**File S1: Cloning descriptions**

All plasmid sequence files are available upon request.

*MRE11* cloning and transformation

pRK1012 contains the *MRE11-3HA-URA3* construct. pRK1036 was based in pRK1012 and contains the *mre11-4A-3HA-URA3* construct. pRK1012 was constructed as follows: the *MRE11* coding sequence and upstream homology region were amplified from yYM256 (Ma and Greider 2009)(primers RW 108, RW 109). The downstream homology region was amplified from the same strain (primers RW 101, RW 104). The 3HA tag was amplified from pFA6α-3HA-*kanMX6* (Bahler *et al.* 1998)(primers RW 110, RW 105) and was fused to the *MRE11* coding sequence by overlap extension Polymerase Chain Reaction (PCR) (Green and Sambrook 2012)(primers RW 105, RW 108). *URA3* was amplified from pRS406 (Sikorski and Hieter 1989)(primers RW 106, RW 103). pRS406 was used as the vector backbone and was opened by PCR (primers RW 102, RW 107). Gibson Assembly of these four pieces yielded pRK1012. pRK1036 was produced by site-directed mutagenesis of pRK1012. Both pRK1012 and pRK1036 were verified by Sanger sequencing and cut with restriction enzymes *Sph*I and *Hind*III to integrate into JHUy761 (Ma and Greider 2009), yielding the strains yRK1006 and yRK1052, respectively.

*RAD50* cloning and transformation

pRK1006 contains the *RAD50-G6-V5-LEU2* construct. pRK1033 contains the *RAD50-G6-V5-TRP1* construct. pRK1035 was based in pRK1033 and contains the *rad50-10A-G6-V5-TRP1* construct. To generate pRK1006, the *RAD50* coding sequence was amplified from yYM256 using phosphorylated primers (YNL250W-A, YNL250W-D) and ligated into *Sma*I-digested pUC19. The *RAD50* coding sequence Gibson Assembly fragment was amplified from *RAD50*/pUC19 (primers RW 80, RW 85). The G6-V5 tag was amplified from pLenti\_Ubc\_V5\_DEST plasmid (Gateway Vector from Life Technologies) (primers RW 81, RW 99) and fused to the *RAD50* coding sequence by overlap extension PCR (primers RW 99 and RW 85). The *RAD50* downstream homology arm was amplified from yYM256 (primers RW 18, RW 19), adding *BsrG*I and *Aat*II cut sites, which were used to directionally clone the insert into pRS405 (Sikorski and Hieter 1989), yielding pRK1000. The pRK1000 vector fragment was linearized by PCR (primers RW 92, RW 100) was combined with the *RAD50-G6-V5* fragment by Gibson Assembly, yielding pRK1006. To generate pRK1033, *TRP1* was amplified from pRS404 (Sikorski and Hieter 1989)(primers RW 223, RW 274). Overlap extension PCR was used to fuse *TRP1* to the *RAD50* downstream homology region, which was amplified from JHUy761 (primers RW 275, RW 276). The *TRP1*-homology region fragment and pRK1006 were cut with restriction enzymes *Aat*II and *Nco*I for directional ligation cloning, yielding pRK1033. pRK1035 was produced by site-directed mutagenesis of pRK1033. Plasmids were sequence verified by Sanger sequencing. pRK1006 was cut with restriction enzymes *Aat*II and *Xho*I to integrate into JHUy761, creating yRK2040. pRK1035 was cut with restriction enzymes *Aat*II and *Xho*I to integrate into JHUy761, creating yRK2082.

*XRS2* cloning and transformation

pRK1028 contains the *XRS2-13myc-hphMX4* construct. pRK1040 was based in pRK1028 and contains the *xrs2-4A-13myc-hphMX4* construct. *XRS2*-*13myc-kanMX6* was amplified with upstream and downstream homology regions from yYM256 (primers YDR369C-A, YDR369C-D). The product was phosphorylated using T4 polynucleotide kinase (NEB M0201) and ligated into *Sma*I-linearized pUC19 vector, yielding pRK1009. The selectable marker was changed to *hphMX4* by cutting pRK1009 with restriction enzymes *Pme*I and *BstE*II and amplifying *hphMX4* from pAG32 (Goldstein and McCusker 1999) (primers RW 195, RW 196). Gibson Assembly of these two products yielded pRK1028. pRK1028 and pRK1040 were validated by Sanger sequencing and cut with restriction enzymes *Xba*I and *Stu*I to isolate the construct for transformation. pRK1028 was transformed into yRK1, yielding yRK60. pRK1040 was transformed into yRK26, yielding yRK56. After transformation, correct integration of all point mutations was confirmed by Sanger sequencing.

