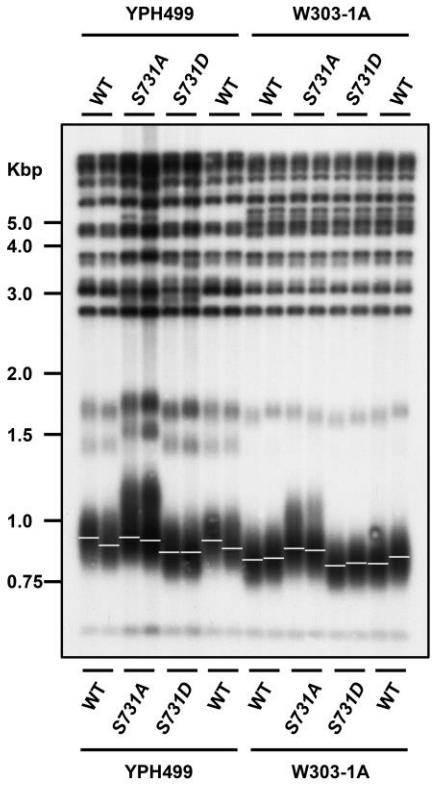
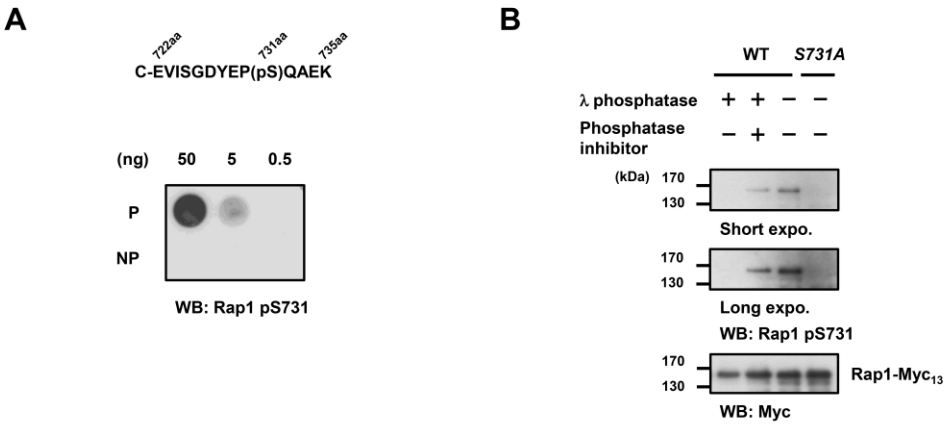
Supplemental Information

Contents



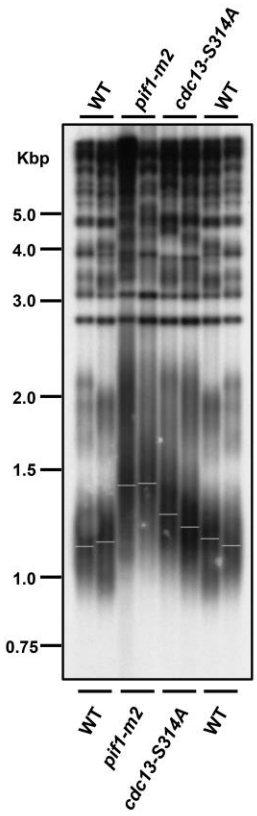
Supplementary Figure S1. Telomere analysis of *rap1* mutants in YPH499 and W303-1A backgrounds.

The genomic DNAs were digested with KpnI, and Southern blotting was performed as described in Figure 1B (n=2).



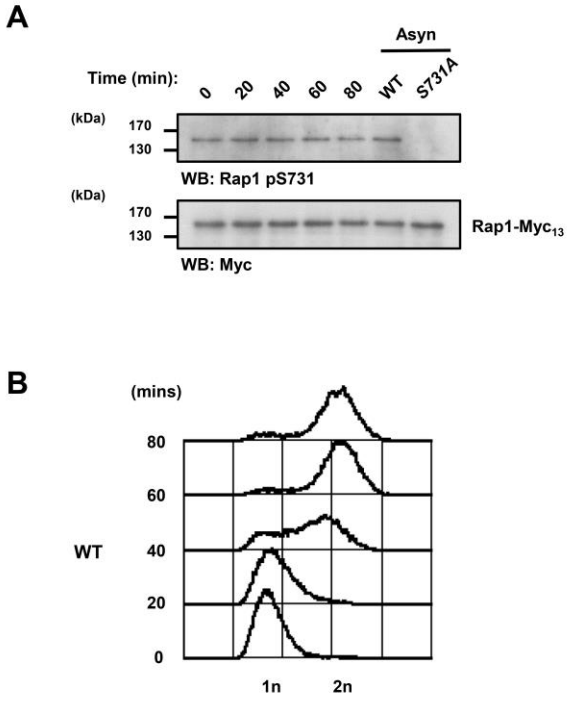
Supplementary Figure S2. Characterization of the phospho-specific antibodies against Rap1 S731.

