Fission Yeast Stn1 Is Crucial for Semi-conservative Replication at Telomeres and Subtelomeres

Supplementary Information

Masahiro Takikawa[1], Yusuke Tarumoto[1, #], and Fuyuki Ishikawa[1, *]

[1] Department of Gene Mechanisms, Graduate School of Biostudies, Kyoto University, Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan

[#] Current address: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, United States of America

[*] Corresponding author

E-mail: fishikaw@lif.kyoto-u.ac.jp (FI)

Table S1.

Strains used in this study. Original sources in the table are (1) and (2). We adapted the gene disruption protocol reported in (3) and used primers are shown below.

rif1 disruption primers

w: 5'-ggtgataagttaataaagggtg-3'

x: 5'-GGGGATCCGTCGACCTGCAGCGTACGAtcgcaacatcagctgttatg-3'

y: 5'-GTTTAAACGAGCTCGAATTCATCGATctaattgtagtcggacaattg-3'

z: 5'-tcttcaatggatgacagaatac-3'

stn1 disruption primers

w: 5'-aagaatgcatttttccacgag-3'

x: 5'-GGGGATCCGTCGACCTGCAGCGTACGAataattgaatttgagagaagatc-3'

y: 5'-GTTTAAACGAGCTCGAATTCATCGATcattgttctatatgcaatattac-3'

z: 5'-gacatagttttatcctcatgac-3'

exo1 disruption primers

w: 5'-cctcatacagctaaattcaatg-3'

x: 5'-GGGGATCCGTCGACCTGCAGCGTACGAcattcgtcaagcaccaaaag-3'

y: 5'-GTTTAAACGAGCTCGAATTCATCGATcacctttctcattatatcacc-3'

z: 5'-agaaatgattcaactaatcctg-3'

mre11 disruption primers

w: 5'-ctaatttaaatctttgttttagtag-3'

x: 5'-GGGGATCCGTCGACCTGCAGCGTACGAcatatctgaggggtcatttg-3'

y: 5'-GTTTAAACGAGCTCGAATTCATCGATaatgtcttcctgaagatagtc-3'

z: 5'-tggtacttttttcacttctttg-3'

sds21 disruption primers

w: 5'-cttgtccctatttggtgctg-3'

x: 5'-GGGGATCCGTCGACCTGCAGCGTACGAataatcgcatcaatatcataatc-3'

y: 5'-GTTTAAACGAGCTCGAATTCATCGATgctaaatgagaactattataaag-3'

z: 5'-ccataacatagtaaatatgcatc-3'

dis2 disruption primers

w: 5'-aaaattgaatcaatttgctagac-3'

x: 5'-GGGGATCCGTCGACCTGCAGCGTACGAggaatccaaatccacatctg-3'

y: 5'-GTTTAAACGAGCTCGAATTCATCGATgtcatttctcaatggtcgatg-3'

z: 5'-caaggaatgccgaaacattg-3'

Supplementary References

1. Miyoshi, T., Sadaie, M., Kanoh, J. and Ishikawa, F. (2003) Telomeric DNA ends are essential for the localization of Ku at telomeres in fission yeast. *J Biol Chem*, **278**, 1924-1931.
2. Kanoh, J. and Ishikawa, F. (2001) spRap1 and spRif1, recruited to telomeres by Taz1, are essential for telomere function in fission yeast. *Curr Biol*, **11**, 1624-1630.
3. Krawchuk, M.D. and Wahls, W.P. (1999) High-efficiency gene targeting in Schizosaccharomyces pombe using a modular, PCR-based approach with long tracts of flanking homology. *Yeast*, **15**, 1419-1427.

