Supplementary Information to

Functionality of the telomerase depends on CPF-CF induced 3'end processing of its RNA component *TLC1* and a novel Nrd1-Nab3 surveillance mechanism

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Supplementary Table 1: Yeast strains

Number	Genotype	Source
HKY36	*ura3-52; leu2Δ1; his3Δ20α*	(1)
HKY82	*mtr10::HIS; ura3; leu2; trp; his3; ade2 α*	(2)
HKY314	*his3Δ1; leu2Δ0; met15Δ0; ura3Δ0 a*	Euroscarf
HKY1028	*rrp6::kanMX4; his3Δ1; leu2D0; lys2Δ0; ura3Δ0 α*	Euroscarf
HKY1112	*trf4::kanMX4 a*	Euroscarf
HKY1293	*tlc1::HIS; ura3-52; lys2-801; trp1-Δ1; his3-Δ200; leu2-Δ1 α*	(3)
HKY1492	*Nrd1-GFP:HISMX6; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0 a*	This study
HKY1776	*mtr10::kanMX4; lys; ura; leu; his a*	This study
HKY2278	*rna15-2; ade2-1; his3-11; leu2-3; trp1-1; ura3-1 a*	(4)
HKY2311	*TLC1:Terminator+N1P0123*:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2407	*TLC1:Terminator+WT:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2409	*TLC1:Terminator+P123*:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2410	*TLC1:Terminator+P0123*:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2411	*TLC1:Terminator+P10123*:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2413	*TLC1:Terminator:SNR13 I:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2414	*TLC1:Terminator:SNR13 II:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2415	*TLC1:Terminator:SNR13 I II:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2421	*TLC1:Terminator+P1012345*:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2445	*TLC1:Terminator+P(-101)AT2345*:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2452	*TLC1:Terminator+N1*:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2456	*rna15-2; TLC1:Terminator+P(-101)AT2345*:URA3; ade2-1; his3-11; leu2-3; trp1-1; ura3-1 a*	This study
HKY2491	*mtr10::kanMX4; TLC1:Terminator+WT:URA3; lys; ura; leu; his a*	This study
HKY2492	*mtr10::kanMX4; TLC1:Terminator+P0123*:URA3; lys; ura; leu; his a*	This study
HKY2493	*mtr10::kanMX4; TLC1:Terminator+N1P0123*:URA3; lys; ura; leu; his a*	This study
HKY2494	*mtr10::kanMX4, TLC1:Terminator+P-1012345*:URA3; lys; ura; leu; his a*	This study
HKY2496	*TLC1:Terminator:N1P-1012345*:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2506	*TLC1:Terminator:N1P0123*:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2508	*TLC1:Terminator:Act1:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2509	*TLC1:Terminator:Npl3:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2557	*TLC1:Terminator:N1P(-101)AT2345*:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study

Number	Genotype	Source
HKY2597	*TLC1:T8Δ:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2609	*TLC1: T86Δ:URA3; ura3-52; leu2Δ1; his3Δ20 α*	This study
HKY2690	*TLC1:Terminator+N1*:URA3 Nrd1-GFP:HISMX6; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0 a*	This study
HKY2705	*TLC1:Terminator+N01*:URA3 Nrd1-GFP:HISMX6; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0 a*	This study

Supplementary Table 2: Plasmids

Number	Genotype	Source
pHK87	*LEU2; CEN; AMP^R*	(5)
pHK88	*URA3; CEN; AMP^R*	(5)
pHK883	*P_{adh1}-GFP-GFP; URA3; CEN*	This study
pHK1469	*SmB-GFP; CEN; URA3*	(6)
pHK1700	*TLC1; CEN, URA3*	(3)
pHK1725	*24xMSL; loxP URA3*	This study
pHK1767	*TLC1:Ter:N12*; CEN, URA3*	This study
pHK1784	*TLC1:Ter:N1P0123*; CEN, URA3*	This study
pHK1785	*TLC1:Ter:P0123*; CEN, URA3*	This study
pHK1827	*TLC1:Ter:N1P-10123*; CEN, URA3*	This study
pHK1828	*TLC1:Ter:P-10123*; CEN, URA3*	This study
pHK1840	*TLC1:Ter:N1P-10P123*; loxP URA3*	This study
pHK1844	*TLC1:Ter:TLC1; loxP URA3*	This study
pHK1845	*TLC1:Ter:P123*; loxP URA3*	This study
pHK1846	*TLC1:Ter:P0123*; loxP URA3*	This study
pHK1849	*TLC1:Ter:P-10123*; loxP URA3*	This study
pHK1855	*SNR13I+I1$_{ter}$; loxP URA3*	This study
pHK1856	*SNR13I$_{ter}$; loxP URA3*	This study
pHK1863	*SmB-GFP; CEN; LEU2*	This study
pHK1866	*TLC1:Ter:P-1012345*; loxP URA3*	This study
pHK1885	*TLC1:Ter:P(-101)AT2345*; loxP URA3*	This study
pHK1888	*TLC1:Ter:N1*; loxP URA3*	This study
pHK1900	*TLC1:Ter:N1P(-101)AT2345*; loxP URA3*	This study
pHK1910	*Npl3$_{ter}$; loxP URA3*	This study
pHK1937	*TLC1:Ter:N01*; loxP URA3*	This study
pHK1911	*Act1$_{ter}$; loxP URA3*	This study
pHK1980	*P_{adh1}-nrd1-GFP(-autoregulation); URA3; CEN*	This study
pHK1986	*P_{adh1}-NAB3-GFP; URA3; CEN*	This study
pHK2011	*P_{adh1}-nrd1-GFP(-autoregulation); LEU2; CEN*	This study

