SUPPLEMENTAL MATERIALS

SUPPLEMENTAL RESULTS

Since the kinase domain of Tel1p is adjacent to its C-terminus, previous groups have placed an epitope tag near the N-terminus or internally to examine the Tel1p kinase function (1, 2). However, in our hands, both of these previously published tagged *TEL1* alleles (1, 2) result in short telomeres (data not shown), suggesting that the epitope tags compromised the functions of Tel1p. In an effort to generate a tagged and functional Tel1p, we inserted 1, 2, or 3 copies of the FLAG tag at the N-terminus of Tel1p at its native chromosomal locus. While *1FLAG-* and *2FLAG-TEL1* resulted in wildtype telomere length when expressed from the *GAL1* promoter or from the native *TEL1* promoter (Figure S1 and 1B), *3FLAG-TEL1* led to slightly shorter telomeres when over-expressed (Figure S1). Since 2FLAG-Tel1p was functional and easier to detect by western blotting (data not shown), we used this version for further studies.

SUPPLEMENTAL REFERENCES

1.   **Mallory, J. C., and T. D. Petes.** 2000. Protein kinase activity of Tel1p and Mec1p, two Saccharomyces cerevisiae proteins related to the human ATM protein kinase. Proc Natl Acad Sci U S A **97:**13749-54.

2.   **Takata, H., Y. Kanoh, N. Gunge, K. Shirahige, and A. Matsuura.** 2004. Reciprocal association of the budding yeast ATM-related proteins Tel1 and Mec1 with telomeres in vivo. Mol Cell **14:**515-22.

Supplemental Table 1. Yeast strains.

Strain name	Genotype
JHUY761	*his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0*
JHUY817	JHUY761 *TEL1/tel1Δ::hygroMX4 MEC1/mec1Δ::kanMX4 SML1/sml1Δ::TRP1*
JHUY870	*his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 MET15/met15Δ0 ura3Δ0/ura3Δ0 XRS2/xrs2Δ::kanMX4*
JHUY877	*mat a-inc/mat α ade2-101/ade2-101 his3-200/his3-200 lys2-801/lys2-801 trp1Δ63/trp1Δ63 ura3-52/ura3-52 leu2Δ1:LEU2-GAL-HO/leu2Δ1 VII-L::ADE2-TG(1-3)-HO site-LYS2 RAD52/rad52Δ::hygroMX4*
yYM21	JHUY761 *TEL1/tel1Δ::kanMX6-P$_{GAL1}$-1FLAG-TEL1*
yYM23	*his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 tel1Δ::kanMX6-P$_{GAL1}$-1FLAG-TEL1*
yYM25	JHUY761 *TEL1/tel1Δ::kanMX6-P$_{GAL1}$-2FLAG-TEL1*
yYM27	*his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 tel1Δ::kanMX6-P$_{GAL1}$-2FLAG-TEL1*
yYM28	JHUY761 *TEL1/tel1Δ::kanMX6-P$_{GAL1}$-3FLAG-TEL1*
yYM94	*his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 tel1Δ::kanMX6-P$_{GAL1}$-2FLAG-tel1-D2612A*
yYM97	*his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 tel1Δ::kanMX6-P$_{GAL1}$-2FLAG-tel1-N2617A*
yYM100	*his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 tel1Δ::kanMX6-P$_{GAL1}$-2FLAG-tel1-D2631A*
yYM103	*his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 tel1Δ::kanMX6-P$_{GAL1}$-2FLAG-tel1-D2612A/N2617A*
yYM147	JHUY761 *TEL1/tel1Δ::kanMX6-P$_{GAL1}$-2FLAG-tel1-D2612A MEC1/mec1Δ::LEU2 SML1/sml1Δ::TRP1*
yYM149	JHUY761 *TEL1/tel1Δ::kanMX6-P$_{GAL1}$-2FLAG-tel1-N2617A MEC1/mec1Δ::LEU2 SML1/sml1Δ::TRP1*
yYM151	JHUY761 *TEL1/tel1Δ::kanMX6-P$_{GAL1}$-2FLAG-tel1-D2631A MEC1/mec1Δ::LEU2 SML1/sml1Δ::TRP1*
yYM153	JHUY761 *TEL1/tel1Δ::kanMX6-P$_{GAL1}$-2FLAG-tel1-D2612A/N2617A MEC1/mec1Δ::LEU2 SML1/sml1Δ::TRP1*
yYM159	*mat a-inc ade2-101 his3-200 lys2-801 trp1Δ63 ura3-52 leu2Δ1:LEU2-GAL-HO VII-L::ADE2-TG(1-3)-HO site-LYS2 rad52Δ::hygroMX4 tel1Δ::kanMX4*
yYM167	JHUY761 *TEL1/tel1Δ::kanMX6-P$_{GAL1}$-2FLAG-TEL1 MEC1/mec1Δ::LEU2 SML1/sml1Δ::TRP1*
yYM188	*his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 tel1Δ::2FLAG-TEL1*
yYM242	JHUY761 *TEL1/tel1Δ::2FLAG-TEL1 MEC1/mec1Δ::LEU2 SML1/sml1Δ::TRP1*
yYM244	JHUY761 *TEL1/tel1Δ::2FLAG-tel1-D2612A MEC1/mec1Δ::LEU2 SML1/sml1Δ::TRP1*
yYM246	JHUY761 *TEL1/tel1Δ::2FLAG-tel1-N2617A MEC1/mec1Δ::LEU2 SML1/sml1Δ::TRP1*
yYM248	JHUY761 *TEL1/tel1Δ::2FLAG-tel1-D2631A MEC1/mec1Δ::LEU2 SML1/sml1Δ::TRP1*

