Supplemental Information for

Active yeast telomerase shares components with catalytic ribonucleoproteins RNase P and RNase MRP

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Supplemental Materials and Methods:

Strains and Plasmids

Yeast strain W3749, a RAD5-derivative of W303-1A (Lisby et al., 2004), was used for most experiments (see Supplemental Table 1 for detailed genotypes of all strains). For telomerase purification and mass spectroscopy analysis, we disrupted *EST1* by replacing the genomic locus by the *KanMX* cassette using a one-step polymerase chain reaction (PCR)-mediated method (Brachmann et al., 1998) in diploid EBY081. The pVL1355 plasmid expressing the Est1-Myc_{18} protein (Evans and Lundblad, 2002) was then transformed into this strain to generate diploid BLY005. BLY005 was sporulated and haploids BLY006 and BLY008 were confirmed to carry *TLC1-10XMS2* and *TLC1wt* respectively with pVL1355 as the sole source of the Est1 protein. Strain EBYH03 containing plasmid pAZ1 was used as the base strain for all *TLC1* variants tested using the pTLC1TRP plasmid (Bah et al., 2004); see Supplemental Table 2 for all plasmids used). For the *NME1* alleles used in viability assays, strain BLY103 was generated by replacing the *NME1* genomic locus by the NatMX cassette in diploid W3749 and was then transformed with the pNME1-URA plasmid. BLY103 was set to sporulate and spore BLY106 was selected and confirmed to harbor the *nme1::NatMX* allele and carry pNME1-URA.

For northern blot analyses after co-immunoprecipitation or telomerase activity assays with the indicated tagged proteins, strains NLYH90, NLYH100 and NLYH200 were used as controls for *TLC1-wt* untagged, *TLC1-wt ProA-EST2* and *tlc1Δ* respectively. Disruption of *TLC1* in NLYH200 was achieved by replacing the genomic locus by *NatMX* marker using standard one-step PCR-mediated gene disruption (Goldstein and McCusker, 1999). Deletion of *TLC1* in YVL3493 was carried out as above to generate strain NLYH494. Tagging of *SME1* and *POP6* with the HA3 epitope in NLYH494 and YVL3493 respectively was achieved by a PCR-based gene targeting using the pFA6a-3HA-KMX plasmid and this generated strain NLYH495 and NLYH496 (Bahler et al., 1998). The strains "WPS" and SXY2 were a kind gift from the Engelke lab (Ghaemmaghami et al., 2003; Xiao et al., 2006). YSW1 was generously provided by the Schmitt lab. In order to study Tlc1 RNA binding to Pop6 and Pop7 proteins, WPS06 and WPS07 were crossed with NLYH068, diploids dissected and haploids obtained. To obtain NLYH600 and NLYH700, deletion of *TRP1* by *NatMX* was done using standard gene replacement strategy. In order to generate NLYH601 and NLYH701, the *SME1* gene was tagged by PCR-based targeting using pFA6a-13Myc-KMX. In order to maintain telomerase activity in these strains, they were transformed with the pAZ1 plasmid. In order to study the different *TLC1* versions, a plasmid shuffle was performed by replacing pAZ1 with the various p*TLC1* variants. Construction of the pTLC1-10xMS2-*Δ*S and pTLC1-10xMS2-*ΔL* was performed by ligating the HpaI-fragment from pTLC1-*Δ*SL (*Δ*S) and pTLC1-*Δ*SL+IL (*ΔL*) to the Hpa1-digested pTLC1-10xMS2 plasmid. In order to study Pop6 and Pop7 binding to the different *TLC1* variants, these plasmids were transformed into NLYH602 and NLYH702 and pAZ1 was then removed by streaking onto FOA-Trp plates. The latter two strains were derived from dissections of NLYD686 and NLYD687 respectively.

