

Supplemental Figures

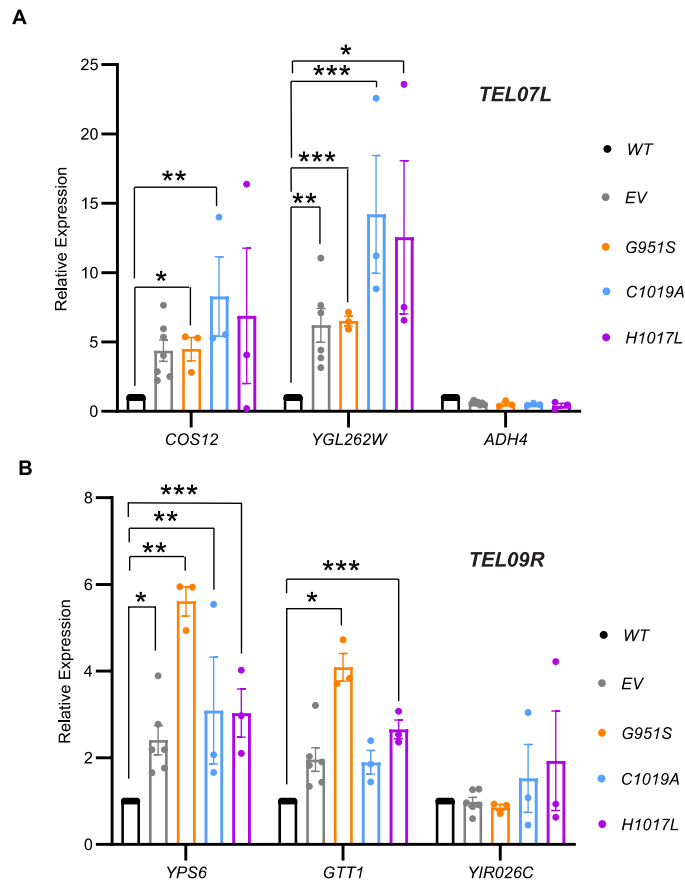


Figure S1. Subtelomeric gene expression in cells expressing Set1 catalytic mutants. RT-qPCR analysis of *TEL07L* (A) and *TEL09R* (B) genes in *set1Δ* cells with an empty vector (EV; yEG647), vector with wildtype FLAG-Set1 (yEG738), or FLAG-Set1 catalytic mutants *G951S* (yEG984), *H1017L* (yEG885), and *C1019A* (yEG886). Expression was normalized to the control gene *TFC1* and is shown relative to *WT*. Error bars represent standard error of the mean (SEM) for a minimum of three biological replicates. Significance was evaluated using two-way ANOVA and Dunnett's multiple comparisons test. *p*-values are indicated as follows: * < 0.05, ** < 0.01, *** < 0.001.

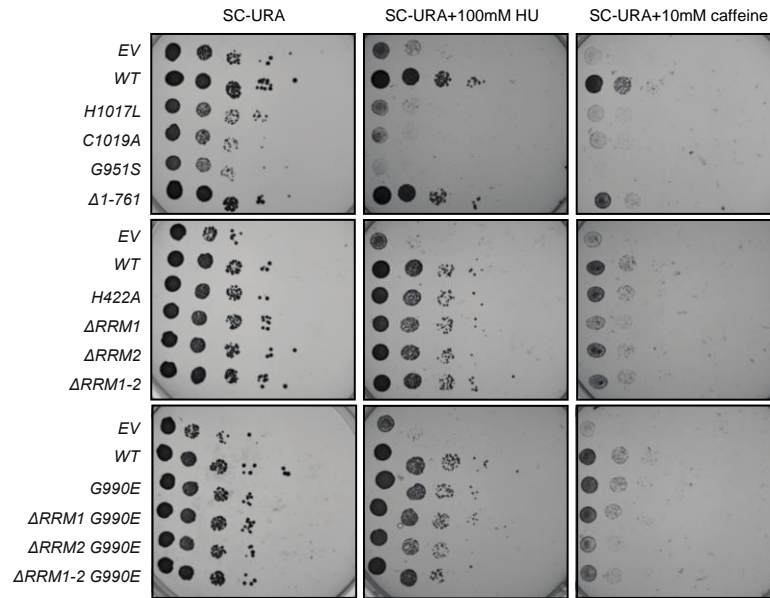


Figure S3. Analysis of Set1 mutants in the presence of DNA damage and replication stress. Ten-fold serial dilutions of saturated yeast cultures were spotted on SC-URA, SC-URA + 100 mM HU, and SC-URA +10 mM caffeine plates and grown at 30°C. Yeast strains are as described for **Figure S2** and **Figure 3**.