S. pombe strains used in this study

	Strain	Genotype	Original source of strain	Construction method
Fig. 1B	JK317	*h⁻ leu1-32 ura4-D18*	(1) in the Supplementary manuscript	
	MT4526	*h⁻ leu1-32 ura4-D18 stn1-3flag::ura4⁺*	This study	JK317 was transformed with pMT842 (*Ustn1-stn1-3flag-Tnmt1-ura4⁺-Dstn1* based on pUC119) DNA fragment.
	MT3982	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::ura4⁺*	This study	JK317 was transformed with pMT843 (*Ustn1-stn1-1-3flag-Tnmt1-ura4⁺-Dstn1* based on pUC119) DNA fragment.
Fig. 1C	MT3967	*h⁻ leu1-32 ura4-D18 stn1-3flag::LEU2*	This study	JK317 was transformed with pMT844 (*Ustn1-stn1-3flag-Tnmt1-LEU2-Dstn1* based on pUC119) DNA fragment.
	MT3982	Fig. 1B		
Fig. 1D	JK317	Fig. 1B		
	MT4526	Fig. 1B		
	MT3982	Fig. 1B		
Fig. 1E	JK317	Fig. 1B		
	JK702	*h⁻ leu1-32 ura4-D18 taz1::ura4⁺*	(2) in the Supplementary manuscript	
	MT3982	Fig. 1B		
Fig. 1F	JK317	Fig. 1B		
	MT3982	Fig. 1B		
Fig. 2B	MT3967	Fig. 1C		
	MT3982	Fig. 1B		
Fig. 2C	MT3982	Fig. 1B		
Fig. 3A	JK317	Fig. 1B		
	MT3982	Fig. 1B		
Fig. 3C	JK317	Fig. 1B		
	MT3982	Fig. 1B		
Fig. 4A	MT3967	Fig. 1C		
	MT4510	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::LEU2*	This study	JK317 was transformed with pMT845 (*Ustn1-stn1-1-3xflag-Tnmt1-LEU2-Dstn1* based on pUC119) DNA fragment.
Fig. 4C	JK317	Fig. 1B		
	MT3982	Fig. 1B		
Fig. 4D	TM2001	*h⁻ leu1-32 ura4-D18 rad11-12myc::ura4⁺*	Lab stock	JK317 was transformed with pMP148 (*rad11-12myc-Tnmt1-ura4⁺* based on pJK202) DNA fragment.
	MT4513	*h⁹⁰ or h⁻ leu1-32 ura4-D18 stn1-1-3flag::LEU2 rad11-12myc::ura4⁺*	This study	MT4512 was mated with TM2001. Isolation by random spore.
Fig. 4E	MT4515	*h⁻ leu1-32 ura4-D18 rad22-12myc::ura4⁺*	This study	JK317 was transformed with pMT846 (*rad52-12myc-Tnmt1-ura4⁺* based on pJK202) DNA fragment.
	MT4516	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::LEU2 rad22-12myc::ura4⁺*	This study	MT4510 was transformed with pMT846 (*rad52-12myc-Tnmt1-ura4⁺* based on pJK202) DNA fragment.
Fig. 5A	JK317	Fig. 1B		
	MT3982	Fig. 1B		
Fig. 5B	JK317	Fig. 1B		
	MT3982	Fig. 1B		
Fig. 5C	MT4530	*h⁺/h⁻ ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18 stn1⁺/stn1::kanMX6*	This study	*stn1* was disrupted in YT1738
	MT4704	*h⁺/h⁻ ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18 pot1⁺/pot1::kanMX6*	This study	*pot1* was disrupted in YT1738
Fig. 5D	MT4506	*h⁻ leu1-32 ura4-D18 chk1-3ha::kanMX6 stn1-1-3flag::ura4⁺*	This study	MT3982 was transformed with pHY840 (*cds1-3ha-Tnmt1-kanMX6* based on pMP43) DNA fragment.
Fig. 5E	MT4508	*h⁻ leu1-32 ura4-D18 cds1-3ha::kanMX6 stn1-1-3flag::ura4⁺*	This study	MT3982 was transformed with pHY841 (*chk1-3ha-Tnmt1-kanMX6* based on pMP43) DNA fragment.
Fig. 6A	MT4518	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::ura4⁺* [pAL-SK]	This study	MT3982 was transformed with pAL-SK.
	MT4519	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::ura4⁺* [pAL-SK-*stn1⁺*]	This study	MT3982 was transformed with pMT847 (One isolated plasmid that contains *stn1⁺*, derived from genomic DNA library).
	MT4520	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::ura4⁺* [pAL-SK-*pmt3⁺*]	This study	MT3982 was transformed with pMT848 (*pmt3⁺-LEU2* based on pAL-SK).
	MT4522	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::ura4⁺ rif1::kanMX6* [pAL-SK]	This study	*rif1* gene was disrupted in MT4518
	MT4524	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::ura4⁺* [pAL-SK-*rif1⁺*]	This study	MT3982 was transformed with pMT849 (*rif1⁺-LEU2* based on pAL-SK).
	MT4527	*h⁻ leu1-32 ura4-D18 stn1-3flag::ura4⁺* [pAL-SK-*rif1⁺*]	This study	MT4526 was transformed with pMT849 (*rif1⁺-LEU2* based on pAL-SK).
	MT4481	*h⁹⁰ or h⁻ leu1-32 ura4-D18 stn1-1-3flag::LEU2 dis2::kanMX6*	This study	MT4512 was mated with MT4463. Isolation by random spore.
	MT4479	*h⁹⁰ or h⁻ leu1-32 ura4-D18 stn1-1-3flag::LEU2 sds21::hphMX6*	This study	MT4512 was mated with MT4461. Isolation by random spore.
Fig. 6B	MT4518	Fig. 6A		
	MT4519	Fig. 6A		
	MT4520	Fig. 6A		
	MT4662	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::ura4⁺* [pAL-SK-*pmt3-AA*]	This study	MT3982 was transformed with pMT850 (*pmt3-AA-LEU2* in pAL-SK)
	MT4629	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::ura4⁺ tpz1-K242R-3ha::kanMX6* [pAL-SK]	This study	MT4518 was transformed with pMT851 (*tpz1-K242R-3ha-Tnmt1-kanMX6* based on pMP43) DNA fragment.
	MT4631	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::ura4⁺ tpz1-K242R-3ha::kanMX6* [pAL-SK-*pmt3⁺*]	This study	MT4520 was transformed with pMT851 (*tpz1-K242R-3ha-Tnmt1-kanMX6* based on pMP43) DNA fragment.
Fig. 6C	MT3982	Fig. 1B		
	MT4520	Fig. 6A		
	MT4522	Fig. 6A		
Fig. 6D	MT4522	Fig. 6A		
Fig. S1A	JK317	Fig. 1B		

