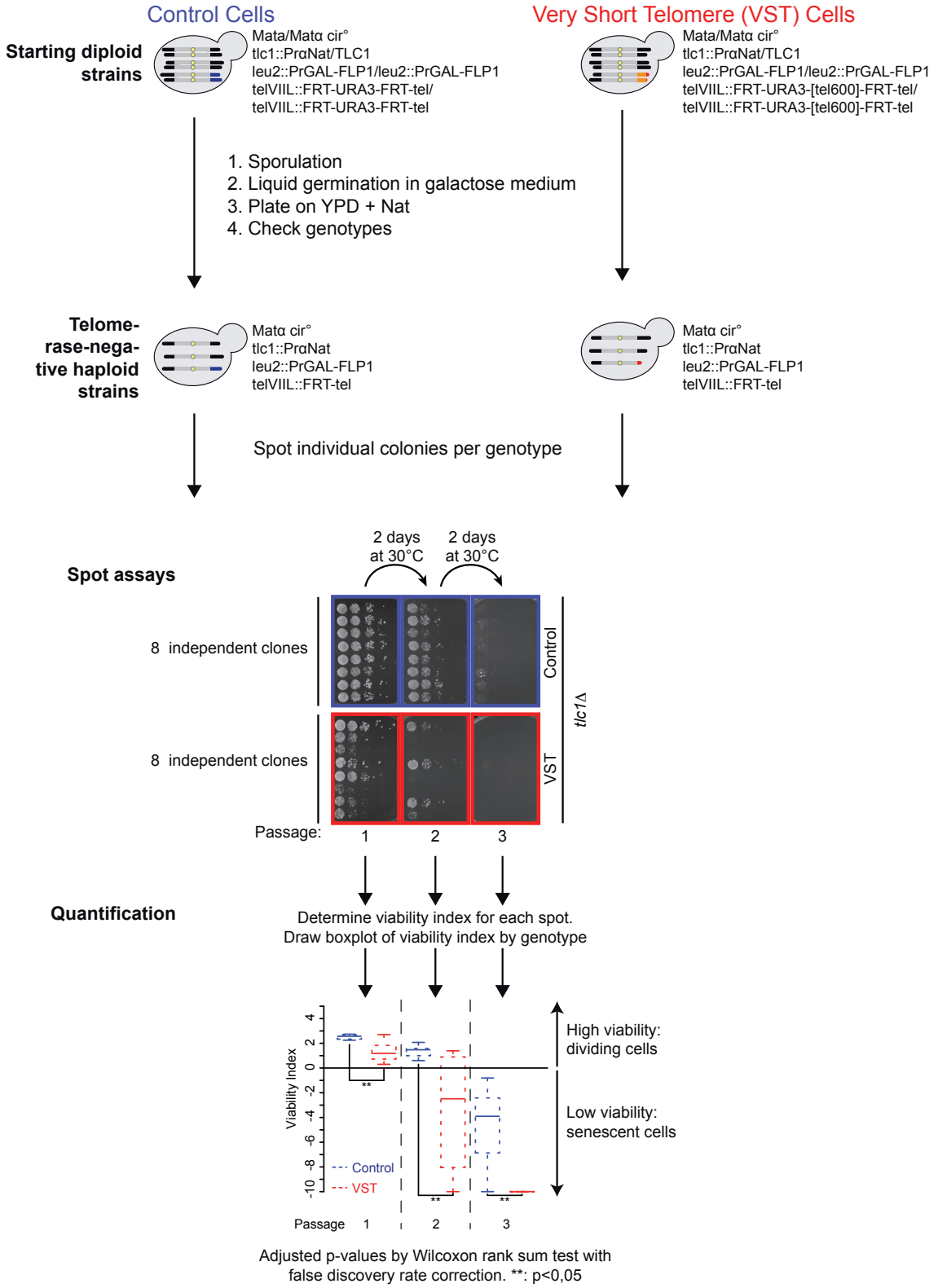
SUPPLEMENTARY INFORMATION

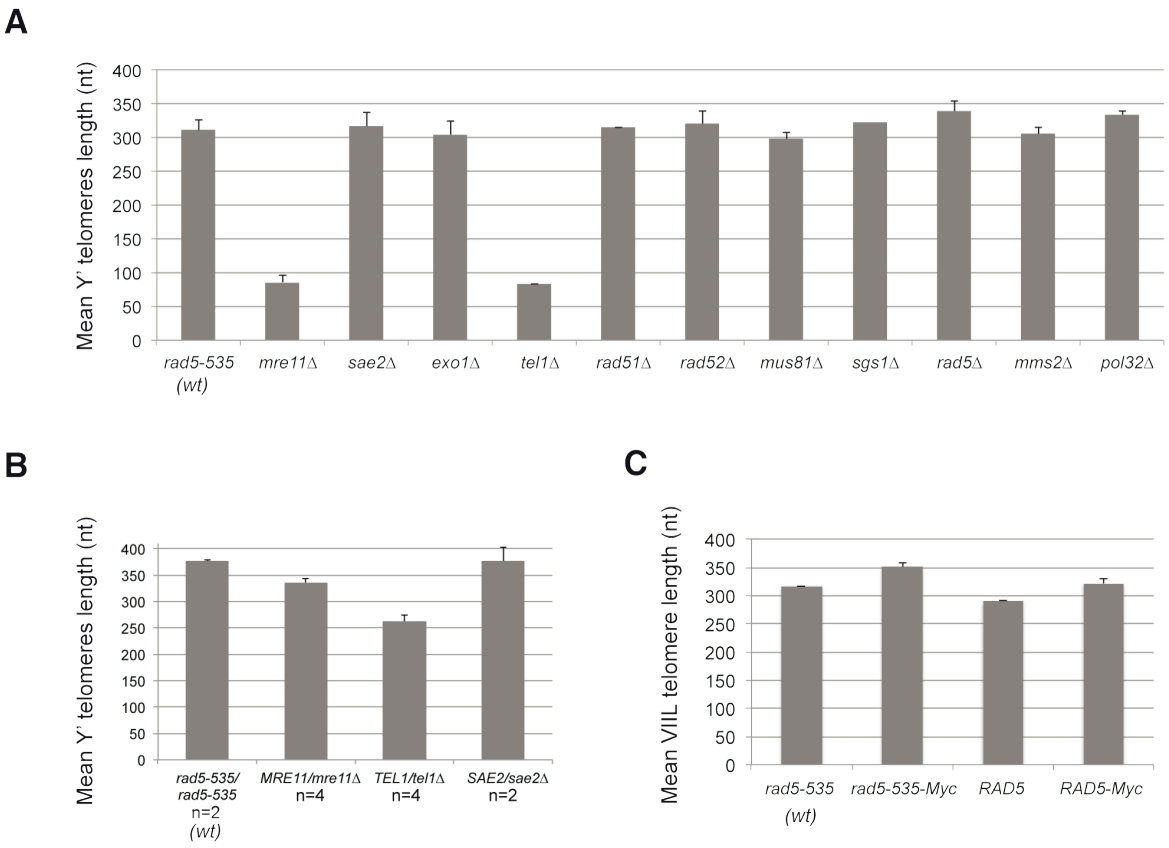
Fallet et al., Figure Supp 1



Supplementary Figure 1: Experimental setup for the study of one single very short telomere in senescence.

In starting diploid strains, one copy of the telomerase template RNA gene TLC1 was replaced by a gene encoding resistance to Nourseothricin under the control of a haploid-specific promoter (*PraNat*; (1,2). These starting diploids also contained at the homozygous state the artificial construct detailed in Figure 1B (either the Control or VST construct) replacing the last 15 kb of both VIIL arms. *FLP1,* encoding the site-specific recombinase, was placed under the control of a promoter inducible by galactose. *cir°* indicates that these strains were deprived of the 2micron to avoid toxicity upon the induction of Flp1 (3). Additional mutations in DNA-processing genes were introduced at the heterozygous state in these diploids using the selectable markers *His3MX6*, *TRP1,* or *KanMX6* (4). These diploids were sporulated, germinated for 6–9 h in galactose-containing liquid media to induce the excision of the region between the two FRT sites (Figure 1B), plated in Nourseothricin-containing media to select for *tlc1Δ* spores and allowed to form colonies for 40 h. Many of these independent telomerase-negative haploid spore colonies were genotyped by streaking part of the colonies on selective media lacking uracil (to check for *URA3* excision) and histidine or tryptophan or containing G418 (to check for deletion of additional mutations in DNA repair pathways). For each experiment, *tlc1Δ ura3* independent colonies containing additional mutations or not were followed by serial spot assays. For each genotype, 8 to 16 independent colonies were studied. A viability index could be determined for each sample at each passage by quantification of digitalized images of the spots. Viability indexes are plotted by genotype for each passage. Adjusted p values were obtained by Wilcoxon rank-sum test with a false discovery rate correction. *: $p < 0.1$; **: $p < 0.05$. See Supplementary Table 2 for detailed p values. Each full experiment was repeated at least twice with independent diploid starting strains and similar results were obtained.