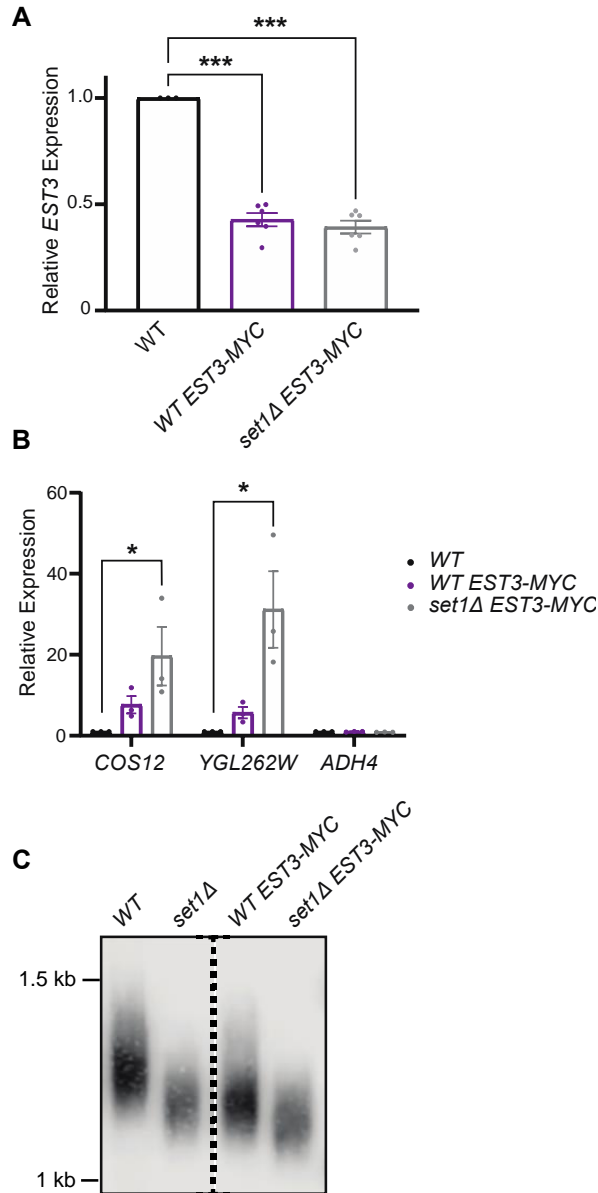


Figure S4. Incorporation of a C-terminal MYC tag disrupts *EST3* mRNA abundance, subtelomeric gene repression, and telomere length. **(A)** RT-qPCR analysis of *EST3-MYC* in WT (yEG1358) and *set1Δ* (yEG1359) cells carrying an integrated allele. **(B)** RT-qPCR analysis of *TEL07L* genes in *EST3-MYC* strains. For **A** and **B**, expression was normalized to the control gene *TFC1* and is shown relative to WT. Error bars represent standard error of the mean (SEM) for a minimum of three biological replicates. Significance was evaluated using one-way ANOVA and Dunnett's multiple comparisons test (**A**) or two-way ANOVA and Sidak's multiple comparisons test (**B**). *p*-values are indicated as follows: * < 0.05, ** < 0.01, *** < 0.001. **(C)** Southern blot showing terminal telomere fragment molecular weight in the indicated strains. The dashed line in the blot indicates samples run on the same gel in which intervening lanes were removed for clarity. All yeast strains described in this figure are derived from the BY4741 background.

Supplemental Tables

Table S1. Yeast strains used in this study.