	MT3981	*h⁻ leu1-32 ura4-D18 stn1ts-1-3flag::LEU2*	This study	See Materials and Methods.
	MT4624	*h⁻ leu1-32 ura4-D18 stn1-I177M-3flag::ura4⁺*	This study	JK317 was transformed with pMT852 (*Ustn1-stn1-I177M-3flag-Tnmt1-ura4⁺-Dstn1* based on pMT8) DNA fragment.
	MT4625	*h⁻ leu1-32 ura4-D18 stn1-M180I-3flag::ura4⁺*	This study	JK317 was transformed with pMT853 (*Ustn1-stn1-M180I-3flag-Tnmt1-ura4⁺-Dstn1* based on pMT8) DNA fragment.
	MT4626	*h⁻ leu1-32 ura4-D18 stn1-V249A-3flag::ura4⁺*	This study	JK317 was transformed with pMT854 (*Ustn1-stn1-V249A-3flag-Tnmt1-ura4⁺-Dstn1* based on pMT8) DNA fragment.
	MT3983	*h⁻ leu1-32 ura4-D18 stn1-I177M M180I V249A-3flag::ura4⁺*	This study	JK317 was transformed with pMT855 (*Ustn1-stn1-I177M M180I V249A-3flag-Tnmt1-ura4⁺-Dstn1* based on pMT8) DNA fragment.
	MT4627	*h⁻ leu1-32 ura4-D18 stn1-I177M V249A-3flag::ura4⁺*	This study	JK317 was transformed with pMT856 (*Ustn1-stn1-I177M V249A-3flag-Tnmt1-ura4⁺-Dstn1* based on pMT8) DNA fragment.
	MT3982	Fig. 1B		
	MT4628	*h⁻ leu1-32 ura4-D18 stn1-M180I V249A-3flag::ura4⁺*	This study	JK317 was transformed with pMT857 (*Ustn1-stn1-M180I V249A-3xflag-Tnmt1-ura4⁺-Dstn1* based on pMT8) DNA fragment.
Fig. S1B	JK317	Fig. 1B		
	YT1738	*h⁺/h⁻ ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18*	Lab stock	
	MT3982	Fig. 1B		
	MT4645	*h⁺/h⁻ ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18 stn1⁺/stn1-1-3flag::ura4*	This study	YT1738 was transformed with pMT843 (*Ustn1-stn1-1-3flag-Tnmt1-ura4⁺-Dstn1* based on pUC119) DNA fragment.
Fig. S2	JK317	Fig. 1B		
	MT3972	*h⁻ leu1-32 ura4-D18 ten1-3ha::LEU2*	This study	JK317 was transformed with pMT859 (*ten1-3ha-Tnmt1-LEU2-Dten1* based on pUC119) DNA fragment.
	MT3967	Fig. 1C		
	MT3982	Fig. 1B		
	MT3977	*h⁻ leu1-32 ura4-D18 stn1-3flag::LEU2 ten1-3ha::ura4⁺*	This study	MT3967 was transformed with pMT858 (*ten1-3ha-Tnmt1-ura4⁺-Dten1* based on pUC119) DNA fragment.
	MT4528	*h⁹⁰ or h⁻ leu1-32 ura4-D18 stn1-1-3flag::ura4⁺ ten1-3ha::LEU2*	This study	MT3972 was mated with MT4535. Isolation by random spore.
Fig. S3B	JK317	Fig. 1B		
	MT3982	Fig. 1B		
Fig. S3C	JK317	Fig. 1B		
lanes 2, 5~8	MT3982	Fig. 1B		
	MT4531	*h⁺ or h⁻ ade6-M210 or ade6-M216 leu1-32 ura4-D18 stn1::kanMX6*	This study	*stn1* was disrupted in YT1738. Isolation by tetrad dissection.
lane 9, 10	MT4510	Fig. 4A		
Fig. S4A	JK317	Fig. 1B		
	MT3982	Fig. 1B		
	MT4532	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::ura4⁺ exo1::kanMX6*	This study	*exo1* was disrupted in MT3982.
	MT4534	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::ura4⁺ mre11::kanMX6*	This study	*mre11* was disrupted in MT3982.
Fig. S4B	MT4532	Fig. S4A		
	MT4650	*h⁹⁰ or h⁻ leu1-32 ura4-D18 stn1-1-3flag::LEU2 rqh1::kanMX6*	This study	MT4512 was mated with TM1159. Isolation by random spore.
	MT4652	*h⁹⁰ or h⁻ leu1-32 ura4-D18 stn1-1-3flag::LEU2 rqh1::kanMX6 exo1::hphMX6*	This study	*exo1* was disrupted in MT4650.
Fig. S5	MT4660	*h⁹⁰ or h⁺ leu1-32 ura4-D18 stn1-1-3flag::LEU2 rad16::ura4⁺*	This study	MT4512 was mated with TN144. Isolation by random spore.
Fig. S6A	JK317	Fig. 1B		
	MT3982	Fig. 1B		
Fig. S6B	JK317	Fig. 1B		
	MT3982	Fig. 1B		
Fig. S6C	MT4520	Fig. 6A		
	MT4522	Fig. 6A		
Fig. S7A	MT4530	Fig. 5C		
	MT4704	Fig. 5C		
Fig. S7B	MT4530	Fig. 5C		
	MT4704	Fig. 5C		
Fig. S8	JK317	Fig. 1B		
lanes 2, 4~6	MT4522	Fig. 6A		
	MT3982	Fig. 1B		
Fig. S10A	TM2241	*h⁻ leu1-32 ura4-D18 pol1-12myc::ura4⁺*	Lab stock	JK317 was transformed with pMP187 (*pol1-12myc-Tnmt1-ura4⁺* based on pJK202) DNA fragment.
	MT4664	*h⁹⁰ or h⁻ leu1-32 ura4-D18 stn1-1-3flag::LEU2 pol1-12myc::ura4⁺*	This study	MT4512 was mated with TM2241. Isolation by random spore.
Fig. S10B	MT4666	*h⁻ leu1-32 ura4-D18 pol2-12myc::ura4⁺*	This study	JK317 was transformed with pMT860 (*pol2-12myc-Tnmt1-ura4⁺* based on pJK202) DNA fragment.
	MT4668	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::LEU2 pol2-12myc::ura4⁺*	This study	MT4510 was transformed with pMT860 (*pol2-12myc-Tnmt1-ura4⁺* based on pJK202) DNA fragment.
Fig. S10C	MT4670	*h⁻ leu1-32 ura4-D18 pol3-12myc::ura4⁺*	This study	JK317 was transformed with pMT861 (*pol3-12myc-Tnmt1-ura4⁺* based on pJK202) DNA fragment.
	MT4672	*h⁻ leu1-32 ura4-D18 stn1-1-3flag::LEU2 pol3-12myc::ura4⁺*	This study	MT4510 was transformed with pMT861 (*pol3-12myc-Tnmt1-ura4⁺* based on pJK202) DNA fragment.
Table S1	MT4461	*h⁻ leu1-32 ura4-D18 sds21::hphMX6*	This study	*sds21* was disrupted in JK317.
	MT4463	*h⁻ leu1-32 ura4-D18 dis2::kanMX6*	This study	*dis2* was disrupted in JK317.
	MT4512	*h⁹⁰ leu1-32 ura4-D18 stn1-1-3flag::LEU2*	This study	JK800 was transformed with pMT845 (*Ustn1-stn1-1-3flag-Tnmt1-LEU2-Dstn1* based on pUC119) DNA fragment.
	MT4535	*h⁹⁰ leu1-32 ura4-D18 stn1-1-3flag::ura4⁺*	This study	JK800 was transformed with pMT843 (*Ustn1-stn1-1-3flag-Tnmt1-ura4⁺-Dstn1* based on pUC119) DNA fragment.