yYM250	JHUY761 *TEL1/tel1Δ::2FLAG-tel1-D2612A/N2617A MEC1/mec1Δ::LEU2 SML1/sml1Δ::TRP1*
yYM256	JHUY761 *TEL1/tel1Δ::2FLAG-TEL1 XRS2/xrs2Δ::XRS2-13myc-kanMX6*
yYM259	JHUY761 *TEL1/tel1Δ::2FLAG-TEL1 XRS2/xrs2Δ::XRS2-13myc-kanMX6*
yYM260	JHUY761 *TEL1/tel1Δ::2FLAG-TEL1 XRS2/xrs2Δ::XRS2-13myc-kanMX6 YKU80/yku80Δ::URA3*
yYM268, 269	JHUY761 *TEL1/tel1Δ::2FLAG-TEL1 XRS2/xrs2Δ::xrs2-834-13myc-kanMX6*
yYM271	JHUY761 *TEL1/tel1Δ::2FLAG-TEL1 XRS2/xrs2Δ::xrs2-D838A/D839A-13myc-kanMX6*
yYM273	JHUY761 *TEL1/tel1Δ::2FLAG-TEL1 XRS2/xrs2Δ::xrs2-D842A/D843A-13myc-kanMX6*
yYM274, 275	JHUY761 *TEL1/tel1Δ::2FLAG-TEL1 XRS2/xrs2Δ::xrs2-K846A/F847A-13myc-kanMX6*
yYM278	JHUY877 *TEL1/tel1Δ::2FLAG-TEL1 pRS416*
yYM279	JHUY877 *TEL1/tel1Δ::2FLAG-tel1-D2612A pRS416*
yYM280	JHUY877 *TEL1/tel1Δ::2FLAG-tel1-N2617A pRS416*
yYM281	JHUY877 *TEL1/tel1Δ::2FLAG-tel1-D2631A pRS416*
yYM282	JHUY877 *TEL1/tel1Δ::2FLAG-tel1-D2612A/N2617A pRS416*
yYM286	JHUY877 *XRS2/xrs2Δ::XRS2-13myc-kanMX6*
yYM288	JHUY877 *XRS2/xrs2Δ::xrs2-834-13myc-kanMX6*
yYM289	JHUY877 *XRS2/xrs2Δ::xrs2-D838A/839A-13myc-kanMX6*
yYM292	JHUY877 *XRS2/xrs2Δ::xrs2-D842A/843A-13myc-kanMX6*
yYM293	JHUY877 *XRS2/xrs2Δ::xrs2-K846A/F847A-13myc-kanMX6*
yYM300	JHUY877 *TEL1/ tel1Δ::2FLAG-TEL1 XRS2/xrs2Δ::xrs2-834-13myc-kanMX6*
yYM301	JHUY877 *TEL1/ tel1Δ::2FLAG-TEL1 XRS2/xrs2Δ::xrs2-K846A/F847A-13myc-kanMX6*
yYM305	JHUY877 *TEL1/ tel1Δ::2FLAG-tel1-D2612A/N2617A XRS2/xrs2Δ::xrs2-K846A/F847A-13myc-kanMX6*
yYM308	JHUY877 *TEL1/ tel1Δ::2FLAG-tel1-D2612A/N2617A XRS2/xrs2Δ::xrs2-834-13myc-kanMX6*
yYM309, 310	JHUY761 *TEL1/tel1Δ::2FLAG-tel1-D2612A/N2617A XRS2/xrs2Δ::XRS2-13myc-kanMX6*
yYM311, 312	JHUY761 *TEL1/tel1Δ::2FLAG-tel1-D2612A/N2617A XRS2/xrs2Δ::xrs2-834-13myc-kanMX6*
yYM319	JHUY877 *TEL1/ tel1Δ::2FLAG-TEL1 XRS2/xrs2Δ::XRS2-13myc-kanMX6*
yYM321	JHUY877 *TEL1/ tel1Δ::2FLAG-tel1-D2612A/N2617A XRS2/xrs2Δ::XRS2-13myc-kanMX6*
yYM324	JHUY877 *TEL1/tel1Δ::HIS3 XRS2/xrs2Δ::XRS2-13myc-kanMX6*
yYM325, 326	JHUY761 *TEL1/tel1Δ::2FLAG-tel1-D2612A/N2617A XRS2/xrs2::xrs2-K846A/F847A-13myc-kanMX6*
yYM327, 328	JHUY761 *TEL1/tel1Δ::kanMX6-PGAL1-2FLAG-tel1-D2612A/N2617A XRS2/xrs2Δ::xrs2-834-13myc-kanMX6*
yYM329, 330	JHUY761 *TEL1/tel1Δ::kanMX6-PGAL1-2FLAG-tel1-D2612A/N2617A XRS2/xrs2Δ::xrs2-K846A/F847A-13myc-kanMX6*
yYM331	JHUY761 *TEL1/tel1Δ::HygroMX4 MEC1/mec1Δ::LEU2 SML1/sml1Δ::TRP1*