Strain Number	Background	MAT	Genotype	Reference
yEG001	BY4741	a	<i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 (WT)</i>	BY4741
yEG230	BY4742	alpha	<i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 (WT)</i>	(Jezek <i>et al.</i> , 2017)
yEG232	BY4741	a	<i>set1Δ::KANMX</i>	(Jezek <i>et al.</i> , 2017)
yEG1108	BY4742	alpha	<i>set1Δ::NATMX</i>	This study
yEG323	BY4742	alpha	<i>tlc1Δ::NATMX</i>	This study
yEG880	BY4743	a/alpha	<i>TLC1/tlc1Δ::NATMX SET1/set1Δ::KANMX</i>	This study
DLY3001	W303	a	<i>RAD5⁺ (WT)</i>	(Holstein <i>et al.</i> , 2014)
yEG1296	W303	alpha	<i>RAD5⁺ set1Δ::NATMX</i>	This study
DLY4557/ yEG1297	W303	alpha	<i>RAD5⁺ cdc13-1 int</i>	(Holstein <i>et al.</i> , 2014)
yEG1298	W303	a	<i>RAD5⁺ set1Δ::NATMX cdc13-1 int</i>	This study
yEG108	S288C	a	<i>MAT a, ura3-52, lys2-801, ade2-101, trp1Δ63, his3Δ200, leu2Δ1, hht1-hhf1::LEU2, hht2-hhf2::HIS, pRS314-HHT2-HHF2 (derived from WZY42)</i>	(Zhang <i>et al.</i> , 1998)
yEG109	S288C	a	<i>MAT a, ura3-52, lys2-801, ade2-101, trp1Δ63, his3Δ200, leu2Δ1, hht1-hhf1::LEU2, hht2-hhf2::HIS, pRS314-HHT2 K4R-HHF2 (derived from WZY42)</i>	(Zhang <i>et al.</i> , 1998)
yEG1342	S288C	a	<i>MAT a, ura3-52, lys2-801, ade2-101, trp1Δ63, his3Δ200, leu2Δ1, hht1-hhf1::LEU2, hht2-hhf2::HIS, pRS314-HHT2-HHF2, set1Δ::KANMX</i>	This study
yEG1343	S288C	a	<i>MAT a, ura3-52, lys2-801, ade2-101, trp1Δ63, his3Δ200, leu2Δ1, hht1-hhf1::LEU2, hht2-hhf2::HIS, pRS314-HHT2 K4R-HHF2, set1Δ::KANMX</i>	This study
yEG100	BY4741	a	<i>spp1Δ::KANMX</i>	(Jezek <i>et al.</i> , 2017)
yEG110	BY4741	a	<i>sdc1Δ::KANMX</i>	(Jezek <i>et al.</i> , 2017)
yEG623	BY4742	alpha	<i>rad6Δ::HIS3MX</i>	(Jaiswal <i>et al.</i> , 2017)
yEG647	BY4741	a	<i>set1Δ::KANMX + p045 (pRS316)</i>	This study
yEG738	BY4741	a	<i>set1Δ::KANMX + p319 (pRS316FLAG-SET1)</i>	This study
yEG740	BY4741	a	<i>set1Δ::KANMX + p342 (pRS316 FLAG-SET1 G990E)</i>	This study
yEG741	BY4741	a	<i>set1Δ::KANMX + p343 (pRS316 FLAG-SET1 H422A)</i>	This study
yEG746	BY4741	a	<i>set1Δ::KANMX + p344 (pRS316 FLAG-SET1 ΔRRM1)</i>	This study

