

Supplementary Materials for

The fission yeast Stn1-Ten1 complex limits telomerase activity via its SUMO-interacting motif and promotes telomeres replication

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Published 16 May 2018, *Sci. Adv.* **4**, eaar2740 (2018)
DOI: 10.1126/sciadv.aar2740

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table S1. Fission yeast strains used in this study.

| Figure | Strain no. | Genotype [§] | Source |
|--------|-----------------------------|---|-------------------------------|
| 1D | SC651 | <i>h⁺ cdc25-22 ts</i> | S. Forsburg |
| | WT | <i>h⁻</i> | P. Russell |
| | SC1124-25 | <i>h⁻ stn1-226</i> | This study |
| 1E | WT | <i>h⁻</i> | P. Russell |
| | SC1124 | <i>h⁻ stn1-226</i> | This study |
| | YTC6733 ^(SC408) | <i>h⁻ stn1::13myc-kanMX6 his3-D1</i> | T. Nakamura |
| | SM1174 | <i>h⁻ stn1-226::13myc-natMX6</i> | This study |
| 1F | WT | <i>h⁻</i> | P. Russell |
| | TN7200 | <i>h⁻ ten1::5Flag-TEV-Avitag-kanMX6 his3-D1</i> | Miyagawa <i>et al.</i> , 2015 |
| | YTC6733 ^(SC408) | <i>h⁻ stn1::13myc-kanMX6 his3-D1</i> | T. Nakamura |
| | SM1174 | <i>h⁻ stn1-226::13myc-natMX6</i> | This study |
| | TN7503 ^(SC1131) | <i>h² ten1::5Flag-TEV-Avitag-kanMX6 stn1::13myc-kanMX6</i> | T. Nakamura |
| | SM1175 | <i>h² ten1::5Flag-TEV-Avitag-kanMX6 stn1-226::13myc-natMX6</i> | This study |
| 2A | SM1174 | <i>h⁻ stn1-226::13myc-natMX6</i> | This study |
| | SM1176 | <i>h² ten1::5Flag-TEV-Avitag-kanMX6 stn1-226</i> | This study |
| | SM1177 | <i>h² est1::V5-kanMX6 stn1-226</i> | This study |
| 2B-C | TN12145 ^(SC1128) | <i>h⁻ tpz1-K242R::hphMX6 his3-D1 ade-M210</i> | Miyagawa <i>et al.</i> , 2015 |
| | SM1178 | <i>h² tpz1-K242R::hphMX6 stn1-226</i> | This study |
| 3A-B | WT | <i>h⁻</i> | P. Russell |
| | SC1124 | <i>h⁻ stn1-226</i> | This study |
| 3C-E | SC838 | <i>h⁺ rhp51Δ::ura4⁺</i> | P. Russell |
| | SM1179 | <i>h² rhp51Δ::ura4⁺ stn1-226</i> | This study |
| 4 | WT | <i>h⁻</i> | P. Russell |
| | SC1124 | <i>h⁻ stn1-226</i> | This study |
| 5 | WT | <i>h⁻</i> | P. Russell |
| | SC1124 | <i>h⁻ stn1-226</i> | This study |
| 5A | YO 001 ^(SC387) | <i>h⁺ rpa1-D223Y</i> | M. Ueno |
| | SM1267 | <i>h⁺ rpa1-D223Y stn1-226</i> | This study |
| 5B-C | CF96 ^(SC388) | <i>h⁺ taz1Δ::ura4⁺</i> | J. Cooper |
| | SM1268 | <i>h⁻ taz1Δ::ura4⁺ stn1-226</i> | This study |
| 5D | TN3485 ^(SC381) | <i>h⁻ rif1Δ::ura4⁺</i> | T. Nakamura |
| | SM1269 | <i>h² rif1Δ::ura4⁺ stn1-226</i> | This study |
| | BAF394 ^(SC1310) | <i>h⁺ rif1-PP1</i> | A. Bianchi |
| | MV1332 | <i>h² rif1-PP1 stn1-226</i> | This study |
| 5E | TN3487 ^(SC392) | <i>h⁻ rap1Δ::ura4⁺</i> | T. Nakamura |
| | SM1331 | <i>h² rap1Δ::ura4⁺ stn1-226</i> | This study |
| 6A-E | WT | <i>h⁻</i> | P. Russell |
| | SC1124 | <i>h⁻ stn1-226</i> | This study |
| | SC1030 | <i>h⁺ exo1Δ::kanMX6</i> | B. Arcangioli |
| | MV1309 | <i>h⁻ exo1Δ::kanMX6 stn1-226</i> | This study |

[§]All fission yeast strains are *leu1-32 ura1-D18*

table S2. Plasmids used in this study.