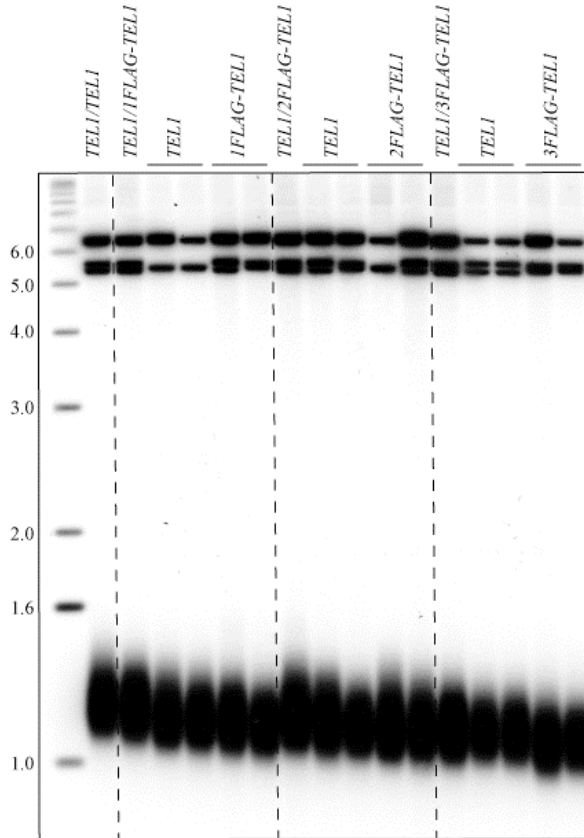|	
	RAD52/rad52Δ::kanMX4
yYM333, 334	JHUY761 *TEL1/tel1Δ::HygroMX4 XRS2/xrs2Δ::XRS2-13myc-kanMX6*
yYM335, 336	JHUY761 *TEL1/tel1Δ::2FLAG-TEL1 XRS2/xrs2Δ::kanMX4*

SUPPLEMENTAL FIGURES

Figure S1. The effect of N-terminal FLAG tagging of *TEL1* on native telomere length.