Fig. S1

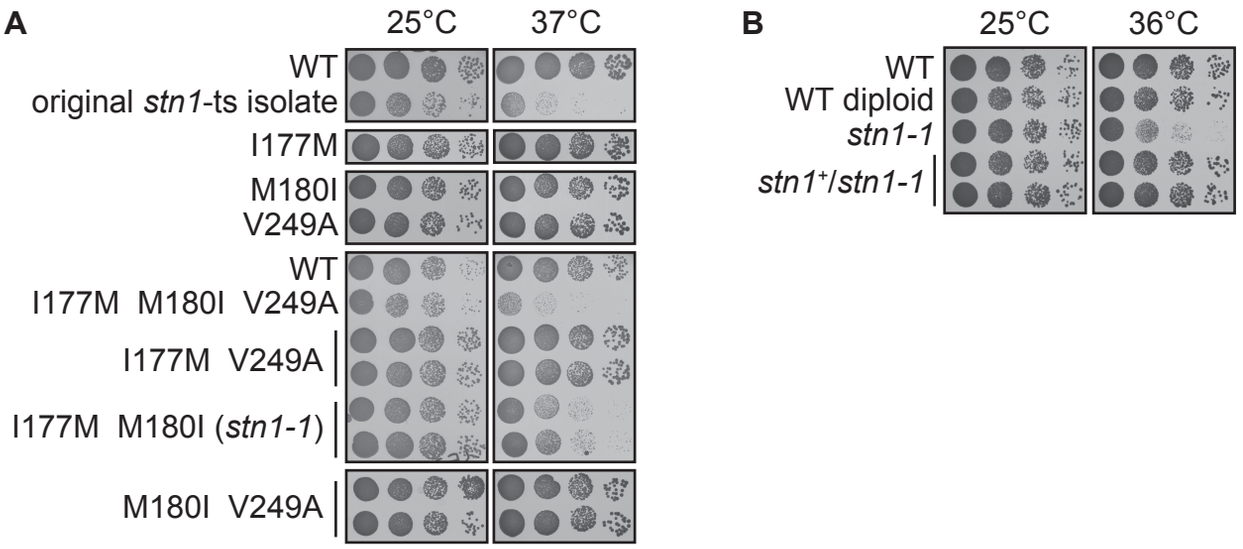

Figure S1.

(A) Ten-fold serial dilutions were spotted onto YES plates and incubated at 25°C or 37°C for 3 days. (B) WT (haploid), WT diploid, *stn1-1* (haploid), and *stn1*$^{+}$/*stn1-1* cells were spotted onto YES plates and incubated at 25°C for 3 days and at 36°C for 2 days.

Fig. S2

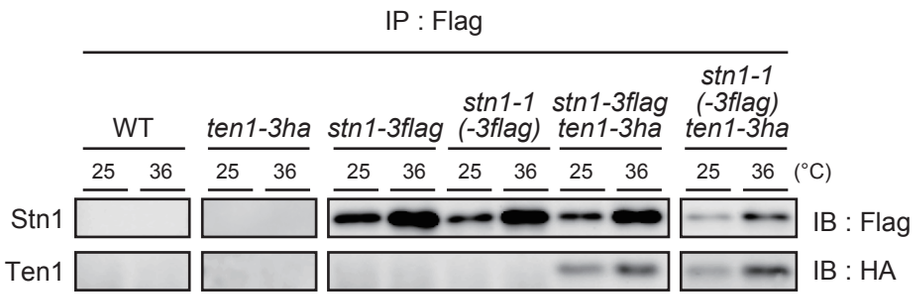

Figure S2.

Flag- or HA-tagged Stn1 or Ten1 expressing strains were used in immunoprecipitation-immunoblotting experiments. Each strain was cultivated at 25°C or 36°C for 16 hr. All the samples were immunoprecipitated with anti-Flag antibodies and immunoblotted with anti-Flag and anti-HA antibodies.