Native protein extracts and immunoprecipitations (IPs) for Mass Spectrometry

Native protein extracts were prepared from strains BLY006 and BLY008 from 1L of cells grown to a density of $1x10^7$ cells/mL in YC-Trp supplemented with 2% raffinose. MS2-ProA was induced by addition of 0.05% galactose to the culture for 45 minutes. Cells were pelleted, washed once with cold water, twice with TMG buffer (10 mM Tris–Cl pH 8.0, 1 mM MgCl2, 10% glycerol), and then resuspended 1:1 (v/v) in TMG-Lysis buffer (TMG supplemented with 200 mM NaCl, 0.2% Triton X-100, 0.2% NP40, 100 mM DTT) supplied with 1:1000 RNasin (Promega) and a cocktail of protease inhibitors (Complete Mini, Roche). Droplets of the suspension were frozen in liquid nitrogen and the resulting "pop-corn" ground in a Freezer/Mill 6850 (SPEX CertiPrep) in liquid nitrogen. The powder was thawed on ice, cleared by centrifugation (16,000xg, 15 min, 4°C), and protein concentrations in the supernatants were determined by Bradford reaction before storage at -80°C for subsequent experiments.

For mass spectrometry-IP experiments, 50-80 mg of protein extracts were diluted in 8 mL of TMG lysis buffer and supplemented with rabbit IgG (Sigma) coated magnetic beads (Dynabeads antibody coupling kit, Life technologies) at 2.4 mg of beads/mL and incubated for 60 minutes at 4°C. Beads were washed four times in TMG-lysis buffer, three times in TMG-Wash buffer (TMG-Lysis buffer without Triton X-100 and NP40) and four times in 20 mM NH_4HCO_3. At end, NH_4HCO_3 buffer was removed and beads were stored at -80°C until mass spectrometry. Half of the IPs were sent to MS facility and a fraction of the IPs were put aside for subsequent telomerase activity assays.

One volume of 2x loading buffer was added to input, IP, and FT, and all samples were boiled for 5 min prior to snap-freezing to -20ºC for subsequent experiments. Samples from 3 independent mass spectrometry-IPs were subjected to trypsin digestion directly on beads and the resulting peptides were separated using a Dionex Ultimate 3000 nanoHPLC system as described in (Drissi et al., 2015). Raw data issued from the MS were processed, searched and quantified using the maxQuant software package version 1.5.1.2 (Cox and Mann, 2008) employing an yeast W303_ALAV00000000 database (12/7/2012). The settings used for the MaxQuant analysis were as described (Drissi et al., 2015). Specific proteins are those that are present in at least 2 mass spectrometry-IPs and identified exclusively in the TLC1-MS2 sample. They were then sorted by sum of peak intensity. The mass spectrometry proteomics data have been deposited to the ProteomeXchange

Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository (Vizcaino et al., 2013) with the dataset identifier PXD003273.

Protein extracts for co-immunoprecipitations

Total protein extracts were prepared as described in (Laterreur et al., 2013). Briefly, 500 mL of cells grown to an OD_{660} of 1.0 were pelleted, washed once with cold water and once with TMG buffer (10 mM Tris-HCl pH 8.0, 1 mM $MgCl_2$, 10% glycerol) supplemented with 200 mM NaCl. The cell pellets were then frozen in liquid nitrogen and lysis was performed by grinding the pellets in the presence of pieces of dry ice in a standard coffee mill (Krups). The cell powder was thawed on ice and 1 pellet volume of TMG (200 mM NaCl, 0.1 mM DTT, 0.2% Triton X-100, 0.2% NP40 and protease inhibitors) was added. Immunoprecipitations were done on 3 mg of total proteins adjusted to 0.5% Tween-20 and to which 40 units of RNasin (Promega, USA) was added per mL of extract. To study TLC1 binding to the Tap-tagged Pop proteins, the adjusted extracts were supplemented with 50 µL rabbit IgG (Sigma) coated magnetic beads (Dynabeads antibody coupling kit, Life technologies). Following an incubation of 3-4 hours at 4°C, the beads were washed twice with 0.5 mL TMG2 (200 mM NaCl, 0.1 mM DTT, Complete mini EDTA free protease inhibitor (Roche) and 0.5 % Tween-20) and twice with 0.5 mL TMG1 (0.1 mM DTT, protease inhibitor and RNasin added at 40U per 0.5 mL of TMG). The washed beads were resuspended in 50 µL TMG3 (0.5 mM DTT, protease inhibitor and 40U of RNasin). For the analysis of telomerase composition in TLC1 variants and in HA-tagged protein extracts of YVL3493, NLYH495 and NLYH496, the extracts were prepared as mentioned above but were supplemented with 50 µL anti-HA conjugated magnetic beads (Life Technologies). The beads were washed and resuspended as described. The immunoprecipitations for the study of Pop6 and Pop7 binding to TLC1 variants were achieved by incubating 3 mg of adjusted extracts with 500 ng of anti-Myc antibody (mouse monoclonal, clone 9E10, Roche Diagnostics, USA), 50 µL of TMG-washed SureBeads™ Protein G Magnetic Beads (BioRad) were added and everything was incubated overnight at 4°C with gentle agitation. Beads were then washed and resuspended in TMG3.