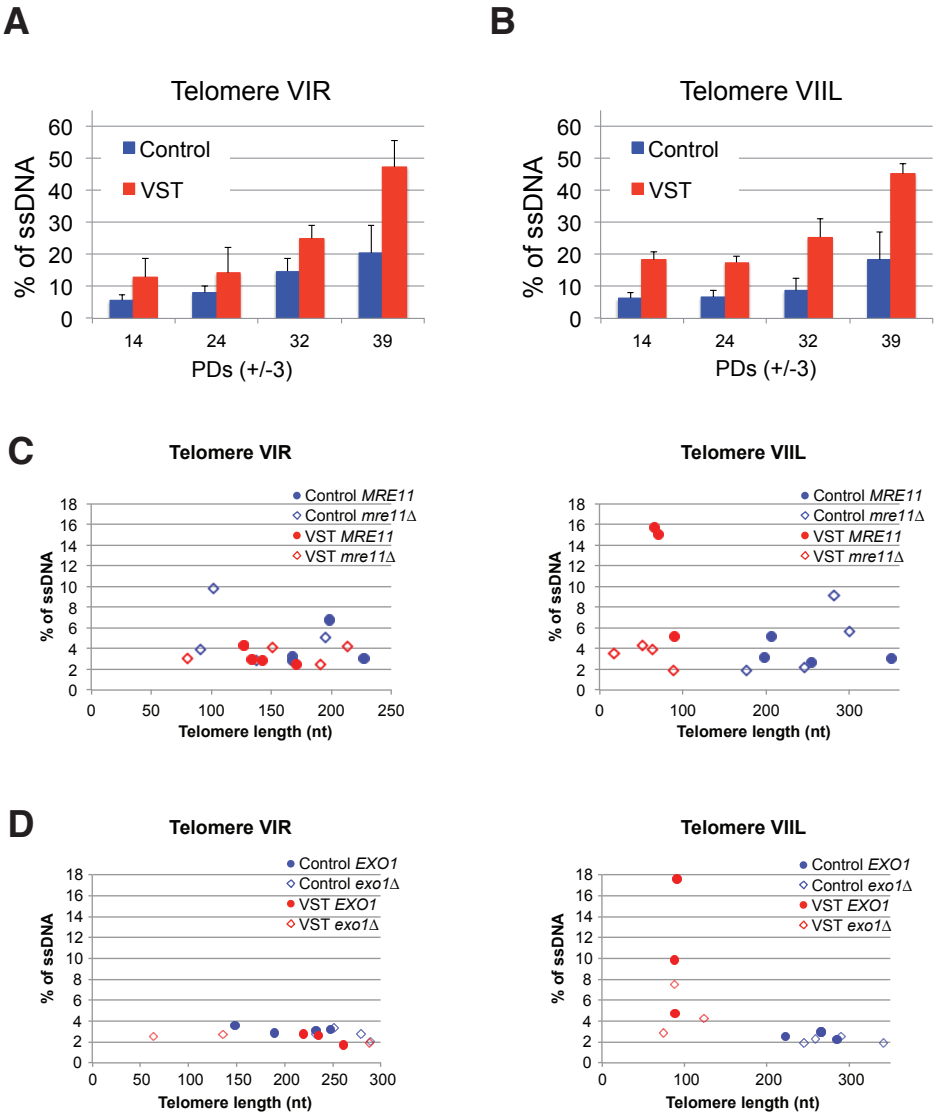
Fallet et al., Supplementary Figure 2



Supplementary Figure 2: Telomere length of various strains used in this study

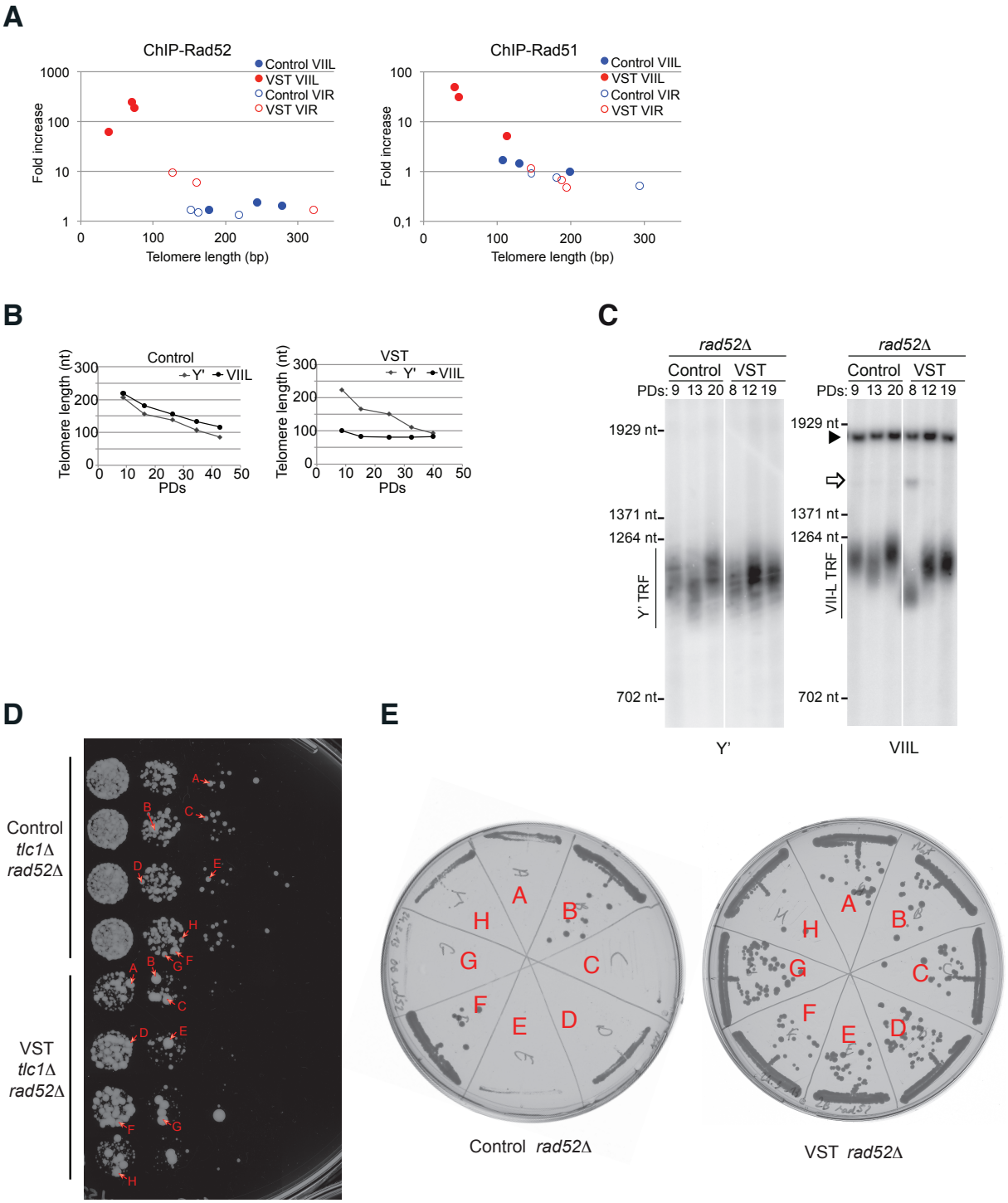
(A) Mean length of Y' telomeres +/- s.d. in TLC1 haploids with the indicated genotypes were determined by telomere-PCR (5). n=2 except for sgs1Δ (n=1) and rad5Δ (n=3). **(B)** Mean length of Y' telomeres +/- s.d. in TLC1/tlc1Δ diploids with the indicated genotypes obtained as in (A). **(C)** Mean length of the VII-L telomere +/- s.d. in TLC1 haploids with the indicated genotypes were determined by telomere-PCR. n=2

