## SUPPLEMENTARY FIGURES

Hsu et al.

#### SUPPLEMENTARY FIGURE LEGEND

# Supp. Fig. 1. K. lactis est $1\Delta$ mutant exhibit senescence and telomere shortening.

(A) The *K. lactis est1* $\Delta$  strain was passaged on YPD medium. Colonies that have undergone the indicated number of generations were re-streaked together on two plates. Some small colonies suggestive of senescence can be observed at 75 and 100 generations (black arrowheads). More severe and uniform growth defects are seen at 175 and 200 generations.

(B) An *est1* $\Delta$  clone and one bearing wild type *EST1* on a plasmid were passaged and their telomeres analyzed after the indicated number of streaks.

#### Fig. S2. Growth defects of K. lactis EST1 mutants

The *K. lactis*  $est1\Delta$  mutant reconstituted with the indicated *EST1* alleles were passaged on YPD medium. Colonies that have undergone the indicated number of generations were re-streaked together.

#### Fig. S3. Characterization of the KlEst1 K467E mutant.

(A) The expression of  $KIEst1_{NTD}$ -TAP and  $KIEst1_{NTD-K467E}$ -TAP (marked by an arrow) in the reconstituted strains was analyzed by IgG-Sepharose pull down and Western using anti-protein A antibodies.

(B) The binding of KIEst1<sub>NTD</sub>-TAP and KIEst1<sub>NTD-K467E</sub>-TAP to Ter1 in cell extracts was analyzed by IgG-Sepharose pull down and RT-PCR. The levels of U1 in the pull down samples were also measured and used as controls for non-specific binding.

2

(C) The *est1* null mutant was transformed with plasmids that contain either *KI*Est1-TAP or *KI*Est1<sub>K467E</sub>-TAP. The resulting strains were passaged in selective media and their telomeres analyzed by Southern after 1, 3 and 5 streaks.

### Fig. S4. KlEst1 interacts with RNA in an RNA concentration- and sequencedependent manner.

(A) Purified Est1 (12.5 nM) was incubated with increasing concentrations of <sup>32</sup>P-labeled Ter1<sub>EBD</sub>, antisense Ter1<sub>EBD</sub>, and CS2a-sub (1, 2, 4, 8, 16 nM), and subjected to the RNA-protein UV crosslinking assay. The products were analyzed by SDS-PAGE and visualized by a PhosphorImager. (B) The relative crosslinking signals from (A) were quantified and plotted.

#### Fig. S5. The KlEst1 residues required for RNA-binding.

A homology model of  $KI\!Est1_{NTD}$  was generated using the I-TASSER server and displayed in surface representations. The TPR subdomain, the DSH subdomain, and the mutation clusters are colored in yellow, green, and red, respectively. The putative peptide-binding groove is labeled "P". The bottom view is related to the top view by a 90° rotation in the x-axis.

### Fig. S6. A conserved basic residue in Est1 is required for telomerase RNAbinding in budding and fission yeast.

An alignment of the TPR domain of selected budding and fission yeast Est1 homologues is displayed. Budding yeast and *S. pombe* Est1residues implicated in RNA-binding are

indicated by filled and open circles, respectively. The locations of  $\alpha$  helices within the TPR domain are designated by cylinders below the alignment.