| Plasmid name | Description | Plasmid n° |
|--------------------|---|------------------|
| pSH18-34 | <i>LexA_{op(x8)}-LacZ, URA3, amp^r</i> | |
| pJG4-5 | acidic activator B42, <i>TRP1</i> , <i>amp^r</i> , HA epitope tag | |
| pEG202 | <i>LexA₍₁₋₂₀₂₎DNA-BD, HIS3, amp^r</i> | |
| pJG4-5-SUMO | B42-Pmt3 | This study, p207 |
| pJG4-5-SUMO-Tpz1 | B42-Pmt3-Tpz1 ²⁴³⁻⁴²⁰ | This study, p238 |
| pJG4-5-Tpz1 | B42-Tpz1 | This study, p194 |
| pJG4-5-Tpz1-K242R | B42-Tpz1-K242R | This study, p448 |
| pJG4-5-Ten1 | B42-Ten1 | This study, p167 |
| pGAD-Ten1 | B42-Ten1 | B. Moser, p423 |
| pEG202-Ten1Stn1 | <i>LexA-Ten1_{Gly5}Stn1</i> | This study, p193 |
| pEG202-NStn1 | <i>LexA-NStn1₁₋₁₅₆</i> | This study, p217 |
| pEG202-CStn1 | <i>LexA-CStn1₁₅₇₋₃₂₅</i> | This study, p219 |
| pEG202-CStn1-195 | <i>LexA-CStn1-195</i> (¹⁹⁵ AAYA ¹⁹⁸) | This study, p242 |
| pEG202-CStn1-226 | <i>LexA-CStn1-226</i> (²²⁶ AAAA ²²⁹) | This study, p243 |
| pEG202-Stn1 | <i>LexA-Stn1</i> | This study, p162 |
| pEG202-Stn1-226 | <i>LexA-Stn1-226</i> (²²⁶ AAAA ²²⁹) | This study, p359 |
| pREP41-Pol1 | | T. Wang, p379 |
| pJK210-Stn1 | <i>ura4, stn1+</i> | This study, p323 |
| pJK210-Stn1-226 | <i>ura4, stn1-226</i> (²²⁶ AAAA ²²⁹) | This study, p331 |
| pTopo-Stn1-myc | <i>stn1-13myc :KanMX6</i> | This study, p348 |
| pTopo-Stn1-195-myc | <i>stn1-195-13myc :KanMX6</i> | This study, p382 |
| pTopo-Stn1-226-myc | <i>stn1-226-13myc :KanMX6</i> | This study, p383 |