RNA-IP and northern blot analyses

Following immunoprecipitation of total protein extracts, TMG3 was removed from the beads and 400 µL of LETS buffer (10 mM Tris-HCl pH 7.5, 100 mM LiCl, 10 mM EDTA pH 8.0, 0.2% SDS) were added to beads, input and flow-through fractions. For total RNA extractions, 10 mL of yeast cells from TLC1-variants strains were grown to OD 0.8, collected, washed, resuspended in 400 µL LETS Buffer and lysed by glassbeads. Samples (RNA-IP and total RNA extracts) were then extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1) and once with chloroform/isoamyl alcohol (24:1). After the addition of NaOAc (final conc. 150 mM) and glycogen (50 µg), the RNA was precipitated with 2 volumes of cold 100% ethanol. Samples were then washed with 70% ethanol, dried and resuspended in 20 µL of nuclease-free water. To analyse RNAs, either 10 µg of total RNA extracts or 5 µL of input, IP and FT were mixed with 1X MOPS (pH 7), 3.7% formaldehyde, 45% formamide and 1X RNA dye. After heating the samples 10 minutes at 60°C, RNAs were separated in a 1.2% agarose-1X MOPS (pH 7)-2% formaldehyde gel by electrophoresis. The gel was then transferred to a Hybond N+ membrane (GE Healthcare). Following UV-crosslinking, the membrane was pre-hybridized and hybridized with a radiolabeled TLC1-specific probe of 694 bp (NcoI-NsiI digestion of pTLC1-TRP) and a 5'-end labeled NME1-specific oligonucleotide probe (5'-GCAATAGAGGTACCAGGTCAAGAAG-3'). Visualization and quantification were performed using a Typhoon FLA9000 apparatus and the Quantity One software.

Western blots

After the removal of TMG3 from IP beads, input and IP samples were mixed with 2X Laemmli loading buffer. The proteins were then denatured for 5 min at 98ºC, separated on 8% SDS-PAGE gels. For Pop6-TAP and Pop7-TAP binding analyses, the samples were run on 12% SDS-PAGE gels. For Est1-Myc_{12} and $FLAG_3$-Myc_{12}- Est2 co-IP with HA-tagged Sme1 and Pop6-HA_3, inputs and IP samples were mixed with 2X Laemmli loading buffer, heated and separated on 10% SDS-PAGE gels. After transfer onto Hybond-ECL nitrocellulose membranes (GE Healthcare), membranes were blocked in 5% milk/PBS-Tween and incubated with a 1:10,000 dilution of mouse monoclonal anti-ProA (Sigma), or with a 1:5,000 rabbit polyclonal anti-ProA (Sigma), or a 1:1,000 dilution of rabbit anti-Myc (Cell Signalling) antibodies. Secondary antibodies were HRP-conjugated sheep anti-mouse IgG and donkey anti-rabbit IgG (GE Healthcare), diluted 1:5,000 in 1% milk/PBS-T. Blots were visualized with a LAS-4000 (GE Healthcare).