Fallet et al., Supplementary Figure 3



Supplementary Figure 3: The accumulation of subtelomeric ssDNA contributes to senescence.

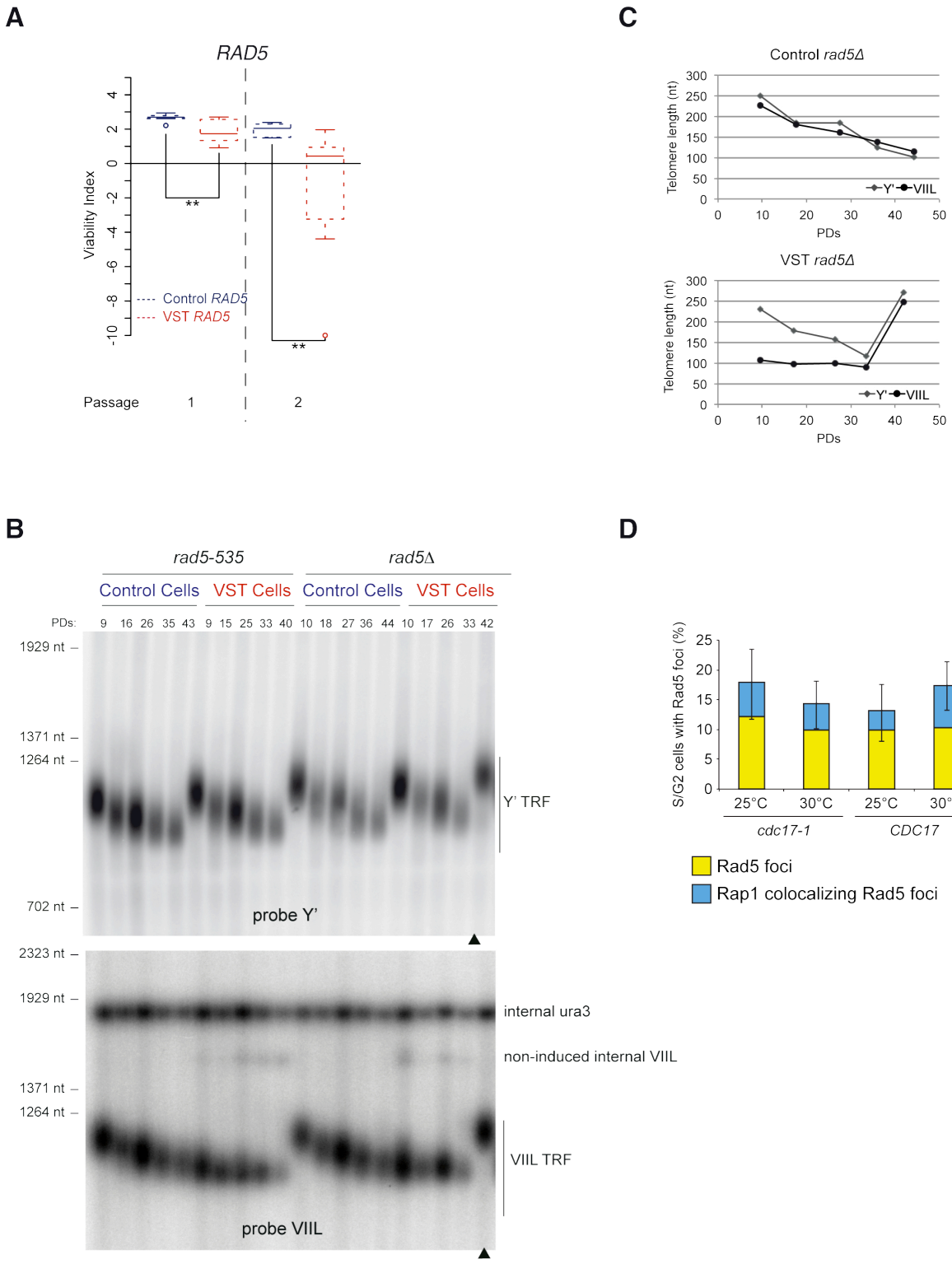
(A–B) The results of ssDNA quantification in Figure 2A represented as histograms. Error bars indicate s.e.m. from 3 independent experiments. **(A)** Telomere VIR. **(B)** Telomere VIIL **(C)** The results of ssDNA quantification for *MRE11/mre11Δ* in Figure 2C represented as a function of telomere length. **(D)** The results of ssDNA quantification for *EXO1/exo1Δ* in Figure 2D represented as a function of telomere length.



