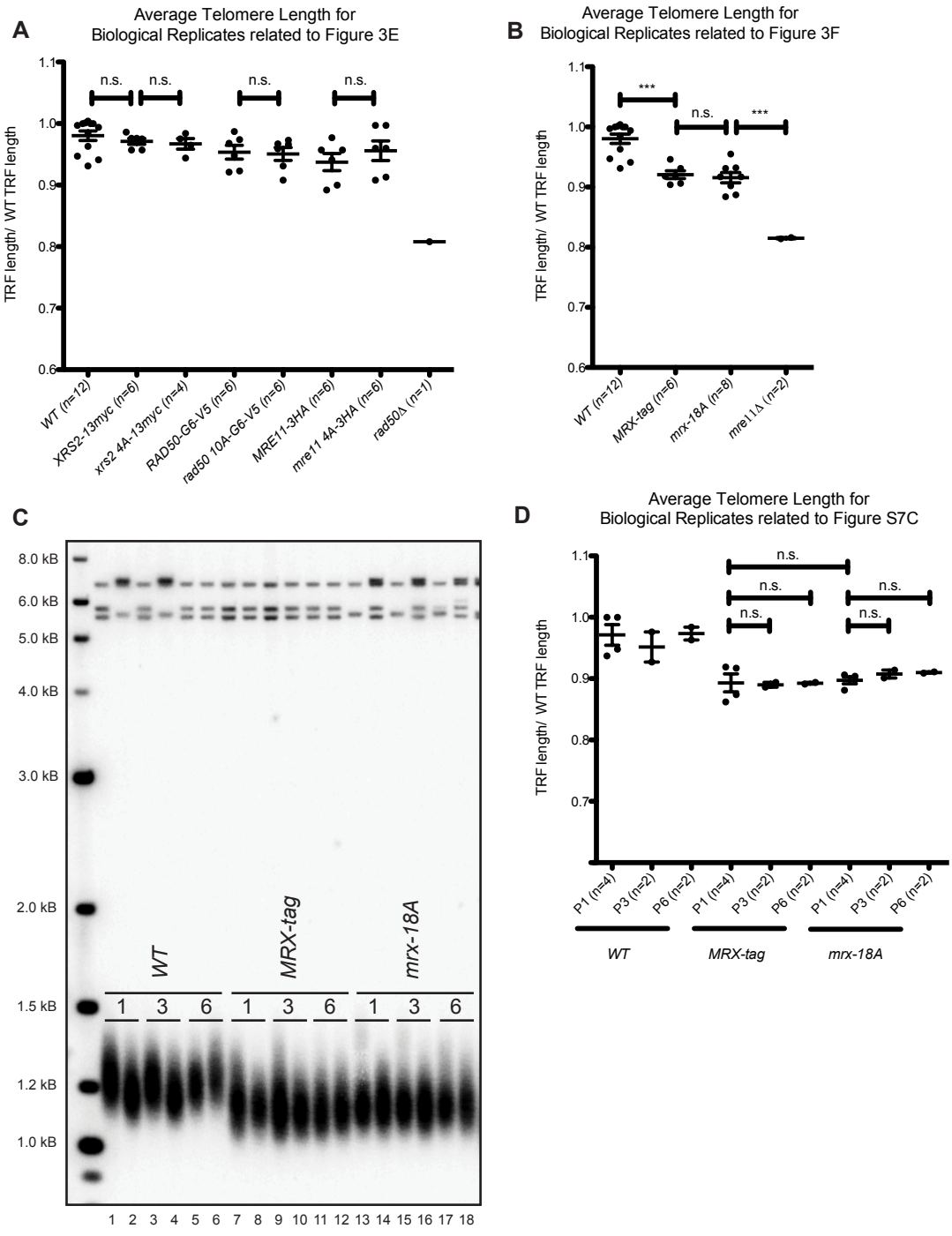
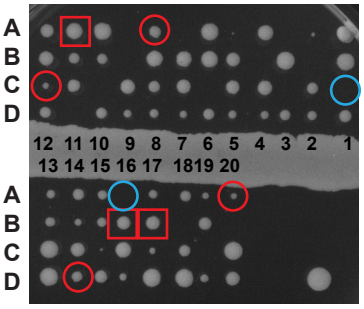

A
Average Telomere Length for
Biological Replicates related to Figure 3E
TRF length/ WT TRF length
n.s.
WT (n=12)
XRS2-13myc (n=6)
xrs2 4A-13myc (n=4)
RAD50-G6-V5 (n=6)
rad50 10A-G6-V5 (n=6)
MRE11-3HA (n=6)
mre11 4A-3HA (n=6)
rad50Δ (n=1)
B
Average Telomere Length for
Biological Replicates related to Figure 3F
***
n.s.
WT (n=12)
MRX-tag (n=6)
mrx-18A (n=8)
mre11Δ (n=2)
C
8.0 kB
6.0 kB
5.0 kB
4.0 kB
3.0 kB
2.0 kB
1.5 kB
1.2 kB
1.0 kB
WT
MRX-tag
mrx-18A
1 3 6 1 3 6 1 3 6
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
D
Average Telomere Length for
Biological Replicates related to Figure S7C
P1 (n=4)
P3 (n=2)
P6 (n=2)
WT
MRX-tag
mrx-18A

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
TEL1-hy909	11A, 16B, 17B
TEL1-hy909 mec1Δ	8A, 12C, 14D, 20A
TEL1-hy909 sml1Δ	6B, 9C, 14A
TEL1-hy909 mec1Δ sml1Δ	1B, 6D, 7C, 10D, 17D, 18A
TEL1-hy909 mre11Δ	4D
TEL1-hy909 mec1Δ mre11Δ	none
TEL1-hy909 sml1Δ mre11Δ	4A, 7D, 9D, 10B, 11B, 18C, 20D
TEL1-hy909 mec1Δ sml1Δ mre11Δ	8D, 12A
WT	7B, 8B, 10A, 18D
mec1Δ	none
sml1Δ	1A, 16C, 20C
mec1Δ sml1Δ	4C, 11C
mre11Δ	1D, 6A, 12B, 14C, 17A
mec1Δ mre11Δ	none
sml1Δ mre11Δ	8C
mec1Δ sml1Δ mre11Δ	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.