Supplementary Table 3: Primers for cloning

Construct	Number	Sequence	Name
Mutagenic PCR	HK3667	5'-ACTTGTGCATCGCTTTCCAAGCGCTTTTGATTGATTGTTCATGACGAGGA-3'	*TLC1 P1** forward

Construct	Number	Sequence	Name
	HK3668	5'-GAACAATCAATCAAAAGCGCTTGGAAAGCGATGCACAAGTACAGTACGCGCGAT-3'	*TLC1 P1** reverse
Mutagenic PCR	HK3669	5'-CATTTTTTTTCCTGATGTATATTTTTTGTATTCTAGAAATCGCGCGTACTG -3'	*TLC1 N1** forward
	HK3670	5'-GAAGGGGGAGTAAAAATAAGTATACCGAAGCTT-3'	*TLC1 N1** reverse
pHK2407	HK4167	5'-CAATTTCACACAGGAAACAGCTATGACCATGATTACGCCACTAGAGAGGAAGATAGGTACCCTATG-3'	*TLC1* forward GA
	HK4168	5'-ctcagATAACTTCGTATAGCATACATTATACGAAGTTATGTAAATATTAAGAGGCATACCTCCGCC-3'	*TLC1* reverse GA
Genomic integration	HK4169	5'-CTAGAGAGGAAGATAGGTACCCTATG-3'	*TLC1* forward integration
	HK4170	5'-TATATTCTAAAAAGAAGAAGCCATTTGGTGGGCTTTATTAGTAAAACGACGGCCAGTGAATTC -3'	*TLC1* reverse integration
Mutagenic PCR	HK4320	5'-GGACAGGCGACAGGCGGAGGTATGCCTCTTAATA-3'	*TLC1 P2** forward
	HK4322	5'-CTCCGCCTGTCCGCCTGTCCTCGTCATGAACAATC -3'	*TLC1 P2** reverse
Mutagenic PCR	HK4323	5'-AGGCGGAGGTATGCCTCTTAACATTTACAAGCAAGCCCACCAAATGGCTT -3'	*TLC1 P3** forward
	HK4324	5'-ATTTGGTGGGCTTGCTTGTAAATGTTAAGAGGCATACCTCCGCCTATCCG -3'	*TLC1 P3** reverse
	HK4327	5'-AACGATCAATTAAAAGCGCTTATAAAGCGATATAGAAGTCGAGTACGCGC-3'	*TLC1 N2** on *N1** reverse
Mutagenic PCR	HK4352	5'-CGATCAATTAAAAGCGCTTATAAAGCGATATAGAAGTCGAGTACGCGCGATTT-3'	*TLC1 N2** on *N1** forward
Mutagenic PCR	HK4403	5'-ATGTCTATTTTTTGTATTGTAGAGATCGCGCGTAC-3'	*TLC1 P0** forward
	HK4404	5'-CGATCTCTACAATACAAAAAATAGACATCAAGAAAAAAAATG-3'	*TLC1 P0** reverse
Mutagenic PCR	HK4410	5'-ATGTCTATTTTTTGTATTCTAGAGATCGCGCGTAC-3'	*TLC1 P0** into *N1** forward
	HK4411	5'-CGATCTCTAGAATACAAAAAATAGACATCAGGAAAAAAAATG-3'	*TLC1 P0** into *N1** reverse
Mutagenic PCR	HK4605	5'-ATGCATTTAGCCAATTTTTGGAAACATTTTTTTTC -3'	*TLC1 P-1** forward

Construct	Number	Sequence	Name
	HK4606	5'-CAAAAATTGGCTAAATGCATCGAAGGCATTAGGAG -3'	*TLC1 P-1** reverse
Mutagenic PCR	HK4607	5'-TTCTTTTTAGAAGCTACAGCGTACAAATAAAAATAAAAAAATA-3'	*TLC1 P4** forward
	HK4608	5'-TTTTATTTGTACGCTGTAGCTTCTAAAAAGAAGAAGCCATTTGGTGG-3'	*TLC1 P4** reverse
pHK1855/56	HK4676	5'-CAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAGACCTTTTAACTTCCCCGTAG-3'	*SNR13* terminator forward GA
pHK1855/	HK4677	5'-ctcagATAACTTCGTATAGCATACATTATACGAAGTTATGCCCAACGTACTAACATCTTT-3'	*SNR13* terminator II reverse GA
pHK1856	HK4678	5'-ctcagATAACTTCGTATAGCATACATTATACGAAGTTATGTACGATACAATGTAAGAAGGATT-3'	*SNR13* terminator I reverse GA
Mutagenic PCR	HK4679	5'-AGCGTACAAAAGAAGAACATCCTAGTAATTGTC-3'	*TLC1 P4Adel** forward
	HK4680	5'-ATGTTCTTCTTTTGTACGCTGTAGCTTCTAAAAAG-3'	*TLC1 P4Adel** reverse
Mutagenic PCR	HK4681	5'-TGAAAGCAATCAAGACATTGATTGGGATTTTTTATTTAG-3'	*TLC1 P5** forward
	HK4682	5'-CAATGTCTTGATTGCTTTCAAGACAATTACTAGGATG-3'	*TLC1 P5** reverse
Mutagenic PCR	HK4738	5'-AACGCTTTCCAAGCGCTTTTGACTGATTGTTCATGACGAG-3'	*TLC1 P1AT** forward
	HK4739	5'-GTCAAAAGCGCTTGGAAAGCGTTGCACAAGTACAGTAC-3'	*TLC1 P1AT** reverse
Mutagenic PCR	HK4740	5'-AAGTCTGCTTTTTGTGCTGTAGAGAACGCGCGTACTGTACTTG-3'	*TLC1 P0AT** forward
	HK4741	5'-GTTCTCTACAGCACAAAAAGCAGACTTCAAGAAAAAAAATG-3'	*TLC1 P0AT** reverse
Mutagenic PCR	HK4742	5'-GTGCACTCAGCCAATTTTTGGAAACGTTTTTTTTCTTGATG-3'	*TLC1 P-1AT** forward
	HK4743	5'-GTGCACTCAGCCAATTTTTGGAAACGTTTTTTTTCTTGAAG-3'	*TLC1 P-1AT** reverse
Genomic integration	HK4749	5'-GCCTTCGATGCATTTAGATAATTTTTGGAAACATTTTTTTGACCTTTTAACTTCCCCGTAG -3'	*SNR13* terminator I integration *TLC1* forward