Cold sensitivity and viability assays

Strain EBYH03 transformed with plasmids used in the cold sensitivity assay were streaked first on 5-FOA plates to select cells that had lost pAZ1. Cells were then streaked on YC-Trp plates and placed at 18°C or 30°C for 5 and 2 days respectively between each streaking (~20 generations/streaking). DNAs from 5 mL liquid cultures were then extracted and subjected to telomere length analysis. For the viability assays with hybrid Nme1 RNAs, strain BLY106 containing pNME1-URA was used as the base strain for all *NME1* variants tested on the pNME1-TRP plasmid. Transformed clones were then streaked on 5-FOA and YC-Ura-Trp plates and allowed to grow at 30°C for 2 days.

***In vitro* telomerase reconstitution and assay conditions**

Telomerase reconstitution assays were performed as reported (Zappulla et al., 2005) with minor modifications. 50 nM of PCR products derived from pKG206 (mini-T) or pTLC1-TRP were combined with 1 µg T7-ProA-Est2 plasmid (Art2-11, both pKG206 and Art2-11 kindly provided by D. Zappulla) and both were added to a 50 µL RRL transcription and translation reaction mix (TNT Quick Coupled, Promega). Reactions were allowed to proceed according to manufacturer's instructions for linearized DNA. When specified, RRL reactions were performed with the addition of 1 µM of recombinant proteins Pop6/Pop7 and/or Pop1. For the assay described in figure 7A, various amount of Pop1 were added; from 0.11µM to 1.0µM. The ProA-Est2/Tlc1 RNP complex was then immunopurified using 0.45 mg of rabbit IgG (Sigma) coated magnetic beads (Dynabeads antibody coupling kit, Life technologies) per RRL reaction for 2 hours at 4°C. Beads were washed four times in 1 mL of TMG buffer and resuspended in 15µL of TMG-30 (TMG at 30% glycerol). To assess protein production and immunopurification, 2.5 µL of beads were boiled in 1x Laemmli loading buffer and separated on 8% SDS-PAGE gels followed by western blot analysis.

Yeast telomerase activity assays were carried out as previously described (Friedman and Cech, 1999; Laterreur et al., 2013) with 10% (in case of native cell extracts), or 30% of the IP-beads from the reconstitution mix above. Briefly, the extension of a telomeric primer (5'-TAGGGTAGTAGTAGGG-3') in the presence of radiolabeled [α-^{32}P] dGTP was monitored to determine telomerase activity. Extension products were separated on 18% polyacrylamide/8M urea electrophoresis gels and visualized using a Typhoon FLA9000 apparatus (GE Healthcare). As internal controls, 12-nt (5'-TTAGGGTTAGGG-3') and 16-nt (5'-TAGGGTAGTAGTAGGG-3') primers were 5'-end labeled and added to the samples before ethanol precipitation at 2000 cpm/µL. Quantification was performed using the Quantity One program (Bio Rad) by adding telomerase extension products signals and dividing them by the loading control signals to give Relative Telomerase Activity (RTA) ratios with respect to wildtype conditions.

Stem IVc RNAs and recombinant Pop1/Pop6/Pop7 proteins

RNA constructs IVc wt, IVc SUB, IVc SA3, IVc ΔCS2, and TeSS were produced by run-off transcription with T7 RNA polymerase using DNA templates based on synthetic oligonucleotides (Milligan et al., 1987). The sequences of the template oligonucleotides were as follows (see also Figure S5A for predicted secondary structures):