A. Upper panel, the sequence of phosphorylated peptides, which was used to raise phospho-specific antibodies against Rap1 pS731. A cysteine residue was added to the N-terminus to facilitate conjugation with a carrier protein for greater immunogenicity. Lower panel, the peptide spotting showed the specificity of anti-Rap1 pS731 antibodies (n=2). **B.** λ phosphatase assay. Immunoprecipitated Rap1-Myc_{13} proteins were treated with λ phosphatase and western blotting was conducted using anti-Myc and anti-Rap1 pS731 phospho-specific antibodies (n=2).



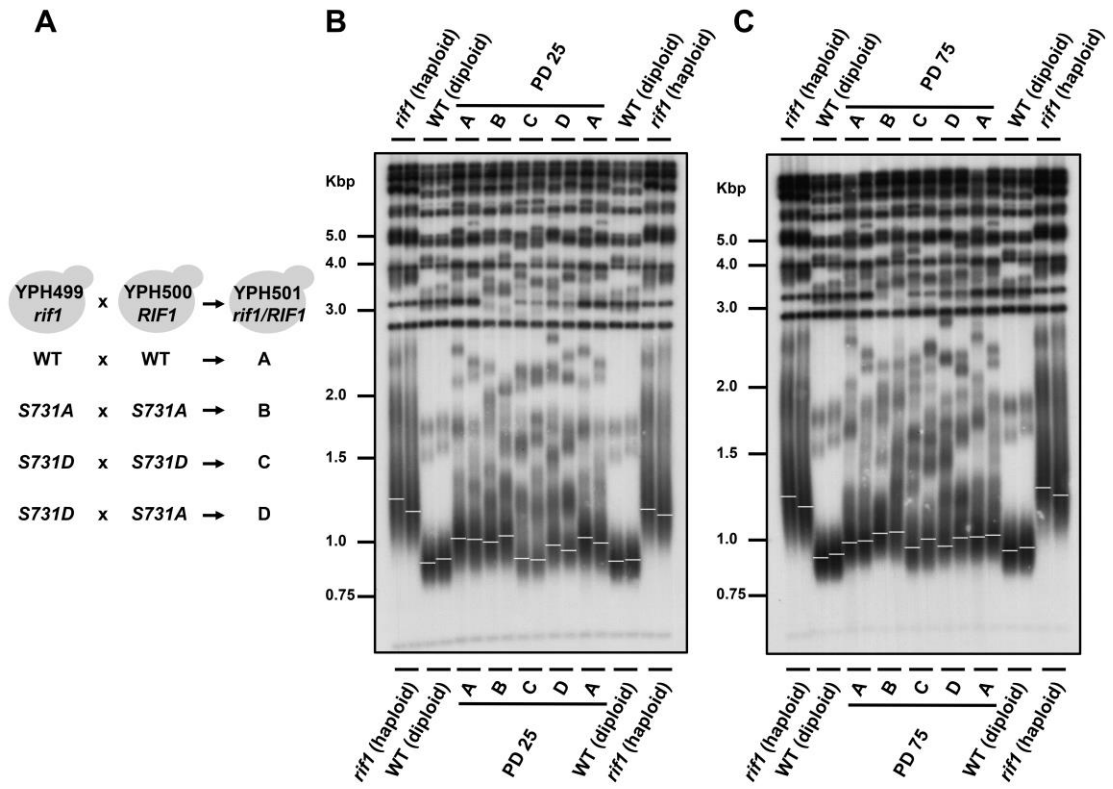
Supplementary Figure S3. Telomere analysis of *pif1-m2* and *cdc13-S314A* mutants containing Myc_{13}-tagged Rap1.

The genomic DNAs were digested with KpnI, and Southern blotting was performed as described in Figure 1B (n=2).