Construct	Number	Sequence	Name
Genomic integration	HK4750	5'-GCCTTCGATGCATTTAGCCAATTTTTGGAAACATTTTTTTGACCTTTTAACTTCCCCGTAG-3'	*SNR13* terminator II integration *TLC1* forward
Mutagenic PCR	HK4838	5'-GTCAGTGAAGATGCCGGTGTGTGCGCAATTTGTGG-3'	*TLC1 N0** first part forward
	HK4839	5'-ACACCGGCATCTTCACTGACACCAGCATACTCGAAATTC-3'	*TLC1 N0** first part revere
pHK1911	HK5015	5'-CAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAATCTCTGCTTTTGTGCG-3' Act1	*Act1* terminator forward GA
	HK5016	5'-ctcagATAACTTCGTATAGCATACATTATACGAAGTTATGGCAATTGCATAAACCTATAAAACTG-3'	*Act1* terminator reverse GA
Genomic integration	HK5017	5'-GCCTTCGATGCATTTAGATAATTTTTGGAAACATTTTTTTAATCTCTGCTTTTGTGCGCG-3'	*Act1* terminator integration to *TLC1* forward
pHK1910	HK5018	5'-CAATTTCACACAGGAAACAGCTATGACCATGATTACGCCACAGGTAAGCCATTTATATAGTTGAG-3'	*Npl3* terminator forward GA
	HK5019	5'-ctcagATAACTTCGTATAGCATACATTATACGAAGTTATGCAAGTTTCCACTGAGTTATCTCAC-3'	*Npl3* terminator reverse GA
Genomic integration	HK5020	5'-GCCTTCGATGCATTTAGATAATTTTTGGAAACATTTTTTTCAGGTAAGCCATTTATATAGTTGAG-3'	*Npl3* terminator integration to *TLC1* forward
Mutagenic PCR	HK5128	5'-TTTGGAAACACTTGATGTATATTTTTTGTATTGTAG-3'	*TLC1 T8 deletion* forward
	HK5129	5'-ATACATCAAGTGTTTCCAAAAATTATCTAAATGCATC-3'	*TLC1 T8 deletion* reverse
Mutagenic PCR	HK5257	5'-GCTGCTCCTTTCCTTTCCAAAGAATTTCGAGTATG-3'	*TLC1 N0** second part forward
	HK5258	5'-TTGGAAAGGAAAGGAGCAGCAGGCTATCAACTGAAAG-3'	*TLC1 N0** second part reverse
Mutagenic PCR	HK5259	5'-TCGGATTGATCCTTCAGTTGATAGCCTGCTGCTC-3'	*TLC1 N0** third part forward
	HK5260	5'-CAACTGAAGGATCAATCCGAAATCCGACACTATCTC-3'	*TLC1 N0** third part reverse
Mutagenic PCR	HK5330	5'-CTTGATGTATAGTATTGTAGAAATCGCGCGTACTGTAC-3'	*TLC1* T6 deletion on T8 forward

Construct	Number	Sequence	Name
	HK5331	5'-GATTTCTACAATACTATACATCAAGTGTTTCCAAAAATTATC-3'	*TLC1* T6 deletion on T8 reverse
Mutagenic PCR	HK5520	5'-CAAAATTTCGTGGCTACCTTGGAATCATTCAAAGATTTG-3'	*NRD1* autoregulation site mutation forward
		5'-CAAGGTAGCCACGAAATTTTGAAAATCGTCGTCCTG-3'	*NRD1* autoregulation site mutation reverse