Southern blotting analysis of telomere length of haploid strains over-expressing 1, 2, or 3

copies of FLAG tagged *TEL1*. Wildtype (*TEL1/TEL1* JHUY761) and parental

heterozygous diploid strains *TEL1/1FLAG-TEL1* (yYM21), *TEL1/2FLAG-TEL1*

(yYM25), and *TEL1/3FLAG-TEL1* (yYM28) were included for comparison. The haploid

strains were independent strains derived from yYM21, 25, and 28. The blot was probed

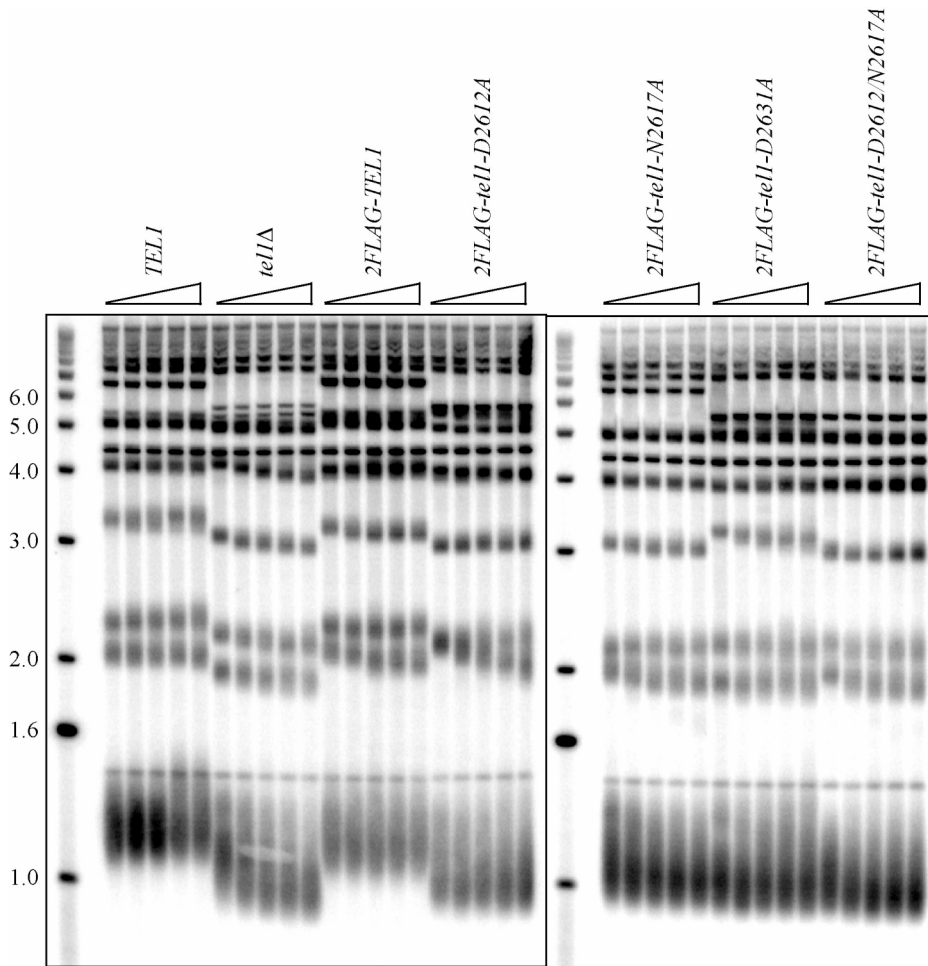with a subtelomeric Y' probe. Sizes of DNA markers (in kb) are indicated on the left.