yEG747	BY4741	a	<i>set1Δ::KANMX + p345 (pRS316 FLAG-SET1 ΔRRM2)</i>	This study
yEG748	BY4741	a	<i>set1Δ::KANMX + p346 (pRS316 FLAG-SET1 ΔRRM1- ΔRRM2)</i>	This study
yEG819	BY4741	a	<i>set1Δ::KANMX + p363 (FLAG-SET1 G990E ΔRRM1)</i>	This study
yEG820	BY4741	a	<i>set1Δ::KANMX + p364 (FLAG-SET1 G990E ΔRRM2)</i>	This study
yEG821	BY4741	a	<i>set1Δ::KANMX + p365 (FLAG-SET1 G990E ΔRRM1- ΔRRM2)</i>	This study
yEG885	BY4741	a	<i>set1Δ::KANMX + p392 (pRS316-FLAG-SET1 H1017L)</i>	This study
yEG886	BY4741	a	<i>set1Δ::KANMX + p393 (pRS316 FLAG-SET1 C1019A)</i>	This study
yEG984	BY4741	a	<i>set1Δ::KANMX + p457 (pRS316 FLAG-SET1 G951S)</i>	This study
yEG1102	BY4741	a	<i>set1Δ::KANMX + p510 (pRS316 FLAG-SET1Δ1-761)</i>	This study
yEG1137	BY4741	a	<i>cdc13::CDC13-MYC::HIS3MX</i>	This study
yEG1138	BY4741	a	<i>set1Δ::KANMX cdc13::CDC13-MYC::HIS3MX</i>	This study
yEG1284	BY4742	alpha	<i>ten1::TEN1-MYC::HIS3MX</i>	This study
yEG1285	BY4741	a	<i>set1Δ::KANMX ten1::TEN1-MYC::HIS3MX</i>	This study
yEG1008	BY4742	alpha	<i>est1::EST1-MYC::HIS3MX</i>	This study
yEG1009	BY4742	alpha	<i>set1Δ::KANMX est1::EST1-MYC::HIS3MX</i>	This study
DLY5761	W303	alpha	<i>RAD5⁺ stn1::STN1-MYC::TRP1</i> <i>RAD5⁺ stn1::STN1-MYC::TRP1</i>	(Holstein <i>et al.</i> , 2014)
yEG1318	W303	a	<i>set1Δ::NATMX</i>	This study
yEG1358	BY4741	a	<i>est3::EST3-MYC::HIS3MX</i>	This study
yEG1359	BY4741	a	<i>set1Δ::KANMX est3::EST3-MYC::HIS3MX</i>	This study
yEG1362	BY4741	a	<i>WT</i>	This study
yEG1363	BY4741	a	<i>set1Δ::KANMX</i>	This study
yEG1364	BY4741	a	<i>est3Δ::HygB</i>	This study
yEG1365	BY4741	a	<i>est3Δ::HygB set1Δ::KANMX</i>	This study

Note: All strains used in this study are derived from the BY4741/BY4742 background unless otherwise indicated in the genotype column.

Table S2. Plasmids used in this study.

Plasmid Number	Description	Reference
p045	pRS316 (URA3)	
p340	pRS316 + FLAG-SET1	This study
p342	pRS316 + FLAG-SET1 G990E	This study
p343	pRS316 + FLAG-SET1 H422A	This study
p344	pRS316 + FLAG-SET1 Δ RRM1	This study
p345	pRS316 + FLAG-SET1 Δ RRM2	This study
p346	pRS316 + FLAG-SET1 Δ RRM1- Δ RRM2	This study
p363	pRS316 + FLAG-SET1 Δ RRM1 G990E	This study
p364	pRS316 + FLAG-SET1 Δ RRM2 G990E	This study
p365	pRS316 + FLAG-SET1 Δ RRM1- Δ RRM2 G990E	This study
p392	pRS316 + FLAG-SET1 H1017L	This study
p393	pRS316 + FLAG-SET1 C1019A	This study
p510	pRS316 + FLAG-SET1 Δ 1-761	This study
p457	pRS316 + FLAG-SET1 G951S	This study

Table S3. Primers used in this study.

Oligo Number	Experiment	ORF/Position	Sequence		
oEG537	SYBR GEX	COS12 - F	CATTACAAATACTCCGGGTATAGACA		
oEG538	SYBR GEX	COS12 - R	GCAGCTGGAA CCATCAAAA		
oEG539	SYBR GEX	YGL262W - F	GAGAATTACTCTGACATTGGAGATGA		
oEG540	SYBR GEX	YGL262W - R	TTGTCATTAC AGAAGCCATC AAC		
oEG541	SYBR GEX	ADH4 - F	CCAATGTACAGCTGGTTTG		
oEG542	SYBR GEX	ADH4 - R	CCTTAGCATTGTCGTGAGCA		
oEG543	SYBR GEX	TFC1 - F	ACACTCCAGGCGGTATTGAC		
oEG544	SYBR GEX	TFC1 - R	CTTCTGCAATGTTTGGCTCA		
oEG1176	SYBR GEX	TLC1 - F	TGTAGAAATCGCGCGTACTG		
oEG1177	SYBR GEX	TLC1 - R	CTATCCGCCTATCCTCGTCA		
oEG1178	SYBR GEX	RIF1 - F	AGTTCGTTGGCTGTTGAAGG		
oEG1179	SYBR GEX	RIF1 - R	TCGCTATCAGACGCATTTTG		
oEG1351	SYBR GEX	CDC13 - F	AAGAGCCTGAGTGTCTCTCA		
oEG1352	SYBR GEX	CDC13 - R	AATTGCACGGGAACCTATTGC		
oEG1353	SYBR GEX	RAP1 - F	TAGCAACGTCAACGACGAAG		
oEG1354	SYBR GEX	RAP1 - R	GAAAAGATGCATTCCCCTCA		
oEG1355	SYBR GEX	EST2 - F	GTCACCTCAATGGCCTCGAT		
oEG1356	SYBR GEX	EST2 - R	CAGGAAGGCATGGTAATGCT		
oEG1357	SYBR GEX	STN1 - F	GCAAGAAGAACGCTTGAAGG		
oEG1358	SYBR GEX	STN1 - R	ACCGAAATGACAAGGAATGC		
oEG1359	SYBR GEX	TEN1 - F	TTCGCATTGGGGTATGGTAT		
oEG1360	SYBR GEX	TEN1 - R	CACGAACGTCATTCTCTGGAT		
oEG1361	SYBR GEX	RIF2 - F	AAGTGTTAGCAGCCCAAAGG		
oEG1362	SYBR GEX	RIF2 - R	CTCTTGCAAGGCCATTGATT		