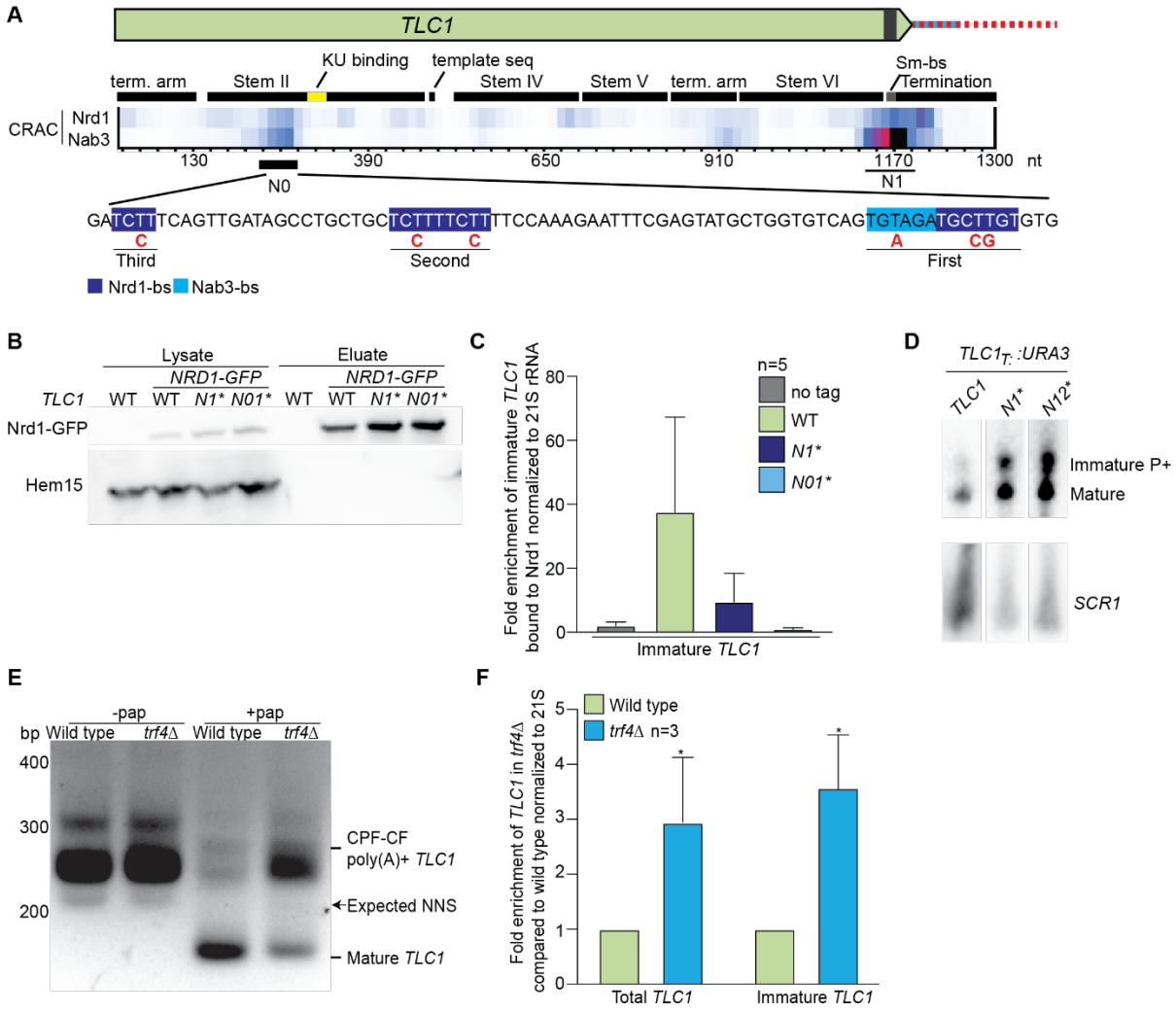
Supplementary Table 4: Primers for qPCR

Number	Sequence	Name
HK1384	5'-GCGGAAGGAACCGTGTGTTC-3'	Immature *TLC1* forward
HK1385	5'-GAAGCCTACCATCACCACACC-3'	Total *TLC1* forward
HK1386	5'-ACAGCGCTTAGCACCGTCTG-3'	Total *TLC1* reverse
HK3089	5'-AGTTACGCTAGGGATAACAGGG-3'	*21S* forward
HK3513	5'-ACGCGCGATTTCTACAATAC-3'	Immature *TLC1* reverse
HK3670	5'-TGACGAACAGTCAAACCCTTC-3'	*21S* reverse
HK5049	5'-ACGCGCGATTTCTAGAATAC-3'	Immature *TLC1 N1** reverse
HK5091	5'-ATCCGCCTATCCTCGTCATG-3'	Readthrough *TLC1* reverse