Fig. S3

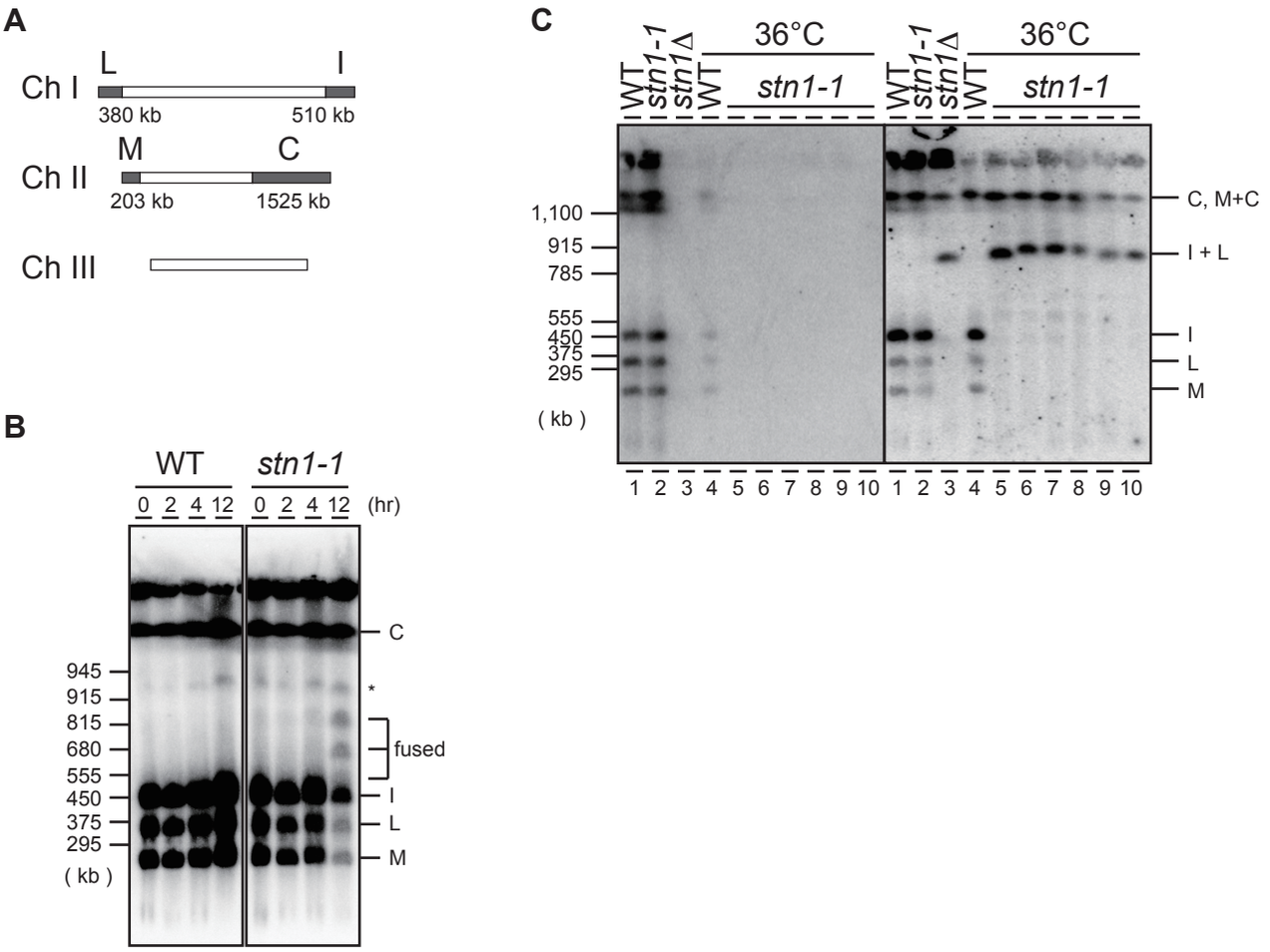

Figure S3.

(A) A model of *S. pombe* chromosomes and the telomere-proximal Not I sites. (B) Wild type and *stn1-1* were cultured at 36°C for the indicated times. Pulsed-field gel electrophoresis was conducted and the membrane was hybridized with I, L, M, and C probes. Non-specific signals are indicated by asterisk. (C) Wild type (25°C), *stn1-1* (25°C), and *stn1*Δ (32°C) were harvested in YES liquid media (lanes 1-3). Before harvesting in YES liquid media at 36°C, wild type and *stn1-1* were sequentially streaked 4 times on YES plates at 36°C. *stn1-1* were picked up from 6 independent colonies (lanes 5-10). Pulsed-field gel electrophoresis was conducted and hybridized with a telomeric probe (left) and I, L, M, and C probes (right).

Fig. S4

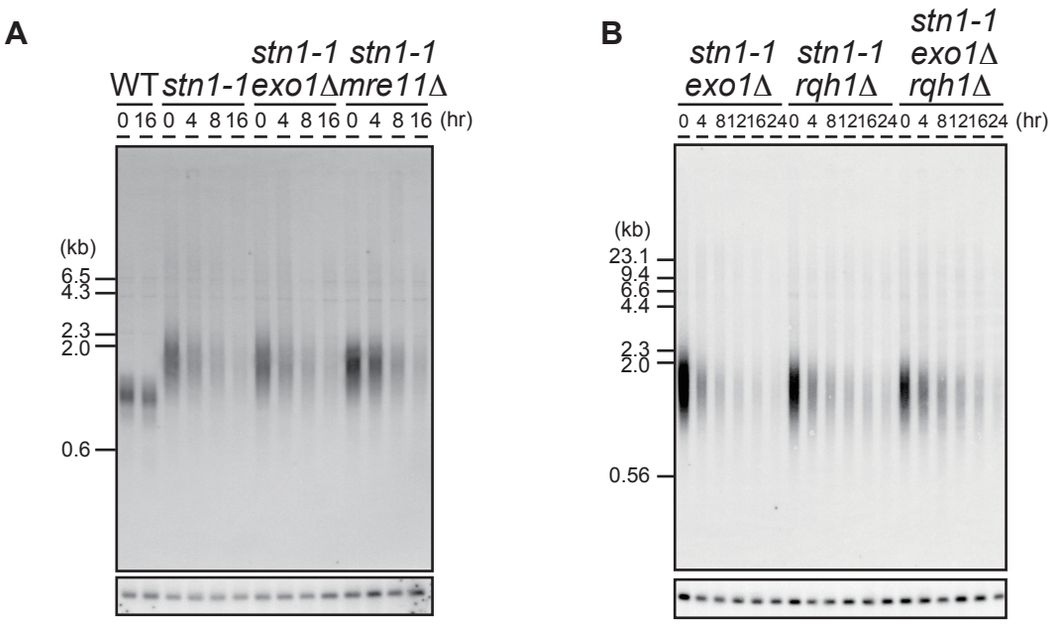

Figure S4.

(A) Wild type, *stn1-1*, *stn1-1 exo1*Δ, and *stn1-1 mre11*Δ, and (B) *stn1-1 exo1*Δ, *stn1-1 rqh1*Δ, and *stn1-1 exo1*Δ *rqh1*Δ were cultured at 25°C and shifted to 36°C for the indicated times. Southern hybridizations were carried out as for Figure 1E. The membrane was hybridized with the telomeric probe (upper) and re-hybridized with the control region probe (bottom).

Fig. S5

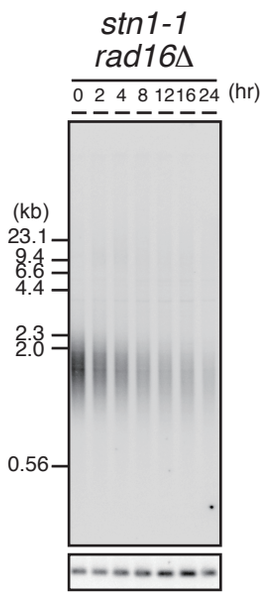

Figure S5.