*TEL1-hy909* cloning and transformation

pRK1065 contains the C-terminal half of the *TEL1* coding sequence and was generated by Gibson Assembly of the following four fragments with the vector backbone: the *TEL1* coding sequence was amplified from JHUy761 (primers RW 403, RW 175); the *cyc1* terminator was amplified from pGAL414 (Mumberg *et al.* 1995) (primers RW 172, RW 173); the *HIS3* selectable marker was amplified from pRS403 (Sikorski and Hieter 1989) (primers RW 180, RW 402); and the *TEL1* downstream homology region was amplified from JHUy761 (primers RW 400, RW 401). The vector backbone was amplified from pRS403 (primers RW 398, RW 399). Plasmids were validated by Sanger sequencing. The *TEL1-hy909* point mutations (Tel1 A2287V, I2336T, K2751R) (Baldo *et al.* 2008) were introduced by site-directed mutagenesis of pRK1065 to yield pRK1088. pRK1088 was cut with restriction enzymes *Not*I and *Aat*II to isolate the construct for transformation. After transformation, correct integration of all point mutations was confirmed by Sanger sequencing. Strains missing A2287V had telomeres longer than wildtype but not as long as strains with all three point mutations (not shown). Strains missing both A2287V and I2336T had telomeres similar to wildtype (not shown). It is critical that all three point mutations are present to observe the *TEL1-hy909* telomere elongation phenotype.

*RAD53* cloning and transformation

The *RAD53-3xFLAG* (pJHU1264) and *rad531-4/9-12AQ-3xFLAG* (pJHU1263) were gifts from the Stern lab (Lee *et al.* 2003). The plasmids were converted for genomic integration using Gibson Assembly to add a *CYC1* terminator and *HIS3* selectable marker downstream of the coding sequence. The parental plasmids were linearized by PCR (primers RW 498, RW 499), the downstream homology region was amplified from pJHU1263 (primers RW 500, RW 501), and the *CYC1-HIS3* cassette was amplified from pRK1065 (primers RW 502, RW 503). Gibson Assembly yielded pRK1083 (based in pJHU1263) and pRK1084 (based in pJHU1264). Plasmids were validated by Sanger sequencing and cut with restriction enzymes *Pac*I and *Not*I to isolate the construct for transformation. pRK1083 was transformed into JHUy937-1 yielding yRK6004 and yRK6005. pRK1083 was transformed into JHUy937-1 yielding yRK6006 and yRK6007. After transformation, correct integration of all point mutations was confirmed by Sanger sequencing.

Literature Cited:

Bahler, J., J. Q. Wu, M. S. Longtine, N. G. Shah, A. McKenzie, 3rd *et al.*, 1998 Heterologous modules for efficient and versatile PCR-based gene targeting in Schizosaccharomyces pombe. Yeast 14**:** 943-951.

Baldo, V., V. Testoni, G. Lucchini and M. P. Longhese, 2008 Dominant TEL1-hy mutations compensate for Mec1 lack of functions in the DNA damage response. Mol Cell Biol 28**:** 358-375.

Goldstein, A. L., and J. H. McCusker, 1999 Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. Yeast 15**:** 1541-1553.

Green, M., and J. Sambrook, 2012 *Molecular Cloning: A Laboratory Manual*

Lee, S.-J., M. F. Schwartz, J. K. Duong and D. F. Stern, 2003 Rad53 Phosphorylation Site Clusters Are Important for Rad53 Regulation and Signaling. Molecular and Cellular Biology 23**:** 6300-6314.

Ma, Y., and C. W. Greider, 2009 Kinase-independent functions of TEL1 in telomere maintenance. Mol Cell Biol 29**:** 5193-5202.

Mumberg, D., R. Muller and M. Funk, 1995 Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. Gene 156**:** 119-122.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics 122**:** 19-27.