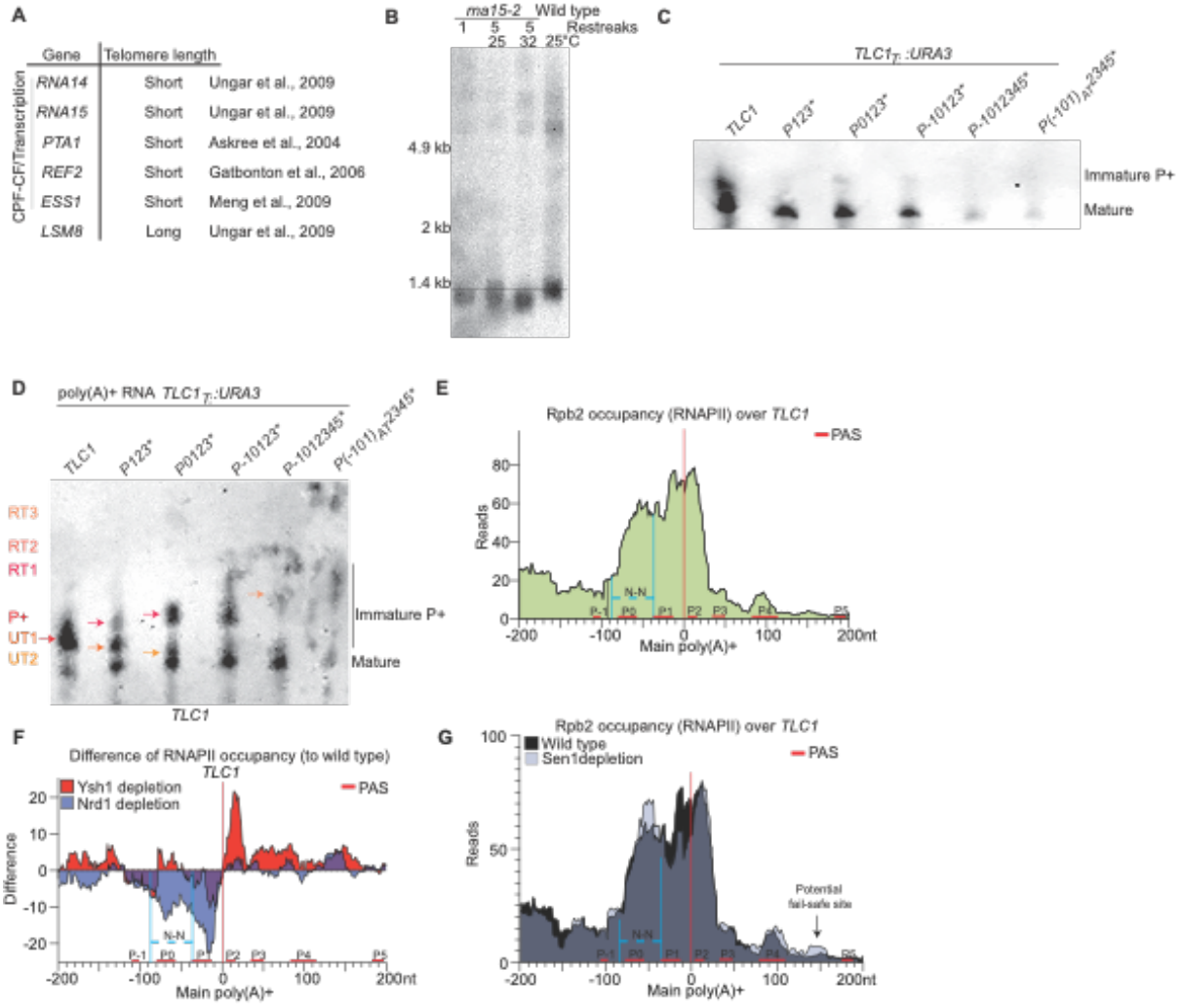
Supplementary Table 5: Primers for analytical PCR and FISH

Number	Sequence	Name
HK940	5'-ATGTGCCCCGTACATCG-3'	*TLC1* forward sequencing
HK1761	5'-CY3-GCGCACACACAAGCATCTACACTGACACCAGCATACTCGAAATTCTTTGG-CY3-3'	*TLC1* Cy3 probe 1
HK1789	5'-CY3-CGATAAGATAGACATAAAGTGACAGCGCTTAGCACCGTCTGTTTGC-CY3-3'	*TLC1* Cy3 probe 2
HK1790	5'-CY3-CCTACTCGTATTTTTCTCTGTCACATCGTTCGATGTACGGGGCACATTTGG-CY3-5'	*TLC1* Cy3 probe 3
HK4375	5'-GTTCATCCATGCCATGTGTAATCC-3'	3'-end PCR adapter reverse
HK4683	5'-TTTGTATAGTTCATCCATGCCATGTGTAATCCTTTTTTTTTTTTTTTTTC-3'	cDNA for 3'end-PCR with adapter
HK4684	5'-TTTGTATAGTTCATCCATGCCATGTGTAATCCTTTTTTTTTTTTTTTTTG-3'	cDNA for 3'end-PCR with adapter
HK4685	5'-TTTGTATAGTTCATCCATGCCATGTGTAATCCTTTTTTTTTTTTTTTTTA	cDNA for 3'end-PCR with adapter