*stn1-1 rad16*Δ was cultured at 25 °C and shifted to 36°C for the indicated times. Southern hybridizations were carried out as for Figure 1E. The membrane was hybridized with the telomeric probe (upper) and re-hybridized with the control region probe (bottom).

Fig. S6

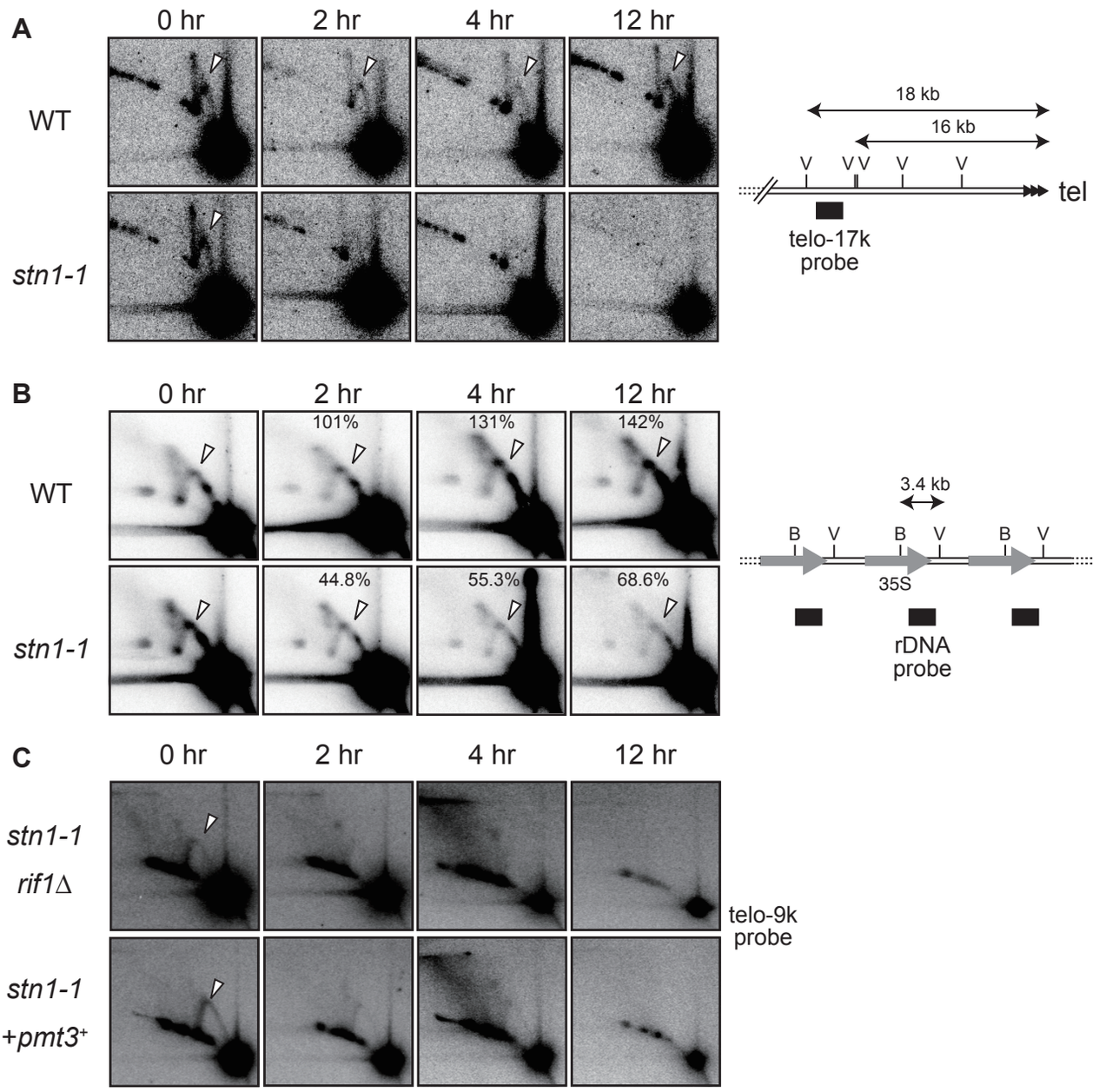

Figure S6.

(A, B) Wild type and *stn1-1* were cultured at 36°C for the indicated times. (A) EcoRV-digested DNA and (B) EcoRV- and Bgl II-digested DNA was resolved with neutral-neutral 2D gel electrophoresis, and hybridization was conducted with (A) telo-17k and (B) rDNA probe, respectively. Replication intermediate signals were quantitated and the changes from 0 hr were described. (C) *stn1-1 rif1*Δ and *stn1-1 pmt3* overexpressing strains were cultivated at 36°C for the indicated times.