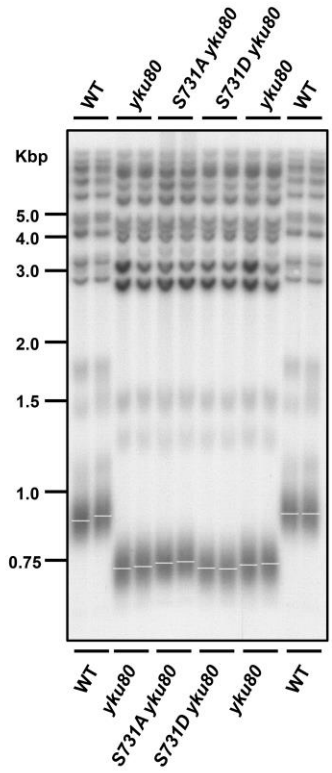
Supplementary Figure S4. Phosphorylation of Rap1 S731 is cell cycle-independent.

The overnight culture was refreshed to log phase in YPAD, arrested at G1 phase by α factor, and released into cell cycle at 24 °C. Samples were collected at 20-min intervals. **A.** Phosphoryalion of Rap1 S731 and total Rap1 amounts were analyzed by western blot analysis. Asynchronized (Asyn) WT and *rap1-S731A* cells were used as controls (n=2). **B.** Aliquots of cells were collected at 0, 20, 40, 60 and 80 minutes for FACS analysis (n=2).



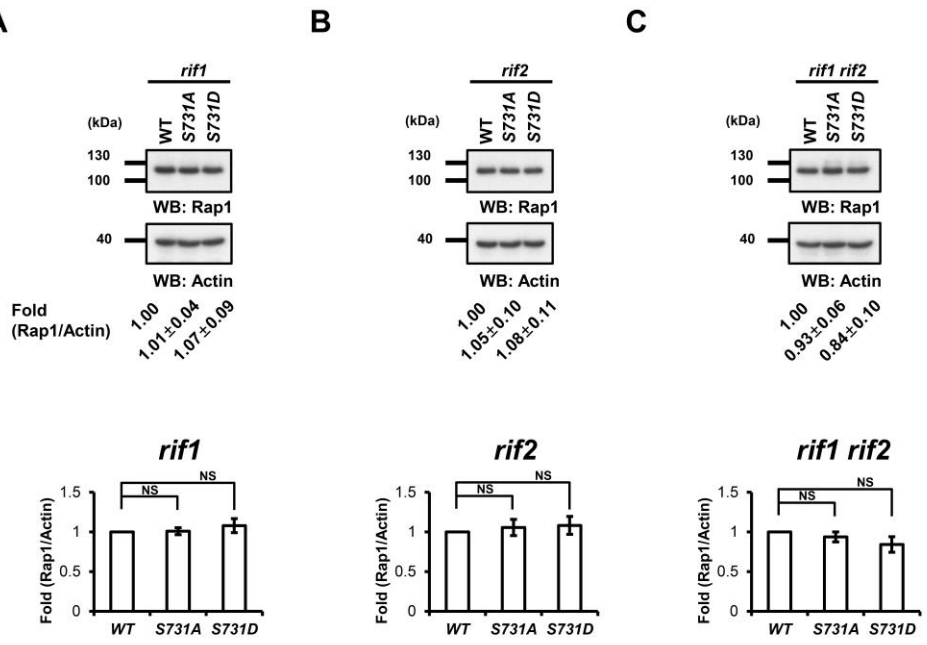
Supplementary Figure S5. Complementation of Rif1 restored the telomeres shortening phenotype of *rap1-S731D*, while the telomeres remained elongated at the initial back-crossing stage.

A. The schematic cartoon represents that the *rap1* mutants in *rif1* strains (YPH499) were back-crossed with *rap1* mutants (YPH500) to obtain different *rap1* mutant hybrids. **B.** Telomere analysis of the descendants at first 25 population doublings (PD25). The diploid hybrid cells were grown in YPAD medium for genomic DNA extraction. The genomic DNAs were digested with KpnI, and Southern blotting was performed as described in Figure 1B. *rif1* (haploid) and WT (diploid) were used as the telomere length controls (n=2). **C.** Telomere analysis of the descendants at 75 generations (PD75). The diploid hybrid cells were serially restreaked on YPAD plates for three times to reach 75 generations. The genomic DNAs were digested with KpnI, and Southern blotting was performed as described in Figure 1B (n=2).