Supplementary Figure 4: Function of HR factors in senescence.

(A) Rad52 and Rad51 association with telomeres is length dependent. The fold increase in the association of Rad52 and Rad51 with telomeres compared to *ARO1* was plotted as a function of telomere length, as determined by telomere PCR, for VIIL (full circles) and VIR (open circles) in Control (blue) and VST (red) cells. Samples are the same as in Figure 3B. **(B)** The lengths of Y' (left

panel) and VIIL (right panel) telomeres in Control and VST cells were measured by Southern blot as shown in Figure 4B. **(C)** Southern blot as in Figure 4B in *tlc1⊿ rad52⊿* Control and VST cells. **(D–E)** Selective pressure in cultures of *rad52Δ tlc1Δ* VST cells. **(D)** Cells from cultures at PD = 8–9 of Control and VST *rad52Δ tlc1Δ* were spotted on selective medium (4000 cells on the most concentrated spot and 10-fold dilutions) and grown for 2 days at 30°C. **(E)** Eight colonies, labelled A to H, were selected from the spots of Control and VST cells, streaked on plates, and grown again for 2 days at 30°C to check for the loss of viability expected after the loss of *TLC1*.



Supplementary Figure 5: Other Rad5 effects.

(A) A very short telomere accelerates senescence in wild-type *RAD5* cells. Quantitative analysis of senescence as in Figure 1C of 8 *RAD5 tlc1Δ* derivatives of yT357 and yT358. **(B)** Rad5-535 does not affect telomere length in the absence of telomerase. *rad5Δ tlc1Δ* Control and VST cells were selected by mass sporulation of yT299 (Control) and yT300 (VST) and plated on galactose medium containing

Nourseothricin but not histidine. Cells were resuspended and inoculated in glucose-rich medium containing Nourseothricin and diluted every day to 10^5 cells/mL. DNA was digested with *Xho* I (Y') or *Stu* I (VIIL), electrophoresed, transferred to a membrane, and probed with a radioactive-labelled Y' or VIIL probe. Terminal restriction fragments (TRFs) are indicated. The VIIL probe also hybridized to the internal *ura3-1* locus as indicated. Arrow heads indicate samples containing post-senescent survivors. **(C)** Telomere length was measured from TRF mobility in electrophoresis and plotted against the number of PDs after the loss of *TLC1* for Y' (diamonds) and VIIL (circles) telomeres. **(D)** Single-stranded G-tails are not the target for Rad5 recruitment to telomeres. Rad5-YFP foci were monitored in wild-type (ML690-16A) and *cdc17-1* mutant (ML690-6B) cells at permissive (25°C) and semi-permissive (30°C) temperatures, at which the *cdc17-1* mutation causes telomerase-dependent overextension of single-stranded G-tails (6).

Supplementary Table 1: Yeast strains used in this study

All strains are in the W303 background. All strains were constructed for this work, except those labelled with *, which were described previously (2).