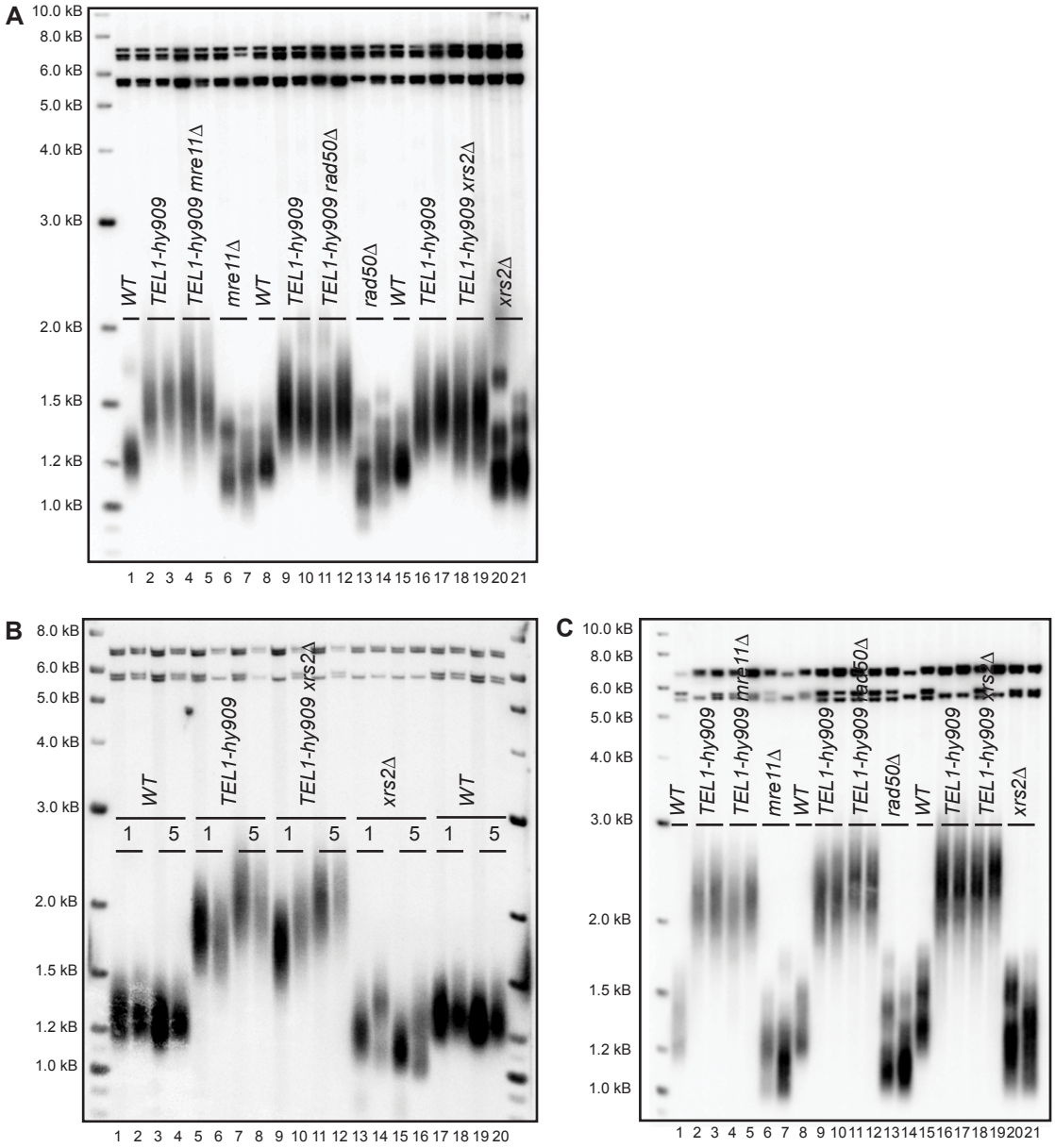


Figure S9 *TEL1-hy909* lengthens telomeres in the absence of the MRX complex in the W303 background

(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 mre11*Δ n=2, *mre11*Δ n=2, *TEL1-hy909 rad50*Δ n=2, *rad50*Δ n=2, *TEL1-hy909 xrs2*Δ n=2, *xrs2*Δ n=2. (B) Haploid cells were passaged on solid media for approximately 120 population doublings. *xrs2*Δ 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 xrs2*Δ n=4, *xrs2*Δ 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 mre11*Δ n=2, *TEL1-hy909 rad50*Δ n=2, *TEL1-hy909 xrs2*Δ n=4, *mre11*Δ n=2, *rad50*Δ n=2, *xrs2*Δ n=4.