oEG1417	SYBR GEX	EST1 - F	GAACAACAGCGCAAAAGAGC
oEG1418	SYBR GEX	EST1 - R	TCTGGGAGCGGAGACAATTT
oEG1419	SYBR GEX	EST3 - F	TGAAGACAACTCGGAGCATG
oEG1420	SYBR GEX	EST3 - R	AATTGTCGGGCTCATATGCG
oEG1421	SYBR GEX	RFA1 - F	AGGGCTGGGAAGAAATTCGA
oEG1422	SYBR GEX	RFA1 - R	GCTTGCTGATTCCATAGGCC
oEG1423	SYBR GEX	RFA2 - F	CGGTGGCTTTGAGAACTCTG
oEG1424	SYBR GEX	RFA2 - R	ATCGTCACAGGTGTCAAGGT
oEG1425	SYBR GEX	RFA3 - F	CAATGATGACGGCGAGCTAG
oEG1426	SYBR GEX	RFA3 - R	CTGTAAAGCAACCACACCGT
oEG1427	SYBR GEX	yKU70 - F	GAGATTCCGGGTCAAAAGCA
oEG1428	SYBR GEX	yKU70 - R	TATCTTGCGCCTCTTGTGGT
oEG1429	SYBR GEX	yKU80 - F	TTCCCGTGACCATCTCCAA
oEG1430	SYBR GEX	yKU80 - R	TGATCGACTAGAACGGACGG
oEG1229	Southern	telomeric TG(1-3) repeats	biotin-CACACCCACACCCACACC
oEG1637	ChIP	EST1 promoter-F	CACAGACGAAGGTGCTTTCA
oEG1638	ChIP	EST1 promote- R	TGAACGCGAAAATCACATTGA
oEG1639	ChIP	EST1 5'-F	GCTCGTGCGCATCTGGATAA
oEG1640	ChIP	EST1 5'-R	AGGAAGCATCTGAACGTGATAT
oEG1641	ChIP	EST1 3'-F	AAACATGCTGCTTCACGAGG
oEG1642	ChIP	EST1 3'-R	ATTGCTTCGTCTGGATATGAGAG
oEG1649	ChIP	EST3 promoter-F	GTTCAATTCCCCGTGCGG
oEG1650	ChIP	EST3 promoter-R	TGGTCAACTTTTGCTGTCTAGT
oEG1651	ChIP	EST3 5'-F	TCATCCCTCTGGCCATGTAA
oEG1652	ChIP	EST3 5'-R	CGAAATGGCACGGATTGGTT
oEG1653	ChIP	EST3 3'-F	TTGGCGATGCTGACTTAGTC
oEG1654	ChIP	EST3 3'-R	ACGGGCACTATTTCTTTGGAC
oEG153	ChIP	5'PMA1-F	TCAGCTCATCAGCCAACTCAAG
oEG154	ChIP	5'PMA1-R	CGTCGACACCGTGATTAGATTG
oEG141	ChIP	TELVIII-F	AGCCCGAGCCTGTACTAAAT
oEG142	ChIP	TELVIII-R	CAAAAGAACTTTTCATGGCA

Supplemental References

Holstein, E.M., Clark, K.R., and Lydall, D. (2014). Interplay between nonsense-mediated mRNA decay and DNA damage response pathways reveals that Stn1 and Ten1 are the key CST telomere-cap components. *Cell Rep* 7, 1259-1269.

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