For IVc wt: 5'-
GGAGTTAACGATAAGATAGACATAAAGTGACAGCGCTTAGCACCGTCTGTTTGCAAATCTAACTTAAACTCCT
ATAGTGAGTCGTATTA -3' ;
For IVc SUB: 5'-
GGAGTTAACGATAAGATAGACATAAAGTGACAGCGCTTAGCACCGTCTGCCCATGGATCTAACTTAAACTCC
TATAGTGAGTCGTATTA -3' ;
For IVc SA3: 5'-
GGAGTTAACGATAAGATAGACATAAACAGACAGCGCTTAGCACCGTCTGTTTGCAAATCTAACTTAAACTCCT
ATAGTGAGTCGTATTA -3' ;
For IVc ΔCS2: 5'-
GGAGTTTAAGATAGACATAAAGTGACAGCGCTTAGCACCGTCTGTTTGCAAATCTAACTTAAACTCCTATAGT
GAGTCGTATTA -3' ;
For TeSS: 5'-
GGTAGACATAAAGTGACAGCGCTTAGCACCGTCTGTTTGCAAATCTACCTATAGTGAGTCGTATTA -3' .

To produce templates for run-off transcription, corresponding template oligonucleotides were annealed in the presence of 10 mM NaCl with an equimolar amount of oligonucleotide T7GG (5'-TAATACGACTCACTATAGG-3') by incubation at 85°C for 2 min followed by incubation on ice for 10 min. The resulting partially double-stranded DNA templates were immediately used in *in vitro* transcription following the standard protocol (Milligan et al., 1987). The product of transcription was purified using 15% denaturing (8M urea) polyacrylamide gels. The proteins were expressed in *E.coli* and purified as previously described (Perederina et al., 2007)(Fagerlund et al., 2015). These proteins had no purification tags.

Gel mobility shift assays and estimation of dissociation constants

Prior to forming complexes with proteins, RNA was refolded by incubation at 85°C for 2 min in 20 mM Tris-HCl (pH 7.5), cooling to room temperature in a styrofoam rack, followed by incubation at 50°C for 10 min in the presence of 5 mM $MgCl_2$, and subsequent cooling to room temperature in a styrofoam rack, followed by the addition of KCl to 50 mM.

For the estimation of the binding constants, 0.5 µg of RNA was 5'-end labeled with ^{32}P, gel-purified, and mixed with 4 µg of cold RNA. Complexes with proteins were formed by mixing the refolded RNA of interest and proteins in a binding buffer (BB) containing 20 mM Tris-HCl (pH 7.5), 50 mM KCl, and 5 mM $MgCl_2$, supplemented with 100 ng/µL of biotinylated BSA and 10 ng/µL of tRNA, followed by incubation at 30°C for 30 min. Mixtures were at a 1:1 molar ratio

(protein:RNA) and RNA concentration varied from 10 nM to 10 µM. Radioactive RNA bands were quantified using PhosphorImager (Molecular Dynamics). To avoid signal saturation, each gel was exposed for 5 min, 1 hr, and 24 hrs. All experiments were repeated at least twice. The ratios of the intensities of the bands corresponding to RNA-protein complexes to the intensities of the bands corresponding to free RNA were averaged, plotted, and interpolated to the 50% binding point. Given the 1:1 stoichiometry of the RNA-protein complexes, and that equimolar amounts of RNA and protein were taken, the apparent dissociation constant was estimated as ½ of the concentration of RNA that had to be initially added to the reaction to result in a 1:1 [free RNA]:[RNA-protein complex] ratio. Indeed, if $[RNA_i]$ is the initial (pre-complex) concentration of RNA, $[Protein_i]$ is the initial (pre-complex) concentration of protein, and [RNA], [Protein], [Complex] are concentrations of free RNA, free protein, and the RNA-protein complex, respectively, then the dissociation constant K_d= [RNA][Protein]/[Complex]. When RNA and protein are added at a 1:1 molar ratio, and form a complex with a 1:1 stoichiometry, $[RNA_i]$= $[Protein_i]$= [RNA]+[Complex]= [Protein]+[Complex]. In an experiment where 50% of RNA forms a complex with protein, we have: [RNA]=[Complex], thus [RNA]=[Protein]=1/2$[RNA_i]$ and K_d= ½$[RNA_i]$. The 1:1 RNA:protein molar ratio throughout the measurement range was chosen to avoid protein precipitation that is observed when Pop6/Pop7 is taken in excess .