Fig. S7

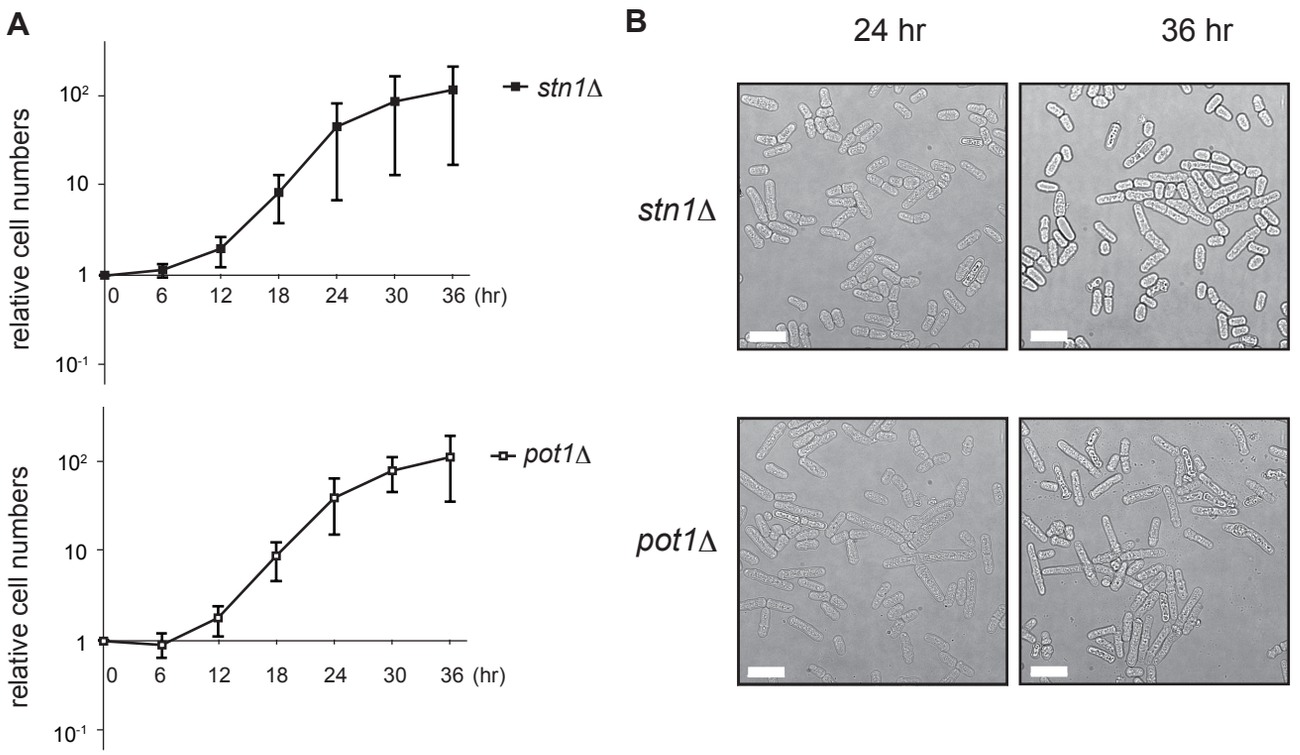

Figure S7.

(A) *stn1+ / stn1*Δ and *pot1+ / pot1*Δ diploid cells were germinated and the relative cell numbers were counted. Germinated *stn1*Δ and *pot1*Δ were cultured up to 36 hours. Error bars represent mean values of three independent experiments with SD. (B) Representative micrographs of germinated *stn1*Δ and *pot1*Δ cells. The scale bar represents 15 μm.

Fig. S8

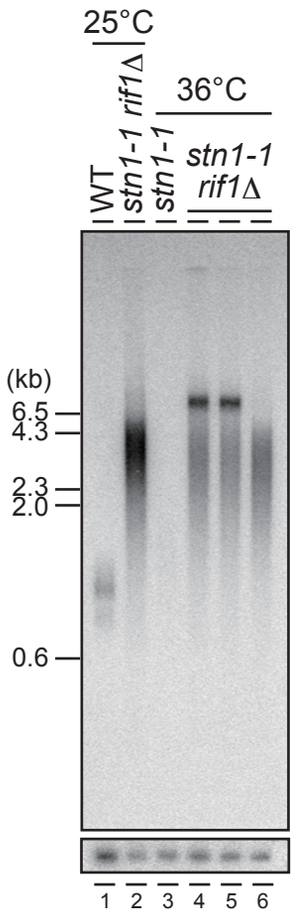

Figure S8.

Wild type and *stn1-1 rif1*Δ were cultured and harvested at 25°C (lanes 1 and 2). Before harvesting in YES liquid media at 36°C, *stn1-1* and *stn1-1 rif1*Δ were sequentially streaked 6 times (over a period of 18 days) on YES plates at 36°C. *stn1-1 rif1*Δ were picked up from 3 independent colonies (lanes 4-6). Southern hybridizations were carried out as for Figure 1E.