Strain	Genotype
yT136*	Mata/α cir° ura3-1/ura3-1 trp1-1/trp1-1 his3-11,15/his3-11,15 can1-100/can1-100 ade2-1/ade2-1 leu2-3,112::Gal-FLP1-LEU2/leu2-3,112::Gal-FLP1-LEU2 rad5-535/rad5-535 tlc1::PrαNat/TLC1 telVIIL::FRT-URA3-FRT-tel/telVIIL::FRT-URA3-FRT-tel
yT138*	Mata/α cir° ura3-1/ura3-1 trp1-1/trp1-1 his3-11,15/his3-11,15 can1-100/can1-100 ade2-1/ade2-1 leu2-3,112::Gal-FLP1-LEU2/leu2-3,112::Gal-FLP1-LEU2 rad5-535/rad5-535 tlc1::PrαNat/TLC1 telVIIL::FRT-URA3-[tel600]-FRT-tel/telVIIL::FRT-URA3-[tel600]-FRT-tel
yT143*	yT136 rad52::kanMX4/RAD52
yT145*	yT138 rad52::kanMX4/RAD52
yT174*	yT136 tel1::HIS3/TEL1
yT176*	yT138 tel1::HIS3/TEL1
yT225	yT136 pol32::TRP1/POL32
yT226	yT138 pol32::TRP1/POL32
yT231	yT136 sae2::TRP1/SAE2
yT232	yT138 sae2::TRP1/SAE2
yT235	yT136 mre11::HIS3/MRE11
yT236	yT138 mre11::HIS3/MRE11
yT257	yT136 sgs1::TRP1/SGS1
yT258	yT138 sgs1::TRP1/SGS1
yT299	yT136 rad5::HIS3/rad5-535
yT300	yT138 rad5::HIS3/rad5-535
yT303	yT136 mms2::HIS3/MMS2
yT304	yT138 mms2::HIS3/MMS2
yT347	yT136 rad51::HIS3/RAD51
yT348	yT138 rad51::HIS3/RAD51
yT355	yT136 mus81::HIS3/MUS81
yT356	yT138 mus81::HIS3/MUS81
yT357	yT136 rad5::HIS3/RAD5
yT358	yT138 rad5::HIS3/RAD5
yT403	yT136 exo1::HIS3/EXO1
yT404	yT138 exo1::HIS3/EXO1
yT567	yT138 rad5::HIS3/rad5-535 rad52::kanMX4/RAD52
yT580	Mata cir° rad5-535 ura3-1 trp1-1 his3-11,15 can1-100 ade2-1 leu2-3,112::Gal-FLP1-LEU2 telVIIL::FRT-tel (lev187 (3) which has excised the URA3-containing circle)
yT581	yT580 rad5-535-myc::HIS3MX6
yT582	yT580 RAD5-myc::HIS3MX6
yT583	yT580 RAD5
ML690-16A	Mat a ADE2 trp1-1 RAD5-YFP RAP1-CFP::LEU2 RAD52-RFP
ML690-16B	ML690-16A *cdc17-1*
ML691-12C	Mat a ADE2 trp1-1 RAD5-YFP RAP1-CFP::LEU2 RAD52-RFP est2::KanMX (pVL291: EST2)

Supplementary Table 2: p-values for the spot assays determined by pairwise Wilcoxon rank sum test with a false discovery rate adjustment

Experiment	Genotype 1	Genotype 2	Passage	p- value
MRE11	Control MRE11	VST MRE11	1	0.00093
Figure 1C	Control MRE11	VST MRE11	2	0.0539
	Control MRE11	Control mre11	1	0.15646
	Control MRE11	Control mre11	2	0.0036
	VST MRE11	VST mre11	1	0.18928
	VST MRE11	VST mre11	2	0.0287
	Control mre11	VST mre11	1	0.08778
	Control mre11	VST mre11	2	0.0033
	Control MRE11	VST mre11	1	0.00373
	Control MRE11	VST mre11	2	1.0000
	Control mre11	VST MRE11	1	0.00093
	Control mre11	VST MRE11	2	0.0033
SAE2	Control SAE2	VST SAE2	1	0,00027
Figure 1D	Control SAE2	VST SAE2	2	0,000035
	Control SAE2	VST SAE2	3	0,02288
	Control SAE2	Control sae2	1	0,00027
	Control SAE2	Control sae2	2	0,2388
	Control SAE2	Control sae2	3	0,00431
	VST SAE2	VST sae2	1	0,02642
	VST SAE2	VST sae2	2	0,0159
	VST SAE2	VST sae2	3	0,19298
	Control sae2	VST sae2	1	0,00064
	Control sae2	VST sae2	2	0,0014
	Control sae2	VST sae2	3	0,00103
	Control SAE2	VST sae2	1	0,000016
	Control SAE2	VST sae2	2	0,0109
	Control SAE2	VST sae2	3	0,16861
	Control sae2	VST SAE2	1	0,97787
	Control sae2	VST SAE2	2	0,000023
	Control sae2	VST SAE2	3	0,00053
EXO1	Control EXO1	VST EXO1	1	7.4e-06
Figure 1E	Control EXO1	VST EXO1	2	8.1e-06
	Control EXO1	VST EXO1	3	0.00022
	Control EXO1	Control exo1	1	0.80913
	Control EXO1	Control exo1	2	0.035
	Control EXO1	Control exo1	3	0.00083
	VST EXO1	VST exo1	1	0.28655
	VST EXO1	VST exo1	2	0.022
	VST EXO1	VST exo1	3	0.74296
	Control exo1	VST exo1	1	0.00013
	Control exo1	VST exo1	2	8.1e-06
	Control exo1	VST exo1	3	2.0e-05
	Control EXO1	VST exo1	1	7.4e-05
	Control EXO1	VST exo1	2	2.8e-05
	Control EXO1	VST exo1	3	0.00010
	Control exo1	VST EXO1	1	1.2e-05
	Control exo1	VST EXO1	2	8.1e-06
	Control exo1	VST EXO1	3	6.8e-05