Supplemental Table S1: Yeast strains used, related to Figures 1 to 7.

Name	Genotype	Reference
NLYH90	*MatA ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 DIA5 (VR-ADE2-T)*	This study
NLYH100	*MatA ura3-52 leu2 trp1 ProA-EST2*	This study
NLYH200	*MatA ura3-52 leu2 trp1 tlc1Δ::NatMX ProA-EST2*	This study
SXY2	*MAT A ade2-1 his3-11,15, leu2-3,112 trp1-1 ura3-1 can1-100 pop1ΔNAT1 RPR1:Kan^R p413-HA$_3$-POP1 p315-S1-RPR1*	(Xiao et al., 2006)
WPS01	*MatA his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 POP1-TAP::HISMX6* (but note that TAP tag autocleaved off from protein)	(Ghaemmaghami et al., 2003)
WPS05[a]	*MatA his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 POP5-TAP::HISMX6*	(Ghaemmaghami et al., 2003)
WPS06[a]	*MatA his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 POP6-TAP::HISMX6*	(Ghaemmaghami et al., 2003)
WPS07[a]	*MatA his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 POP7-TAP::HISMX6*	(Ghaemmaghami et al., 2003)
WPS12[a]	*MatA his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 RMP1-TAP::HISMX6*	(Ghaemmaghami et al., 2003)
WPS13[a]	*MatA his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 RPP1-TAP::HISMX6*	(Ghaemmaghami et al., 2003)
YSW1	*MatA pep4:LEU2 nuc1::LEU2 sep1::URA3 trp-1 his3-11,15 can-100 ura3–1 leu2–3,112 POP4::TAP::TRP1ks*	(Gill et al., 2004)
NLYH068	RWY11 *ProA-EST2*	(Bajon et al., 2015)
NLYD686	WPS06 x NLYH068	This study
NLYD687	WPS07 x NLYH068	This study
NLYH600	*Matα ura3 tlc1Δ::LEU2 trp1Δ::NatMX POP6-TAP :HISMX6 ProA-EST2*	This study
NLYH601	NLYH600 *SME1-MYC$_{13}$ ::KMX*	This study
NLYH602	*Matα ura3 trp1 tlc1Δ::LEU2 POP6-TAP :HISMX6*	This study
NLYH700	*Matα ura3 tlc1Δ::LEU2 trp1Δ::NatMX POP7-TAP :HISMX6 ProA-EST2*	This study
NLYH701	NLYH700 *SME1-MYC$_{13}$ ::KMX*	This study
NLYH702	*MatA ura3 trp1 tlc1Δ::LEU2 POP7-TAP :HISMX6*	This study
YVL3493	*MatA leu2 trp1 ura3-52 prb$^-$ prc$^-$ pep4-3 EST1-Gly$_6$-MYC$_{12}$ FLAG$_3$-MYC$_{12}$- Gly$_6$-EST2*	(Lubin et al., 2012)
NLYH494	*MatA leu2 trp1 ura3-52 prb$^-$ prc$^-$ pep4-3 EST1-Gly$_6$-MYC$_{12}$ FLAG$_3$-MYC$_{12}$- Gly$_6$-EST2 tlc1Δ::NatMX*	This study
NLYH495	NLYH494 *SME1-HA$_3$:KMX*	This study
NLYH496	*MatA leu2 trp1 ura3-52 prb$^-$ prc$^-$ pep4-3 EST1-Gly$_6$-MYC$_{12}$ FLAG$_3$-MYC$_{12}$- Gly$_6$-EST2 POP6-HA$_3$:KMX*	This study
EBY081	*MatA*/α *TLC1/tlc1Δ::TLC1-10xMS2 his3-11/HIS3:GAL1p-PRA-MS2*	(Bajon et al., 2015)
BLY005	*Mat*A/α *TLC1/tlc1Δ::TLC1-10xMS2 his3-11/HIS3:GAL1p-PRA-MS2* est1Δ::KMX/EST1 pVL1355 (EST1-myc$_{18}$)	This study
BLY006	*Mat*A *tlc1Δ::TLC1-10xMS2 HIS3:GAL1p-PRA-MS2 est1Δ::KMX* pVL1355 (EST1-myc$_{18}$)	This study
BLY008	*Mat*A *his3-11/HIS3:GAL1p-PRA-MS2 est1Δ::KMX* pVL1355 (EST1-myc$_{18}$)	This study