Figure S2. Southern blotting analysis of telomere length of strains with *TEL1* and *tel1* alleles (parental strains are JHUY817, yYM242, 244, 246, 248, and 250). Cells were streaked on YPD plates from spore colonies every 48 hours. Genomic DNA was prepared from streaks # 1, 4, 7, 10, and 13 (symbolized by gradient triangles) and analyzed by Southern blotting. The blot was probed with a poly (dA-dC)•(dG-dT) probe. Sizes of DNA markers (in kb) are indicated on the left.
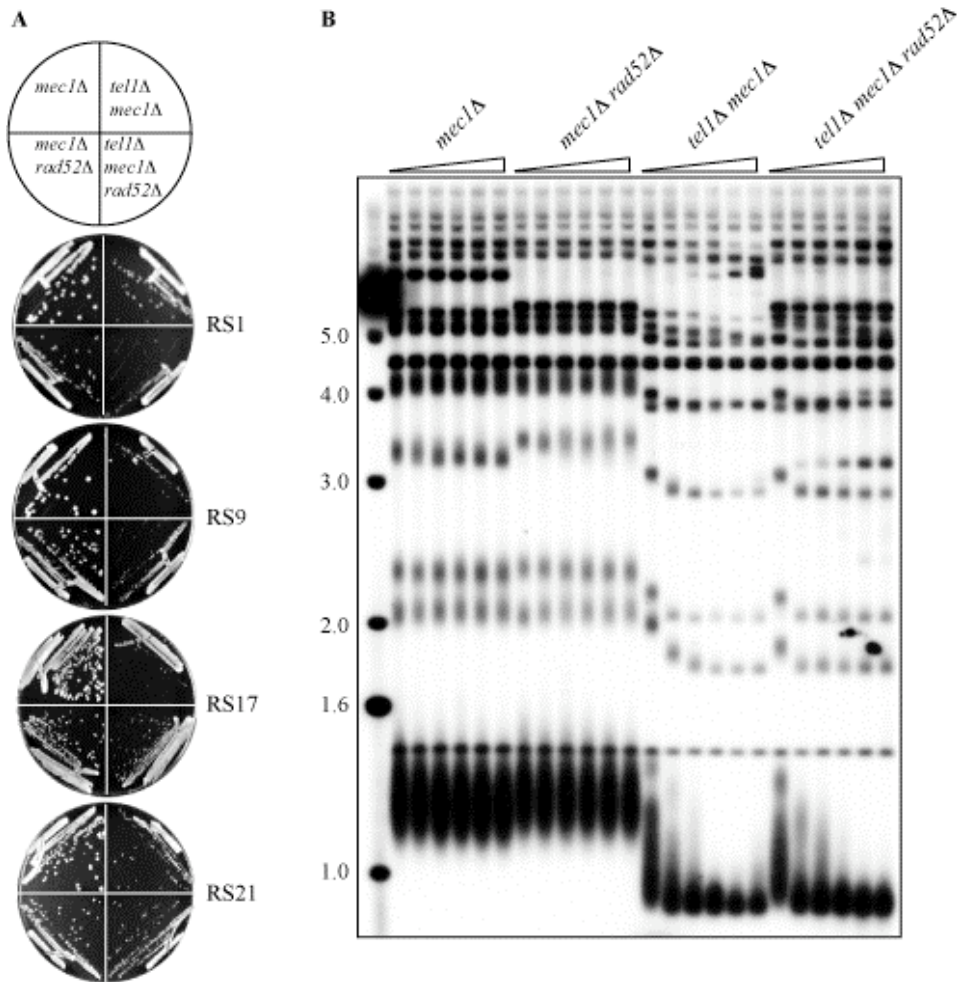
Figure S3. *tel1Δ mec1Δ* or *tel1Δ mec1Δ rad52Δ* strains do not seem to generate survivors. (A). Cells were streaked on YPD plates from spore colonies (dissected from yYM331) every 48 hours. Re-streak (RS) numbers are indicated. *SML1* is deleted (not labeled) from all the strains to rescue the *mec1Δ* lethality. (B). Genomic DNA was prepared from re-streaks # 1, 5, 9, 13, 17, and 21 (symbolized by gradient triangles) and analyzed by Southern blotting. The blot was probed with a poly (dA-dC)•(dG-dT) probe. Sizes of DNA markers (in kb) are indicated on the left.