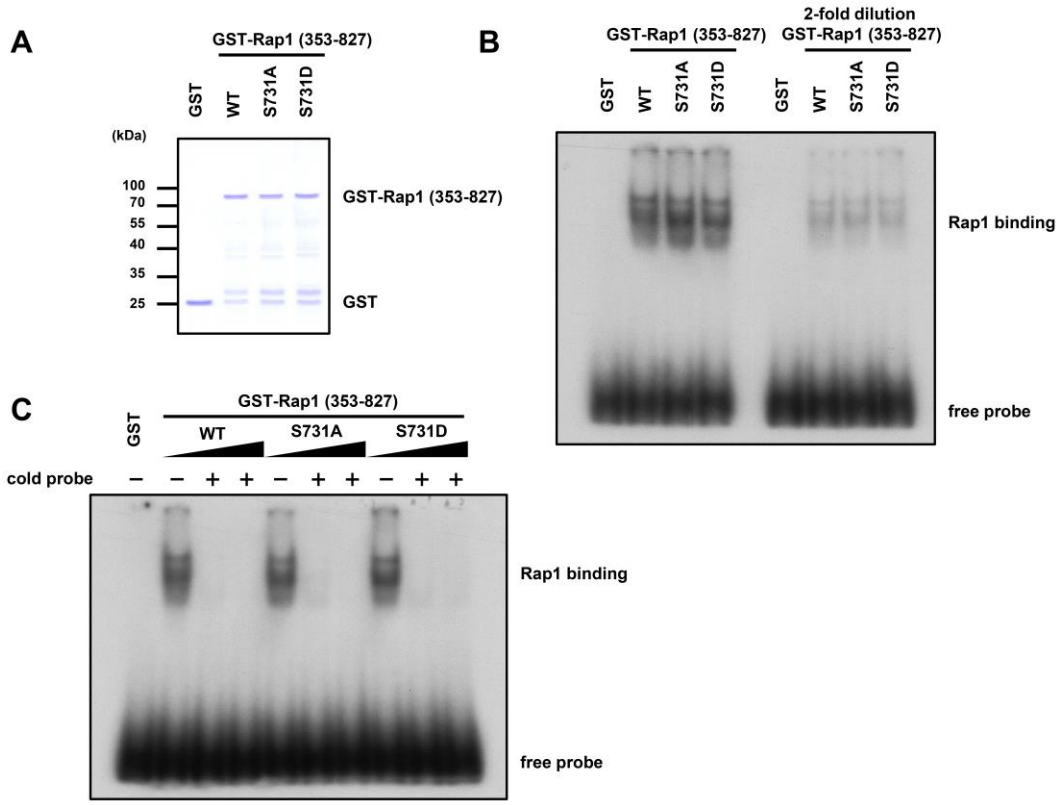
Supplementary Figure S6. Telomere analysis of WT, *rap1-S731A,* and *rap1-S731D* in the *yku80* background.

The genomic DNAs were digested with KpnI, and Southern blotting was performed as described in Figure 1B (n=2).



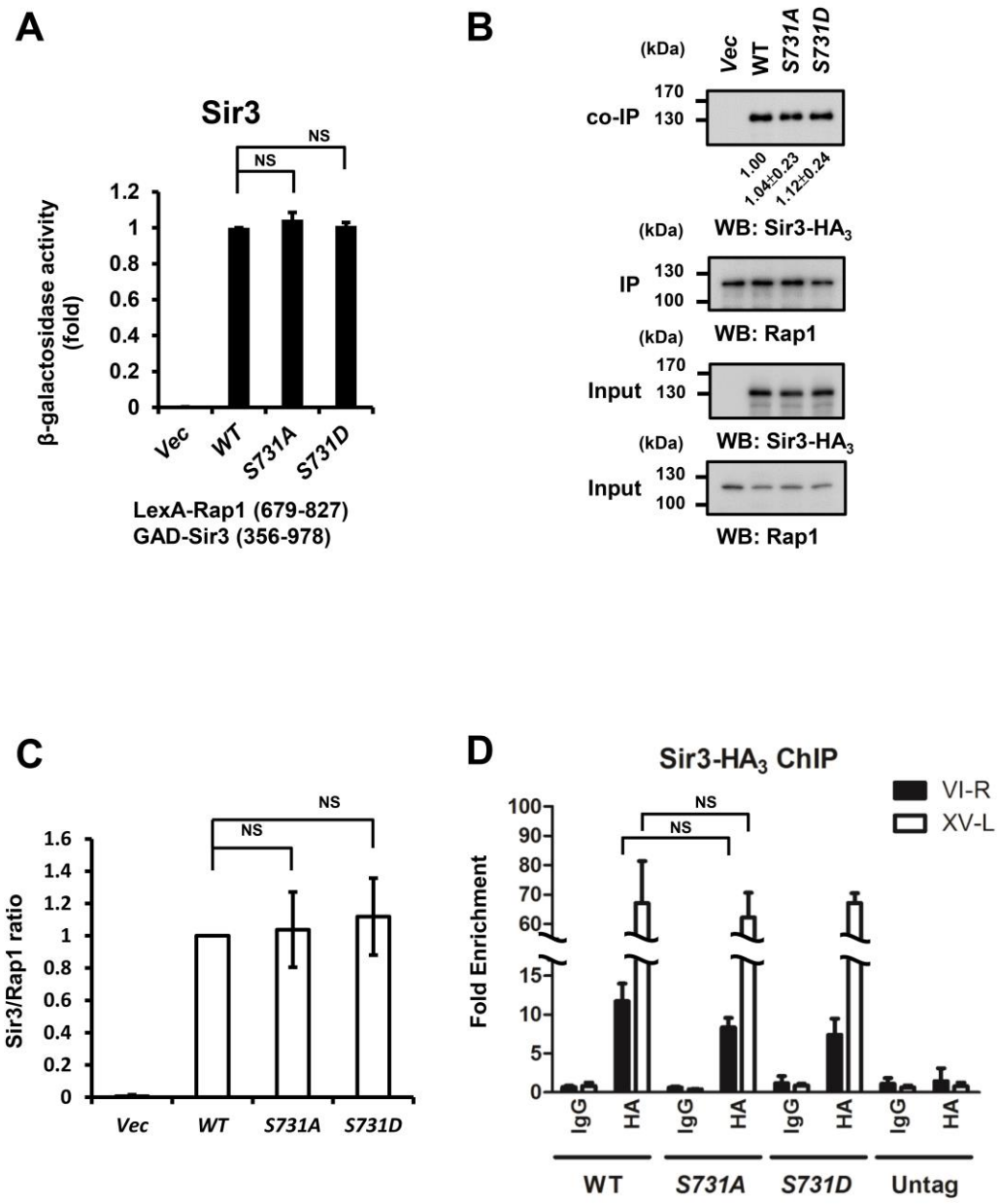
Supplementary Figure S7. The total Rap1 amounts are comparable between WT, *rap1-S731A* and *rap1-S731D* cells in the *rif1*, *rif2* and *rif1 rif2* backgrounds.