RAD51		Control RAD51	VST RAD51	1	0,027
Figure 3A		Control RAD51	VST RAD51	2	0,05889
		Control RAD51	Control rad51	1	0,000052
		Control RAD51	Control rad51	2	0,01433
		VST RAD51	VST rad51	1	0,011
		VST RAD51	VST rad51	2	0,00028
		Control rad51	VST rad51	1	0,014
		Control rad51	VST rad51	2	0,00273
		Control RAD51	VST rad51	1	0,0000044
		Control RAD51	VST rad51	2	0,00016
		Control rad51	VST RAD51	1	0,393
		Control rad51	VST RAD51	2	0,2093
POL32		Control POL32	VST POL32	1	0.01329
Figure 3B		Control POL32	VST POL32	2	0.00062
		Control POL32	Control pol32	1	0.00591
		Control POL32	Control pol32	2	0.00062
		VST POL32	VST pol32	1	0.01329
		VST POL32	VST pol32	2	0.38899
		Control pol32	VST pol32	1	0.00093
		Control pol32	VST pol32	2	0.67498
		Control POL32	VST pol32	1	0.00093
		Control POL32	VST pol32	2	0.00062
		Control pol32	VST POL32	1	0.77887
		Control pol32	VST POL32	2	0.77887
rad5-535/	rad5	Control rad5-535	VST rad5-535	1	4.8e-06
Figure 5A		Control rad5-535	VST rad5-535	2	0.00047
		Control rad5-535	Control rad5	1	1.9e-05
		Control rad5-535	Control rad5	2	0.00227
		VST rad5-535	VST rad5	1	0.077
		VST rad5-535	VST rad5	2	0.07506
		Control rad5	VST rad5	1	2.8e-05
		Control rad5	VST rad5	2	0.00227
		Control rad5-535	VST rad5	1	6.0e-07
		Control rad5-535	VST rad5	2	5.7e-05
		Control rad5	VST rad5-535	1	0.021
		Control rad5	VST rad5-535	2	0.13167
MMS2		Control MMS2	VST MMS2	1	3.6e-06
Figure 5B		Control MMS2	VST MMS2	2	6.8e-06
		Control MMS2	Control mms2	1	0.44630
		Control MMS2	Control mms2	2	0.6823
		VST MMS2	VST mms2	1	0.00041
		VST MMS2	VST mms2	2	0.0052
		Control mms2	VST mms2	1	8.3e-08
		Control mms2	VST mms2	2	1.4e-05
		Control MMS2	VST mms2	1	8.3e-08
		Control MMS2	VST mms2	2	1.4e-05
		Control mms2	VST MMS2	1	1.0e-05
		Control mms2	VST MMS2	2	6.8e-06

MUS81	Control MUS81	VST MUS81	1	0.07795
Figure 7A	Control MUS81	VST MUS81	2	0.0422
	Control MUS81	Control mus81	1	0.72090
	Control MUS81	Control mus81	2	0.5054
	VST MUS81	VST mus81	1	0.07795
	VST MUS81	VST mus81	2	0.1086
	Control mus81	VST mus81	1	0.00093
	Control mus81	VST mus81	2	0.0027
	Control MUS81	VST mus81	1	0.00093
	Control MUS81	VST mus81	2	0.0027
	Control mus81	VST MUS81	1	0.07795
	Control mus81	VST MUS81	2	0.0295
SGS1	Control SGS1	VST SGS1	1	1.1e-07
Figure 7B	Control SGS1	VST SGS1	2	4.6e-06
	Control SGS1	Control sgs1	1	4.5e-05
	Control SGS1	Control sgs1	2	0.51
	VST SGS1	VST sgs1	1	0.71
	VST SGS1	VST sgs1	2	0.94
	Control sgs1	VST sgs1	1	4.0e-08
	Control sgs1	VST sgs1	2	4.6e-06
	Control SGS1	VST sgs1	1	4.0e-08
	Control SGS1	VST sgs1	2	4.6e-06
	Control sgs1	VST SGS1	1	3.3e-05
	Control sgs1	VST SGS1	2	4.6e-06
rad5-535	Control rad5-535	VST rad5-535	1	0.0047
Figure S1	Control rad5-535	VST rad5-535	2	0.0047
	Control rad5-535	VST rad5-535	3	0.0015
RAD5	Control RAD5	VST RAD5	1	0,013
Figure S3A	Control RAD5	VST RAD5	2	0,0027