Figure S1

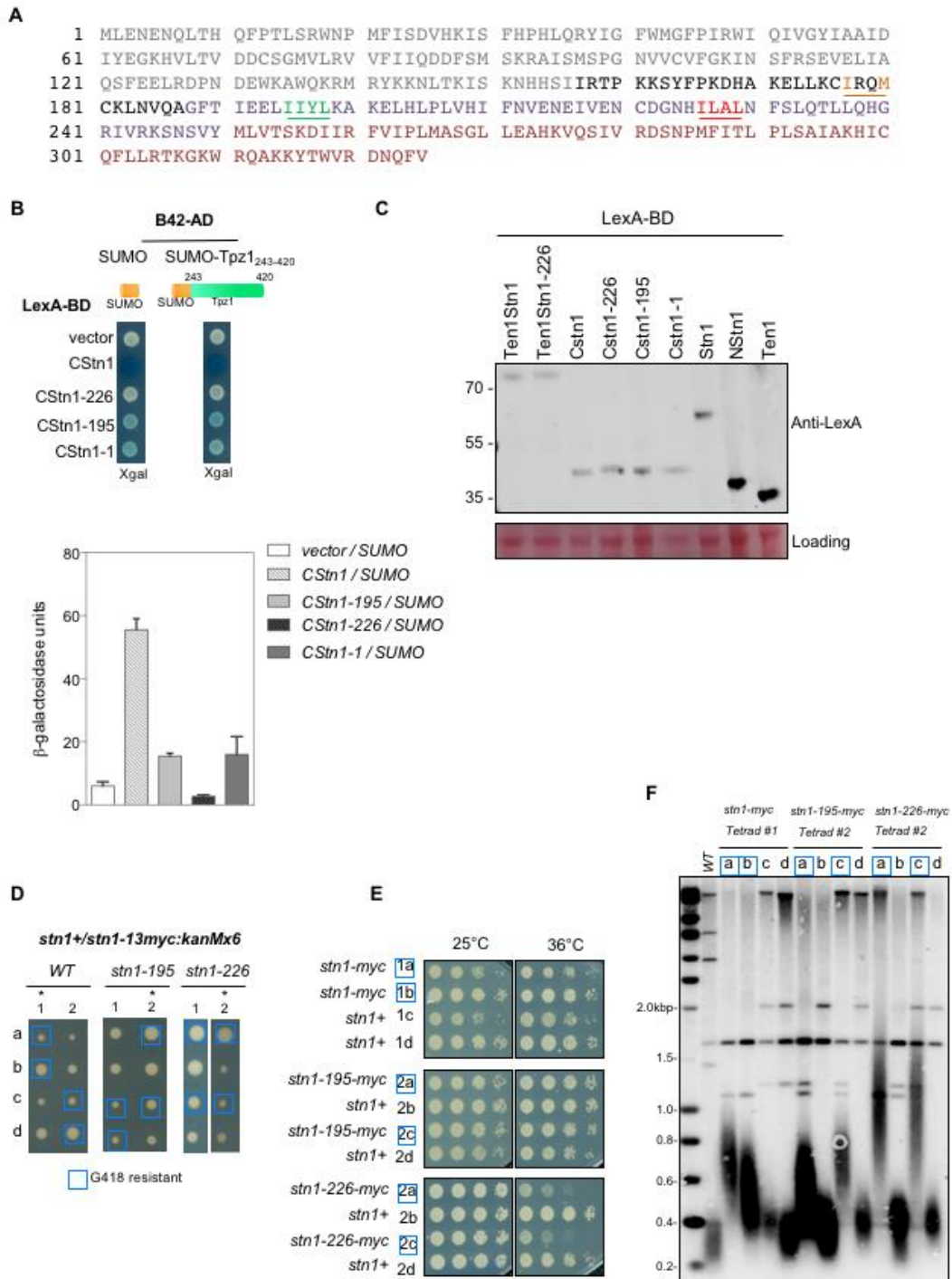
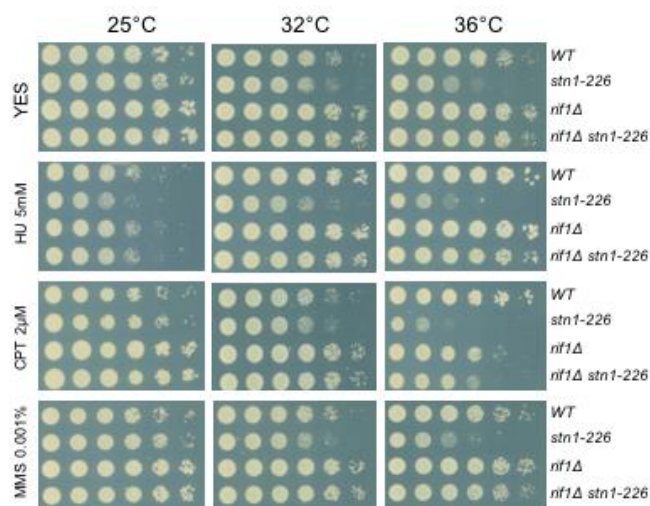


fig. S1. Comparison of Stn1-226 with other Stn1 mutants. (A) Fission yeast Stn1 protein sequence: N-terminal domain (1-155) in grey and the C-terminal domain (156-325), including the winged-helix-turn-helix domain (WH1, 188-251) in blue and the WH2 domain (252-325) are represented. The ¹⁷⁷IRQM, ¹⁹⁵IYYL and ²²⁶ILAL that

are mutated in *stn1-1*, *stn1-195* and *stn1-226* alleles are underlined, respectively. **(B)** Interaction of Stn1 fragments with SUMO and SUMO-Tpz1 analyzed by Y2H. Y2H interaction was quantified by measurement of β -galactosidase activity. **(C)** Level of LexA fusion proteins used in this study (Anti LexA (N-19) antibody, Santa Cruz Biotechnology). **(D)** Tetrad dissections at 25°C of *stn1+/*stn1-myc**, *stn1+/*stn1-195-myc** and *stn1+/*stn1-226-myc** heterozygous diploid strains. Spore colonies with the indicated genotypes are shown. **(E)** Five-fold serial dilutions of the indicated cell cultures. **(F)** Telomere length analysis of the indicated spore colonies. *Apal*-digested genomic DNA was analyzed by Southern blotting with a radiolabelled telomeric probe.

Figure S2

A



B

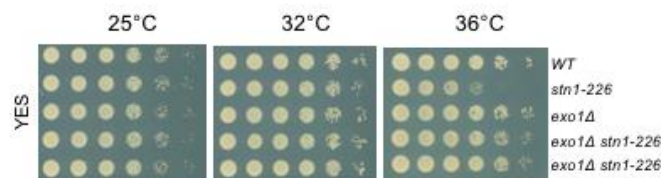


fig. S2. Deletion of *rif1+* and *exo1+* restores viability of *stn1-226* allele. (A, B)

Analysis of viability of the indicated strains at different temperatures. Cells were grown at 25°C, then serially diluted and plated on YES rich medium supplemented by the indicated amounts of CPT, MMS and HU.