A. Total Rap1 in the *rif1* background. **B.** Total Rap1 in the *rif2* background. **C.** Total Rap1 in *rif1 rif2* background. Upper panel, cell lysates were precipitated by trichloroacetic acid (TCA) and analyzed by western blotting. Endogenous Rap1 protein was detected by polyclonal anti-Rap1 antibodies. Actin was detected by polyclonal anti-actin antibodies as a loading control. The fold of Rap1 over actin compared to that of WT is shown below. Data were expressed as the mean ± s.d.. Lower panel, the normalized data revealed that Rap1 proteins are comparable between WT, *rap1-S731A* and *rap1-S731D* cells in the *rif1*, *rif2* and *rif1 rif2* backgrounds (n=4, NS, non-significant, Student's *t*-test, two-tailed). Bars, s.d.



Supplementary Figure S8. EMSA assay of Rap1 fusion proteins showed no noticeable change of the Rap1 binding affinity to telomeric DNA sequence.

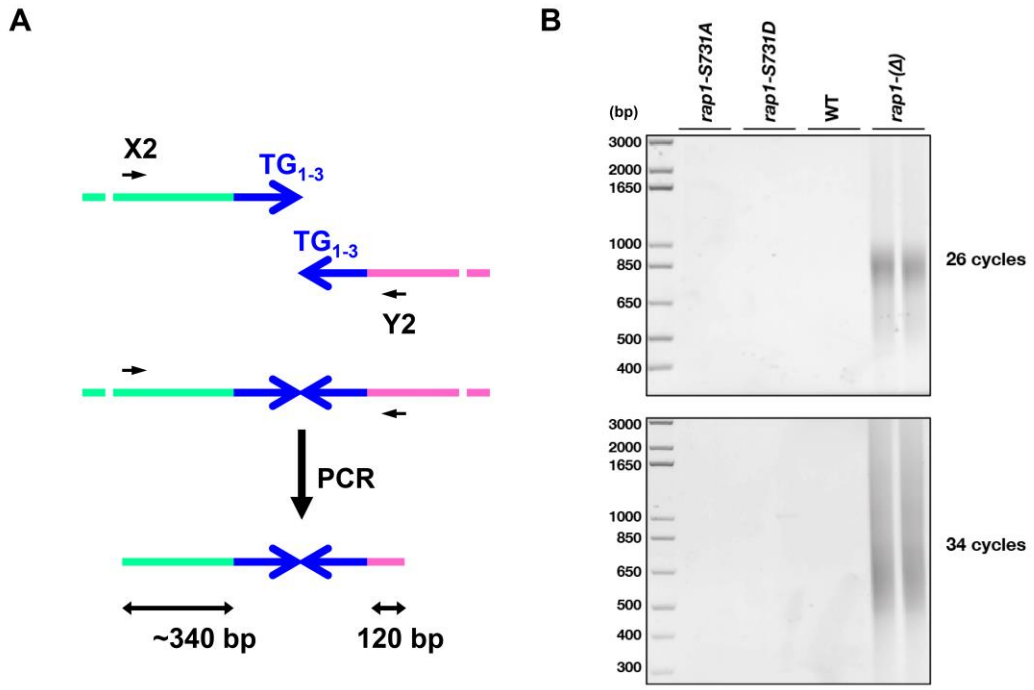
A. The aliquots of eluted GST and GST-Rap1(353-827) fusion proteins were resolved on a 10 % SDS-PAGE and stained with Coomassie blue as the EMSA protein loading control. **B.** Left part, the aliquots of eluted GST and GST-Rap1(353-827) fusion proteins were incubated with the ^{32}P-labeled Scer19 double-stranded oligonucleotides and the reactions were resolved on a 6 % non-denaturing polyacrylamide gel for autoradiography detection. Right part, the 2-fold dilution of fusion proteins were used to perform EMSA (n=2). **C.** Non-labeled Scer19 double-stranded oligonucleotides were added in 3 and 9 times molar excess as cold probes to compete for the Rap1 fusion protein binding (n=2). The minus-labelled (–) lanes contain no cold probe.