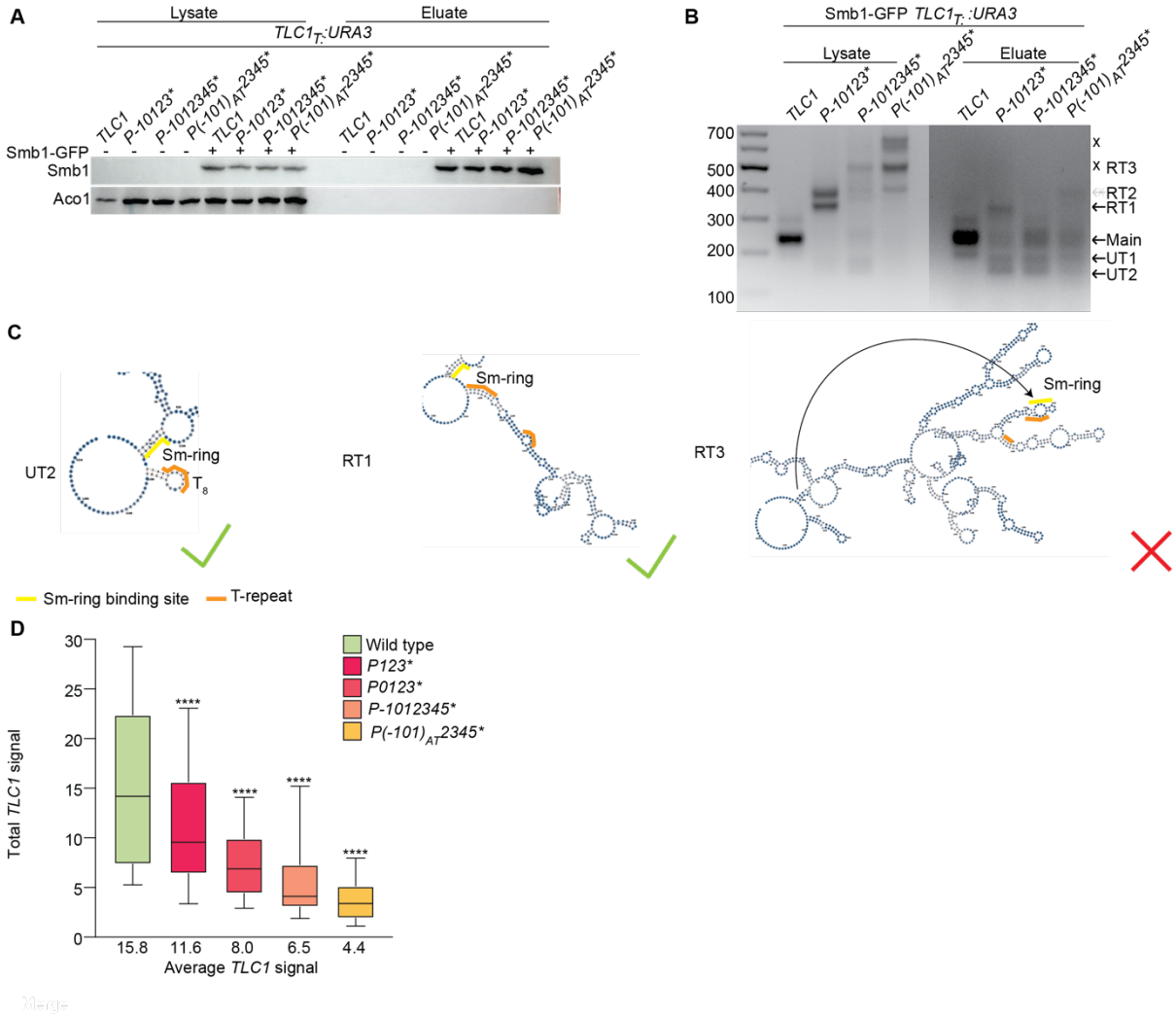
Number	Sequence	Name
	-3’	
HK5104	5’-GAAAAGGAAGAGCAATCCTG-3’	*TLC1* forward
HK5540	5’-GAGAGGAAGATAGGTACCCTATG-3’	*TLC1* digoxygenin probe 1 forward
HK5541	5’-TAATACGACTCACTATAGGGGCAGCAGGCTATCAACTGAA-3’	*TLC1* digoxygenin probe 1 reverse + T7 promotor
HK5542	5’-GTATGCTGGTGTCAGTGAAG-3’	*TLC1* digoxygenin probe 2 forward
HK5543	5’-TAATACGACTCACTATAGGGGGTCGAGAAGAGGATCGGTAC-3’	*TLC1* digoxygenin probe 2 reverse + T7 promotor
HK5544	5’-CTACCATCACCACACCCAC-3’	*TLC1* digoxygenin probe 3 forward
HK5545	5’-TAATACGACTCACTATAGGGGATAAGATAGACATAAAGTGACAGCG-3’	*TLC1* digoxygenin probe 3 reverse + T7 promotor
HK5546	5’- GAAAAAGAACGTCAGGGAACATG-3’	*TLC1* digoxygenin probe 4 forward
HK5547	5’-TAATACGACTCACTATAGGGCTACTCGTATTTTTCTCTGTCACATC-3’	*TLC1* digoxygenin probe 4 reverse + T7 promotor
HK6067	Cy5-AATGCATGTCGACGAGGTCCGAGTGTAA-Cy5	FLAPY Cy5 probe
HK6068	TTACACTCGGACCTCGTCGACATGCATTGCGCACACACAAGCATCTACACTGACACCAGCATACTCGAAATTCTTTGGTTACACTCGGACCTCGTCGACATGCATT	smiFISH anchor *TLC1* 1
HK6069	TTACACTCGGACCTCGTCGACATGCATTCGATAAGATAGACATAAAGTGACAGCGCTTAGCACCGTCTGTTTGCTTACACTCGGACCTCGTCGACATGCATT	smiFISH anchor *TLC1* 2
HK6070	TTACACTCGGACCTCGTCGACATGCATTCCTACTCGTATTTTTCTCTGTCACATCGTTCGATGTACGGGGCACATTTGGTTACACTCGGACCTCGTCGACATGCATT	smiFISH anchor *TLC1* 3
HK6071	TTACACTCGGACCTCGTCGACATGCATTGCAAACGCAACAGCCATTGACATTTTCATAGGGTACCTATCTTCCTCTCTTACACTCGGACCTCGTCGACATGCATT	smiFISH anchor *TLC1* 4
HK6072	TTACACTCGGACCTCGTCGACATGCATTGGTAGGCTTCCCATGGTAATTAAAAGGTTAGGTCGAGAAGAGGATCGGTACGTTACACTCGGACCTCGTCGACATGCATT	smiFISH anchor *TLC1* 5
HK6073	TTACACTCGGACCTCGTCGACATGCATTCTCAAATTACGTTCTTGATCTTGTGTCATTGTTCAGTTACTGATCGCCCGCTTACACTCGGACCTCGTCGACATGCATT	smiFISH anchor *TLC1* 6