Fig. S9

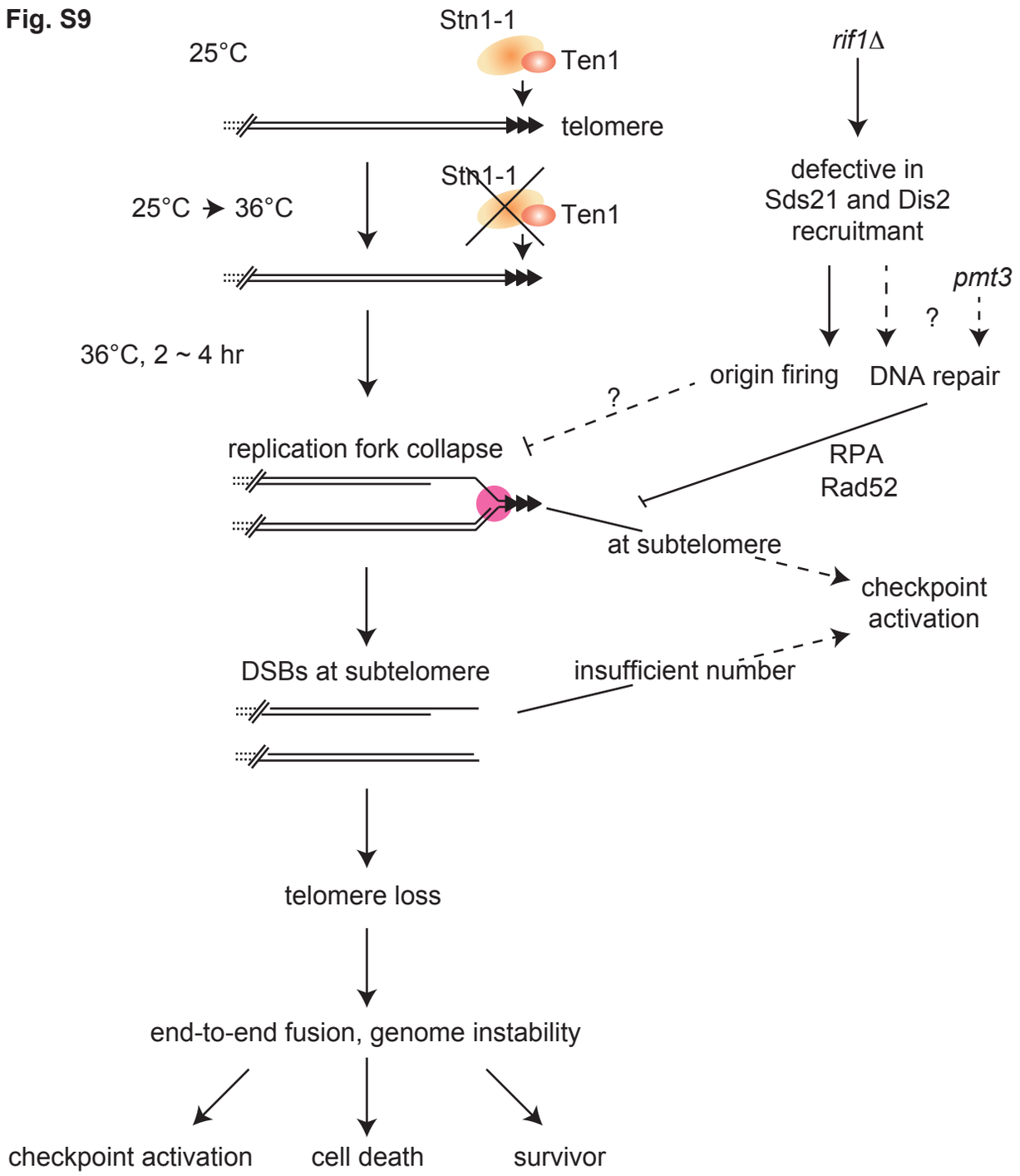

Figure S9.

Schematic model based on this study. Immediately after the temperature is shifted to the restrictive temperature, Stn1-1 loses function, and replication forks frequently collapse at subtelomeres. Fork collapse results in DSB formation and telomere loss. Severe DNA damage at telomeres and subtelomeres is spontaneously suppressed via Pot1 accumulation.

Fig. S10

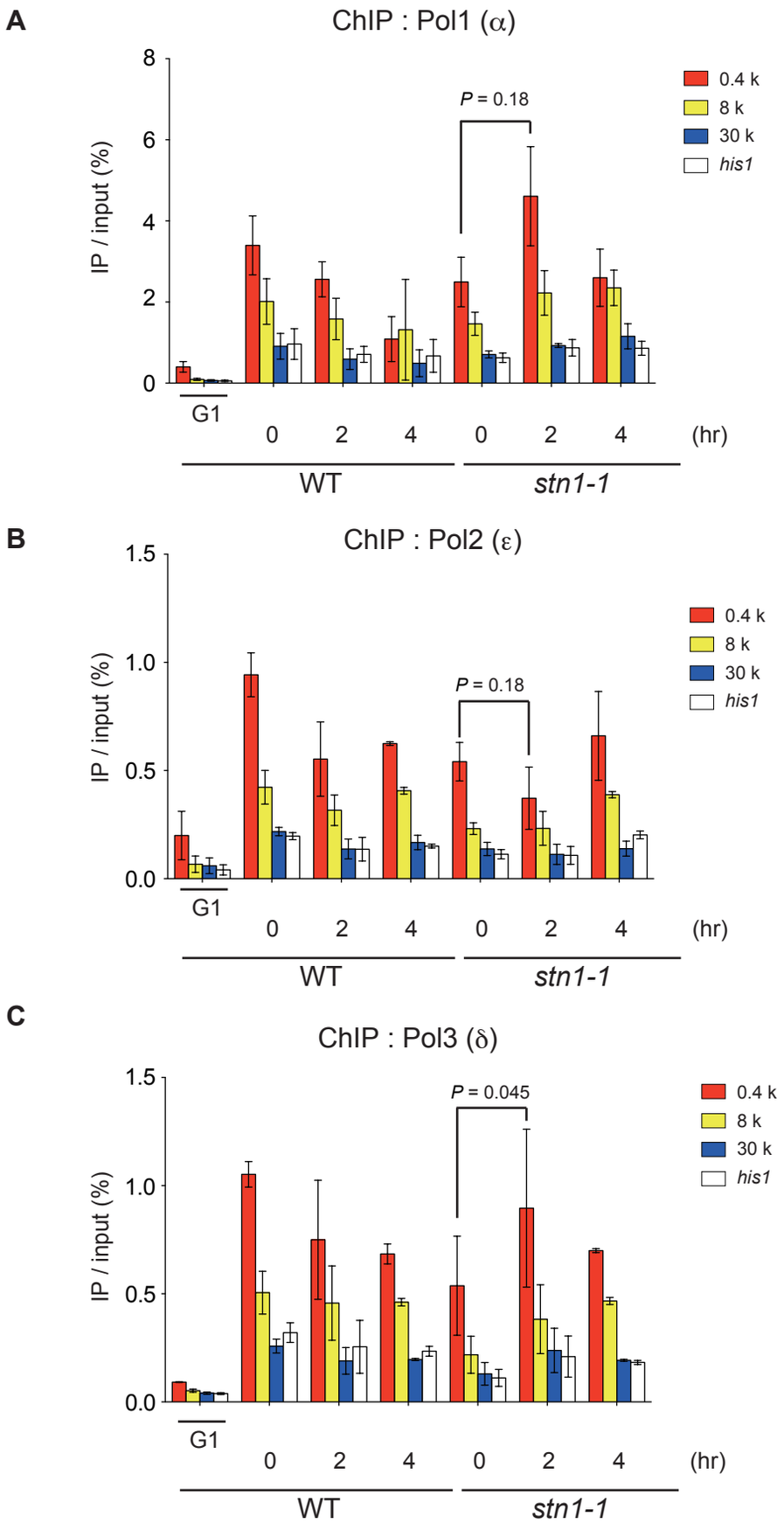

Figure S10.

(A, B, C) C-terminally myc-tagged (A) Pol1 (α), (B) Pol2 (ε), and (C) Pol3 (δ) expressing wild type and *stn1-1* were cultured at the indicated times and analyzed by ChIP-qPCR. Error bars show mean values of three independent experiments with SD. Each *P* value was calculated with a two-tailed Student' s t-test.