Supplementary Figure S9. Absence of phosphorylation does not change the Rap1-Sir3 interaction.

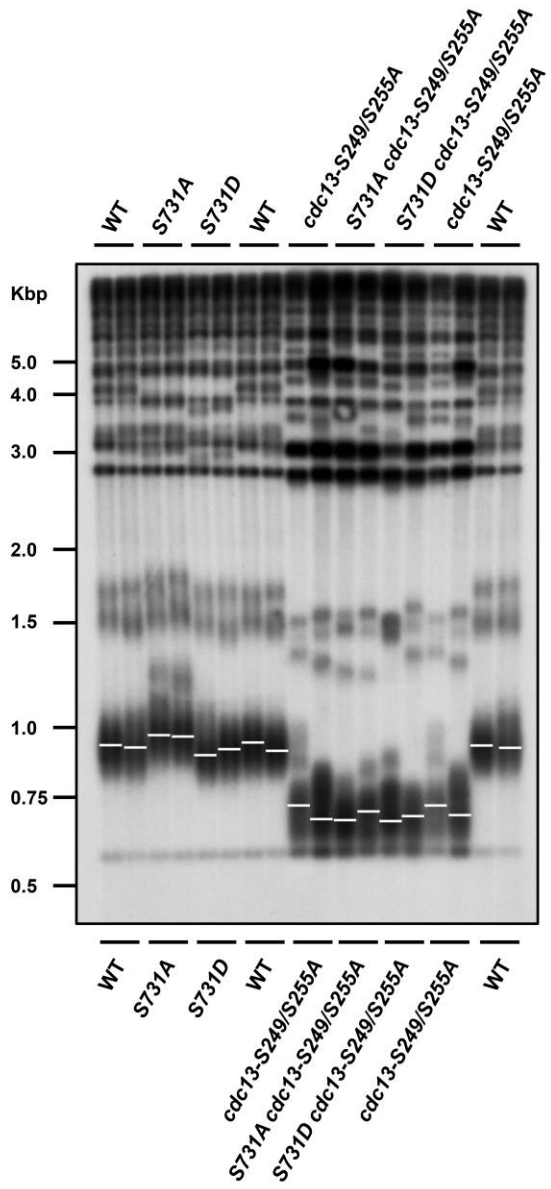
A. Yeast two-hybrid assay revealed that both *rap1-S731* mutations do not alter the Rap1-Sir3 interaction (n=4, NS, non-significant, Student's *t*-test, two-tailed). The Y axis means

the relative β-galactosidase activity fold. Bars, s.d. **B.** Co-IP assay demonstrated that the interaction between Rap1 and Sir3 is not changed in the absence of S731 phosphorylation. Endogenous Rap1 was immunoprecipitated from cells overexpressing Sir3-HA_3. Immunoprecipitates were separated by SDS-PAGE. Western blotting was conducted using anti-Rap1 and anti-HA antibodies. WT cells carrying the empty vector were used as a negative control. **C.** Quantification data of Sir3 and Rap1 co-IP (n=4, NS, non-significant, Student's *t*-test, two-tailed). Bars, s.d. **D.** ChIP data indicated that Sir3 binding to VI-R or XV-L telomeres is not disturbed by the lack of Rap1 S731 phosphorylation (n=3, NS, non-significant, Student's *t*-test, two-tailed). Strains expressing HA-tagged proteins and an untagged strain were immunoprecipitated with anti-HA or anti-normal mouse IgG antibodies. The data were presented as in Figure 5A.