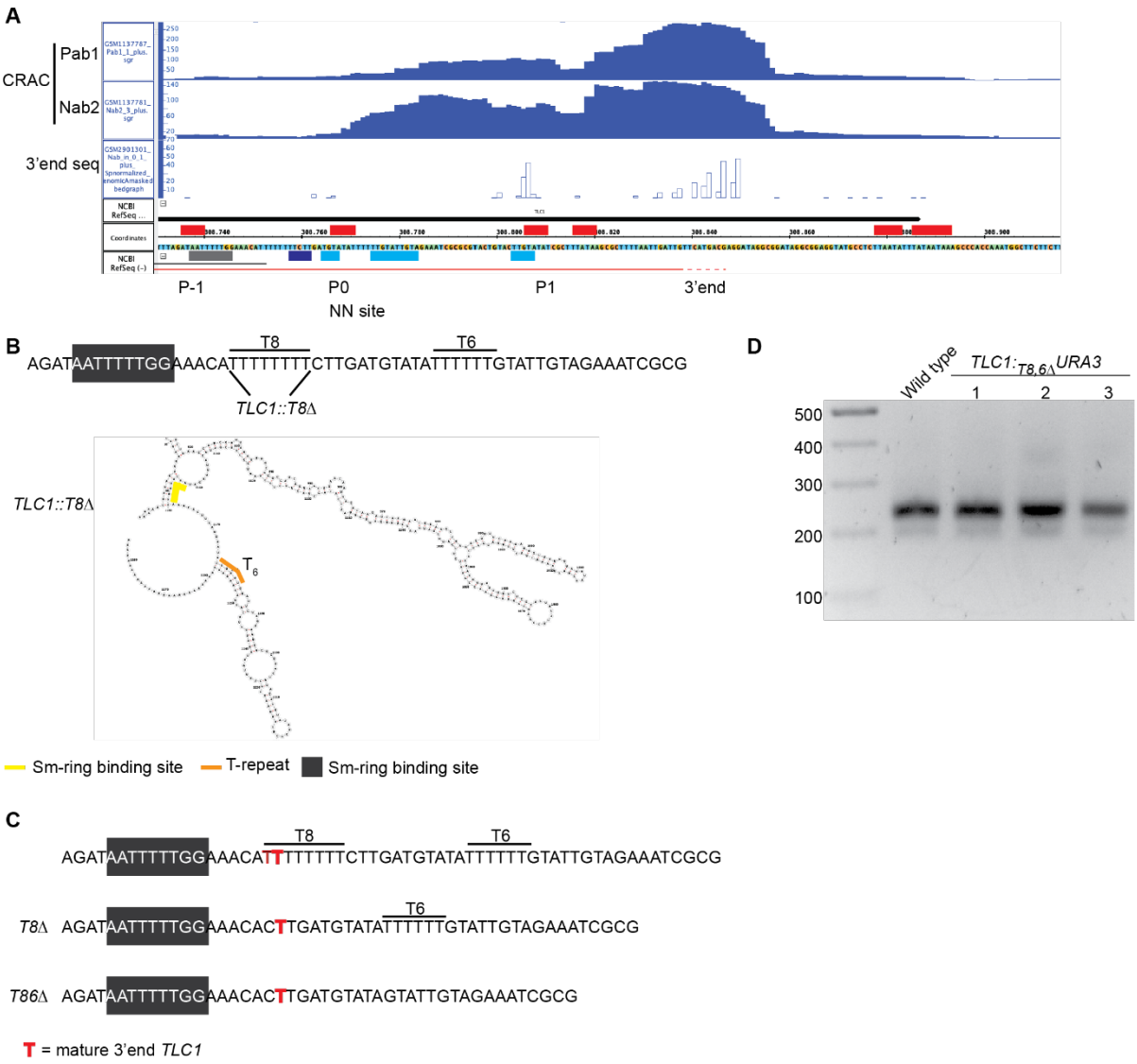
Sup. Figure 1. Interruption of Nrd1 Nab3 binding results in increased *TLC1* levels similar to *trf4*Δ. (A) Nrd1 and Nab3 bind *TLC1* at two main sites. CRAC data from (7) was evaluated and binding of Nrd1 and Nab3 displayed over *TLC1*. Site N0 is close to the 5'end and site N1 at the 3'end of *TLC1*. Mutations used for *N0** are shown. (B) The pull-down of Nrd1-GFP is shown in the indicated mutant strains in Western blots. Hem15 was used as a washing control. (C) Nrd1-RIPs show decreased binding to *TLC1* upon mutation of the N1 and N01 site. The isolated RNA of Nrd1-GFP pull-down was analyzed via qPCR amplifying immature *TLC1* normalized to *21S rRNA*. Enrichment in the eluate fraction is displayed for all strains. *n=5* (D) Immature and mature *TLC1* is enriched upon mutation of the Nrd1 and Nab3 binding sites. Northern blot is shown of total RNA isolated from the indicated strains for *TLC1* and *SCR1* as a control. *n=3* (E) No specific NNS-terminated *TLC1* transcripts are present in *trf4*Δ. 3'-end PCR of non- and Poly(A) Polymerase (pap)-treated RNA is shown in wild type and *trf4*Δ. (F) Total and immature *TLC1* is enriched in *trf4*Δ. qPCR analysis using primers that amplify total and immature *TLC1* is shown.