Supplementary Table 3: Oligonucleotides used in this study

Name	Use	Sequence 5'-3'
OMK_pol32_F1	POL32 F1	ACAACCAGAAATAGGCTTTAGTTAACTCAATCGGTAATTACAGCTGAAGCTTCGTACGC
OMK_pol32_R1	POL32 R1	GATCTATTTTTCTATCACGTAAGTTGACATTTGTATTATAGGCCGATTCATTAATGCAGG
oT193	MRE11 F1	TTAAGAGAATGCAGACAATTGACGCAAGTTGTACCTGCTCAGATCCGATAAAACTCGACTcggatccccgggttaattaa
oT194	MRE11 R1	TCGCGAAGGCAAGCCCTTGGTTATAAATAGGATATAATATAATATAGGGATCAAGTACAAgaattcgagctcgtttaaac
oT197	SAE2 F1	TATCGTTCACATACCTGCATTTCCATCCATGCTGTAAGCCATTAGGTGTTTGTATGTGAGcggatccccgggttaattaa
oT198	SAE2 R1	TTCAACCATACCAAAAAAAATGTATTTGAAGTAATGAATAAAGAATGATGATCGCTGGCGgaattcgagctcgtttaaac
oT220	SGS1 F1	ATTATTGTTGTATATATTTAAAAAATCATACACGTACACACAAGGCGGTAcggatccccgggttaattaa
oT221	SGS1 R1	GCGAATGGTGTCGTAGTTATAAGTAACACTATTTATTTTTCTACTCTTCAgaattcgagctcgtttaaac
oT230	RAD5 F1	TACAAAGTTACATTATCAAAAGGCCTTAGAAACACACCTAAAGTCTTACAGTATCACAATcggatccccgggttaattaa
oT231	RAD5 R1	AGTTCTTTCGGGTTGAAAATAATAATAAATAAAGTCTTTATATATGAGTATGTGGTATGAgaattcgagctcgtttaaac
oT354	RAD5 F2	GACACAGACGAAGACGAGAGAAGAAAAAGGAGAATTGAAGAAATCCAGATGCTGTTTGAAcggatccccgggttaattaa
oT234	MMS2 F1	GTCGTGGTGAAATTCTTATTCTGTATATGCAACGTAGAAGAAAGCAGCGTTTACACAAAAcggatccccgggttaattaa
oT235	MMS2 R1	TTGCTTGTATATATGAGTGGCTTGGAATGCTGCAAATACTGTTTAGGAAAAAGTAGATAAgaattcgagctcgtttaaac
oT319	MUS81 F1	AAACAAAGTTTCAAAGGATTGATACGAACACACATTCCTAGCATGAAAGCcggatccccgggttaattaa
oT320	MUS81 R1	ATCACTTTTTTCTTTATAAAACCTTGCAGGGATGACTATATTTCAAATTGgaattcgagctcgttaaac
oT586	EXO1 F1	CAGGTATATCTATATGCTCTCATAGAATTATATTTGATATTGCTTTTTGGcggatccccgggttaattaa
oT587	EXO1 R1	TTTACTGGGCATTGATTTTTTAATTCTTGTCTTGAGGCATTTCGACGAGAgaattcgagctcgtttaaac
oT323	RAD51 F1	TTCTTCTATCTTCCGTAGTTTCCATATACTAGTAGTTGAGTGTAGCGACAcggatccccggttaattaa
oT324	RAD51 R1	GGATGGAAATGAAGATAAAAATGTACGGAACGCAACCTAAGAAAAAGAGGgaattcgagctcgtttaaac
oT316	Y' probe	GAGTTTTTCAGCGTTTGCGTTCCA
oT318	Y' probe	TTACTCGCGCTGTCACACCTTACC
169	teloPCR	CGGGATCCGGGGGGGGGGGGGGGGG
oT182	teloPCR Y'	TACATGAGGGCTATTTAGGGCTATTTAGGG
oT156	teloPCR VIIL	TTGTTGAAAGCCATACCTGCC
oT531	teloPCR VIR	AGGACTGGGTCATGGGGCGC
oT310	qPCR VIIL F	TGATATGTGTTACGCAGAATAC
oT311	qPCR VIIL R	TGAGAAGCACCGCAATG
oT312	qPCR VIR F	ATCATTGAGGATCTATAATC
oT313	qPCR VIR R	CTTCACTCCATTGCG

oT314	qPCR ARO1 F	TCGTTACAAGGTGATG
oT315	qPCR ARO1 R	AATAGCGGCAACAAC
oT570	qPCR Y' F	GGCTTGGAGGAGACGTACATG
oT571	qPCR Y' R	CTCGCTGTCACTCCTTACCCG
oT588	VIIL QAOS forward	CACCGCAATGCAAGGCAATTTCAC
oT589	VIIL QAOS reverse	AACCAGCGCAGCGGCATGTGT
oT590	VIIL QAOS tagging	AACCAGCGCAGCGGCATGTGTGTTACGCAGAATAC
oT591	VIR QAOS forward	TGAGGATTATAGGTAAATGGCAAGGGTAA
oT592	VIR QAOS reverse	TGCCCTCGCATCGCTCTCCTT
oT593	VIR QAOS tagging	TGCCCTCGCATCGCTCTCCTTCACTCCATTG

Suplementary references

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112. Adams Martin, A., Dionne, I., Wellinger, R.J. and Holm, C. (2000) The function of DNA polymerase alpha at telomeric G tails is important for telomere homeostasis. Mol. Cell. Biol., **20**, 786-796.