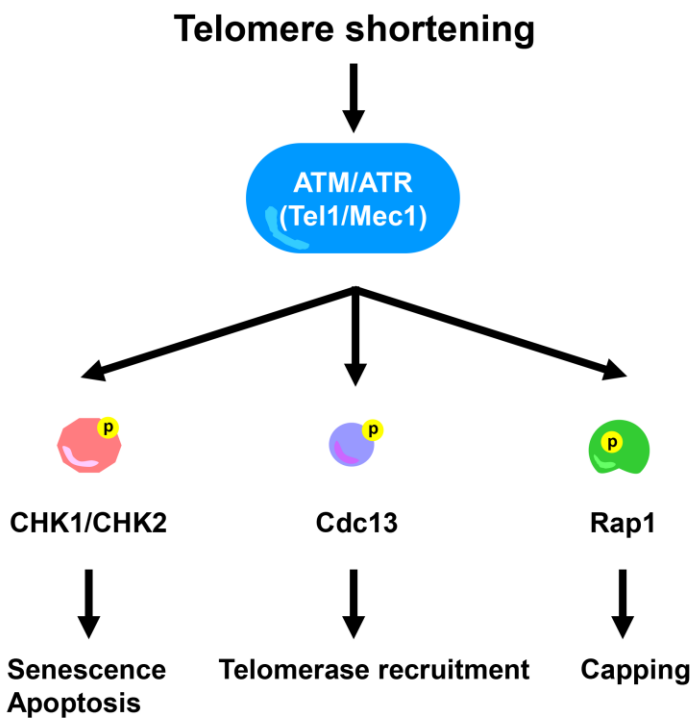
Supplementary Figure S10. Rap1 S731 phosphorylation does not modulate telomere-telomere fusions.

A. Schematic figure of the positions of the primers used for PCR amplification assay. **B.** Telomere fusions occur in neither *rap1-S731A* nor *rap1-S731D* stationary phase mutants. Two independent colonies of WT, *rap1-S731A*, *rap1-S731D* and *rap1-(Δ)* mutant cells were incubated to stationary phase (6 days) at 30 °C. The genomic DNA was extracted, and X2 and Y2 primers were used to amplify the telomere fusions by PCR. The *rap1-(Δ)* was used as a control (n=2).



Supplementary Figure S11. Telomere lengthening phenotype of *rap1-S731A* was abrogated in *cdc13-S249/S255A* cells.

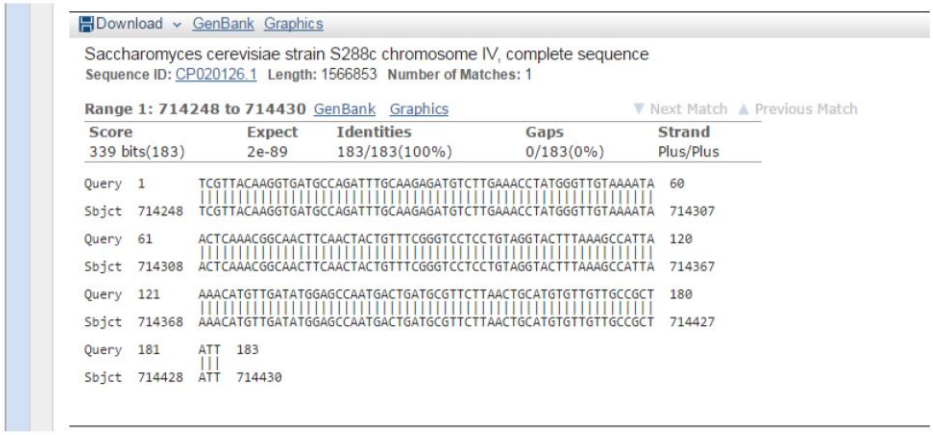
Telomere analysis of WT, *rap1-S731A,* and *rap1-S731D* in the *cdc13-S249/S255A* mutation background. The telomere length of each colony derived from the dissected spores was analyzed at 75 generations (PD75). The genomic DNAs were digested with KpnI, and Southern blotting was performed as described in Figure 1B (n=2).



Supplementary Figure S12. ATM/ATR kinases activate different downstream signaling pathways to circumvent progressive telomere shortening puzzle.