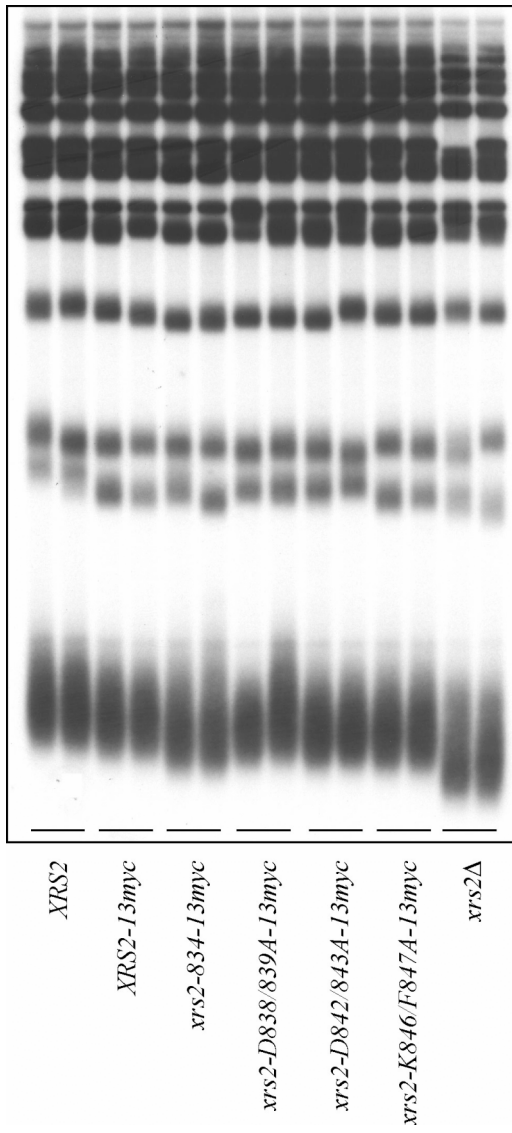
Figure S4. Southern blotting analysis of *xrs2* C-terminal mutants. Genomic DNA was prepared from untagged (*XRS2* and *xrs2Δ*) and tagged *XRS2* strains (derived from yYM259, 269, 271, 273, 274, and JHUY870). The blot was probed with a poly (dA-dC)•(dG-dT) probe.
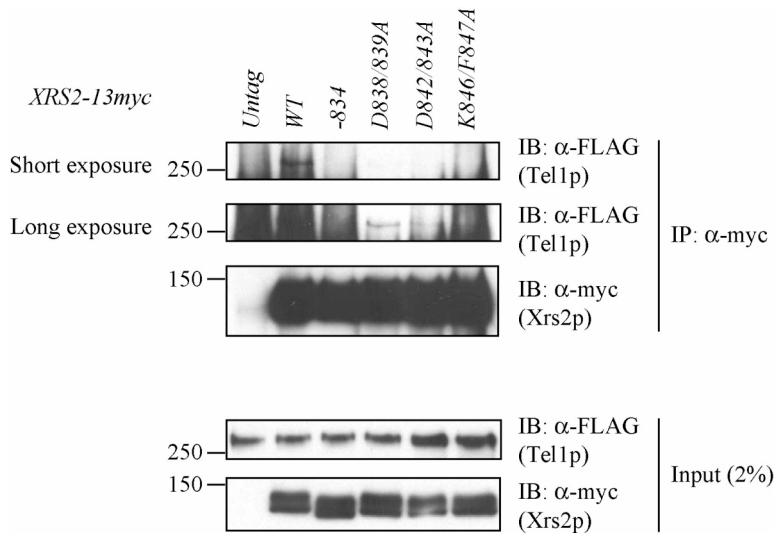
Figure S5. Xrs2p interacts with Tel1p via the C-terminal 20 amino acids of Xrs2p and residues K846 and F847 of Xrs2p are required. Strains expressing the indicated wildtype or mutant Xrs2p-13myc and 2FLAG-Tel1p (derived from yYM260, 269, 271, 273, and 274) were immunoprecipitated with an α-myc antibody. 2% of the input material and the immunoprecipitate were examined by western blotting for Tel1p (α-FLAG) and Xrs2p (α-myc). For the detection of Tel1p in the immunoprecipitate, the short and long exposures of the same blot are shown.