W3749	MatA/α *can1-100/can1-100 ura3-1/ura3-1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 bar1Δ::LEU2/bar1Δ::LEU2*	(Lisby et al., 2004)
BLY103	W3749a/α + *NME1/nme1Δ::NatMX* + pNME1-URA	This study
BLY106	*Matα can1-100 ura3-1 his3-11,15 leu2-3,112 trp1-1 bar1Δ::LEU2 nme1Δ::NatMX* + pNME1-URA	This study
EBYH03	*Mat*A *can1-100 ura3-1 his3-11,15 leu2-3,112 trp1-1 bar1Δ::LEU2 tlc1Δ::NatMX* + pAZ1	This study

[a]: ATCC 201388 (BY4741)

Supplemental Table S2: Plasmids used, related to Figures 2 to 7.

Name	Description	Reference
pAZ1	*URA3, CEN, TLC1*	(Gravel and Wellinger, 2002)
pTLC1TRP	*TRP1, CEN, TLC1*	(Bah et al., 2004)
pTLC1-ΔSL (ΔS)	*TRP1, CEN, TLC1 Δ 615-630::GAATTC*	(Gunisova et al., 2009)
pTLC1-ΔSL+IL (ΔL)	*TRP1, CEN, TLC1 Δ 602-642::GAATTC*	(Gunisova et al., 2009)
pTLC1-SA3	*TRP1, CEN, TLC1 (A633T, C634G)*	(Laterreur et al., 2013)
pTLC1-SUB	*TRP1, CEN, TLC1Δ605-611 ::CCAUGGG*	(Gunisova et al., 2009)
pTLC1-10xMS2	*TRP1, CEN, TLC1-10xMS2*	(Gallardo et al., 2011)
pTLC1-10xMS2-ΔS	*TRP1, CEN, TLC1-10xMS2 Δ 615-630::GAATTC*	This study
pTLC1-10xMS2-ΔL	*TRP1, CEN, TLC1-10xMS2 Δ 602-642::GAATTC*	This study
pTLC1-P3NME1	*TRP1, CEN, TLC1Δ601-643::NME1(31-76)*	This study
pTLC1-P3RPR1	*TRP1, CEN, TLC1Δ601-643::RPR1(32-84)*	This study
pTLC1-P3NME1-11	*TRP1, CEN, TLC1Δ601-643::NME1(31-76Δ45-64)*	This study
pNME1-URA	*URA3, CEN, NME1*	This study
pNME1-TRP	*TRP1, CEN, NME1*	This study
pNME1-P3TLC1	*TRP1, CEN, NME1Δ31-76::TLC1(601-643)*	This study
pNME1-ΔP3	*TRP1, CEN, NME1Δ31-76*	This study
pKG206	*T7-Mini-T(500)*	(Zappulla et al., 2005)
Art2-11	*T7-ProA-Est2*	(Zappulla et al., 2005)
pVL1355	*TRP, CEN, EST1-myc18*	(Evans and Lundblad, 2002)
pFA6a-13Myc-KMX	13Myc-KanMX6 cassette	(Bahler et al., 1998)
pFA6a-3HA-KMX	3HA-KanMX6 cassette	(Bahler et al., 1998)

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