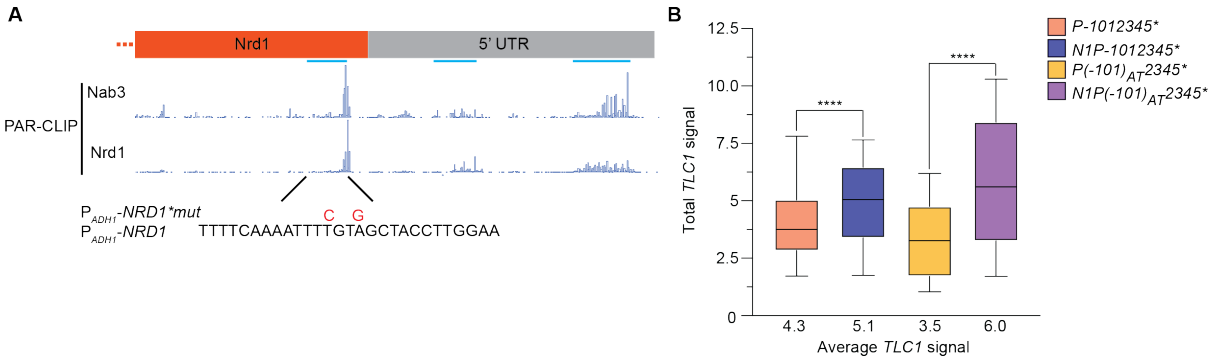
Sup. Figure 2. *TLC1* 3'end processing and transcription termination depends on CPF-CF factors. (A) High through put analyses identified several CPF-CF factor mutants that affect telomere length. (B) Telomere length is shortened in the CPF-CF mutant *rna15-2* as shown in Southern blots at the semi-permissive temperature after five re-streaks. Telomere ends were detected after XhoI digestion of the genomic DNA using a Dig-labeled TG repeat specific probe. (C) *TLC1* levels are decreased in PAS mutants. Northern blot experiment is shown of total RNA isolated from the indicated strains for *TLC1*. *n=3* (D) CPF-CF mediated 3'end processing occurs at upstream and downstream located PAS in different PAS mutants. Northern blot is shown of poly(A) enriched RNA isolated from the indicated strains for *TLC1*. *n=2* (E) RNAPII profile around the main cleavage site of *TLC1*. RNAPII occupancy was analyzed from PAR-CLIP data of Rbp2 (8) 200 nt around their main poly(A)+ 3'end of *TLC1*. (F) Transcription termination of *TLC1* depends on the CPF-CF complex. PAR-CLIP data of Rbp2 (8) upon anchor away of Ysh1 or Nrd1 in comparison to wild type is shown 200 nt around their main poly(A)+ 3'end of *TLC1*. (F) RNAPII profile around the main cleavage site of *TLC1*. RNAPII occupancy of wild type and Sen1 depleted cells was analyzed from PAR-CLIP data of Rbp2 (8) 200 nt around their main poly(A)+ 3'end of *TLC1*.



Sup. Figure 3. Folding is predicted to differ of 3'elongated *TLC1*. (A) The pull-down of Smb1-GFP is shown in the indicated mutant strains in Western blots. Aco1 was used as a washing control. (B) Same as Figure 4A. Smb1-RIPs show binding of the shorter poly(A)+ transcripts generated by the indicated PAS mutants, but not for the longer readthrough transcripts RT2 and RT3. The isolated RNA of Smb1-GFP pull-downs was analyzed in 3'-end PCRs with one internal and one adapter primer that amplifies polyadenylated *TLC1*. The RNA was not artificially polyadenylated. Signal was strengthened for the eluate fraction. (C) Readthrough *TLC1* transcripts indicate folding defects for the longest transcripts. Usually the poly(A) tail of *TLC1* folds back onto a T-rich region in its 3'end (T8+T6), creating a long stem. The longest forms fold differently and miss the linear stem. RNA folding was predicted using the "RNAfold" WebServer (13). (D) *TLC1* shows decreased signal upon PAS mutation. Signal of smeFISH experiments of Figure 4B was quantified for the total cell. Density values are displayed in thousand.



Sup. Figure 4. Efficient maturation of *TLC1* requires a poly(U)-stretch downstream of the Sm-ring binding site. (A) Nab2 and Pab1 bind to the 3'ends of P0 and P1 3'end processed transcripts. Nab2 and Pab1 CRAC experiments (14) and 3'end seq data (15) are shown for the *TLC1* terminator region. (B) Deletion of the T8-stretch does not result in a shift of the Sm-ring binding site. RNA folding was predicted using the "RNAfold" WebsServer (13). (C) The mature transcript was sequenced and the position of the mature 3'end is shown. (D) Transcript termination is not affected in the *T86*Δ mutant. 3'-end PCR of non Poly(A) Polymerase (pap)-treated RNA is shown in wild type and 3 biological replicates of *T86*Δ.



Sup. Figure 5. Nrd1 and Nab3 surveil CPF-CF mediated 3'end processing for *TLC1*. (A) Mutation of Nrd1 autoregulation sites. Nrd1 and Nab3 binding sites are shown for Nrd1 from PAR-CLIP data (16). 5' UTR is not included in the used plasmid and binding sites in the ORF were mutated as indicated. (B) *TLC1* shows increased signal upon loss of quality control in PAS mutants. Signal of smeFISH experiments of Figure 6H was quantified for the total cell.

Supplementary References

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