## Supp. Fig. 2



## Supp. Fig 3



RT-PCR

Supp. Fig. 4







## Supp. Fig. 6

S_pom S_cer K_lac S_jap S_oct C_lus C_tro E_gos	1 1 1 1 1 1	M S Q Q L S
P_sti H_sap_c	1	MA H S S D
S_pom S_cer K_lac S jap	38 48 44 39	IAIEIEV-EKK <mark>U</mark> N
s_oct C_lus	38 40	CTISRRF - EDDILKI - KE - NH D TVYRADFLAWTYCH KKULKLI
C_tro E_gos P_sti	5 40	
H_sap_c	38	D L Y Q - K M U Y D M E - Y A L D K K V Q I - TT E Q D L M N H A F K N Q I - TT
S_pom S_cer	86 109	HPRHVUEKKKTKKVFFKELKTCAIFVQTCISELISKFQLDSYREF-EC-KWTSSATVSSTISNDEMS SNRKKFRVFRIFQKKIGFKITHRYVYDIEHLCAKVDMNSVISNALF-AKONLMQYTDGLSTHEKI SNRKFRVFRIFQKKIGFFELTHYVYDIEHLCAKVDMNSVISNALF-AKONLMQYTDGLSTHEKI
S_jap S_oct	51	KCLLQGCT-SFPULKKNIRRNYEKCLKSATKFYQLFIQTUIKQFDLFIDVSWUHERGL-KQLRIKTDPEIYS NKKHYVERKHRKKETTELSSCTAFYENCINEIIIGSCVFQLNPVLQNWLW-ERTRATLTCKTQN
C_lus C_tro E gos	105 93 41	P K K G S K S K F K S V M K K I N E Y F M S B K C V I S F YD K VM Q Y V A G S F T N - S L I P S F P F - K E L E I E I S S S Q N E K C Q K E G R P G E F K V R P V M K K I N D N F M K Y I K E I Y Q F Y G K L L K Y F T K Y K N - P N I P D K F L - E F R F T V S G N A I E A E G G P O E R T Y I S F R M N K K I N I I F K I I R O F Y N G L V Q O H F D R YD F G K L P A G V I - R E M N V O S G G E L O T
P_sti H_sap_c	111 72	RCVNEGQPGKFKVKPUBSEKLHDQLOKFTKQLFNFUSLLNYFTHUDI-PLPRKET-EHFNFQPNPKAIR- LQGQAKNRANPNRSEVQANLSIFLEAASGFUTQLLQELCTVFNVDLPCRVKSSQLGIISNKQTHTSA
S pom	151	SIPEASYSRNHMEALECVYN OF IYD CDMARWSSTCLK
S_cer K lac	176	ILNTSNPLTFSIVISLOR CVINLGSTHFYKTLLNKPSNKPKSVEGPEKSIRVLNIASL VLKPEDSESIDCVMASOR CLUYIGCSORVKIIMEH
S_jap S_oct	121 150	- SLFEE TD KEHVLNCVYRCLICLGDTÂRYFVMCSH
C_lus C_tro	171 163	– A S – D S – – NFLN ST K V I L Y Q CLL G L GN V T R H S A Q I D I S Y V Q P C K S M A S Y Q L – Y T K N E K P – I A N L K E K V M P L L Y S K C I Q – C Q – D D – – NFL G H V I H L S H K C C L C L G D M L R N Q A F I D T N Y V P C L S N K E F F K F K S – – S P N K – R N H M G S Y V K A I Q Y Y N L C I M
E_gos P_sti H_sap_c	$107 \\ 181 \\ 139$	- LDDSSYFTILCVMSLQRCLLYLGCCHRYKCCCSKLSKR-FHISDDDSMRMFMLAKQ - TS-NTNDQANVLFLCHKCLVILGDISRHKSKIENSFVVPCSSNKAFFNNRELNTIQKSLLEEFTYEKATOFMKLCIV - L
54F_6	100	
S_pom S_cer K_lac S_jap	205 234 231 172	TLPGNGMHRNOIAVVWASDECIVESIYMFSSALCSEDPP-KSÄLLNL(KOLIAF
C_lus C_tro E_gos	245 236 163	LFPNM TDPYNHIGVI YN SVOEKFLAVEWFSRAQL TSSKKRDIAKYNMATIFTKPWLEETYSRVLKTSE LIPALSEPYNOIGVI YN SVDDKFNAIYWFLRSHFSRLSEH QLGFANMSAIL KKHWFTTAL VDI VN GNSE IVPSVGETYLOEGLV YV QTGNYGHAAYAFMRGSLSRMPT-DAGIPNLESI VADASSGLFAKHQGI LKRLRSKESDS
P_sti H_sap_c	257 181	LIPALNEPYNHIGMICNTLNKQFEACIMFLRSTOTRIGNFQVGMNNFKSIIRKKPFVAKONRINSDRFDGGRSK LVPSNGQPYNQLAILASSKGDHLTTIFYYCRSIAVKFPF-PAASTNLQKALSKALESRDEVK