In mammalian cells, progressive telomere shortening triggers ATM/ATR kinases to phosphorylate CHK1 and CHK2, activate downstream p53 signaling pathway, and finally cause cellular senescence and/or apoptosis to suppress tumorigenesis. In budding yeast, shortened telomeres induce Tel1/Mec1 to phosphorylate Cdc13 for telomerase recruitment and Rap1 for telomere capping.

A



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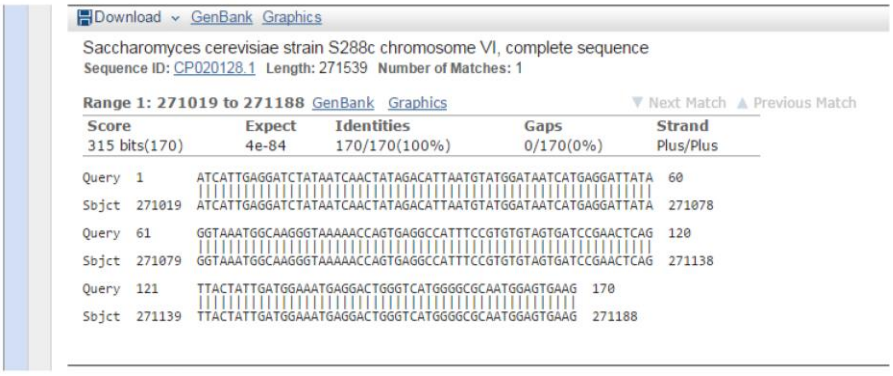
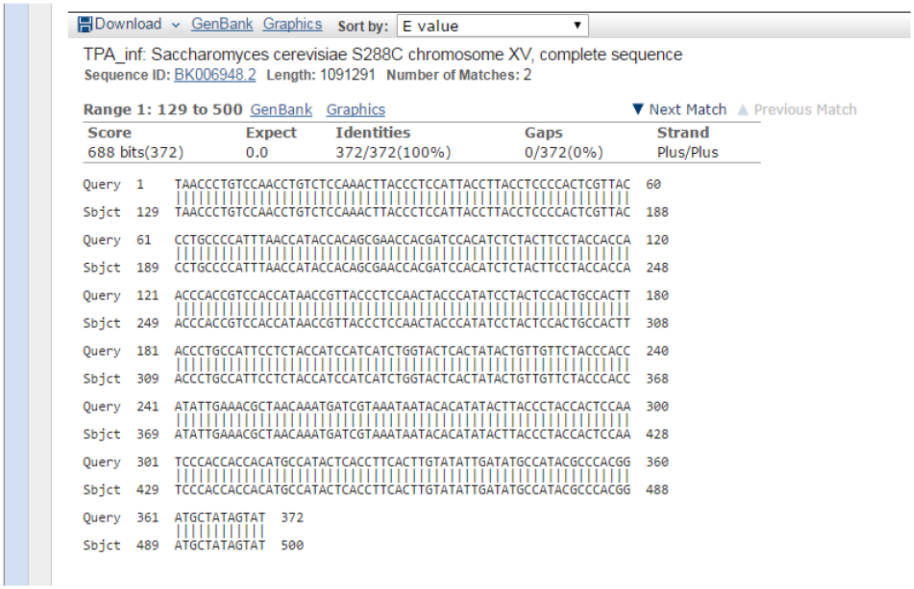


C

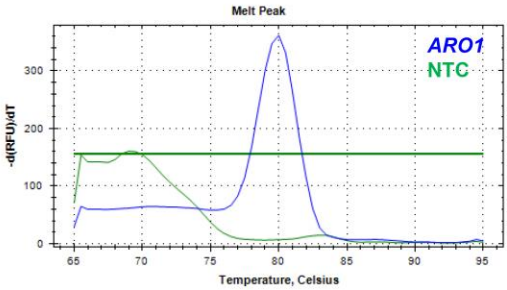


Supplementary Figure S13. *In silico* specificity screens of ChIP primers.

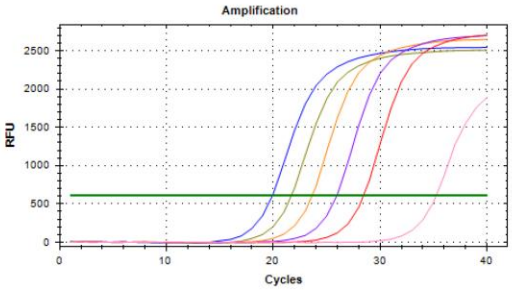
In silico specificity of PCRs was assessed by BLAST (NCBI) for **A.** *ARO1*-ChIP primers. **B.** VI-R-ChIP primers. **C.** XV-L-ChIP primers.

A

Melt Analysis for *ARO1*-ChIP primer

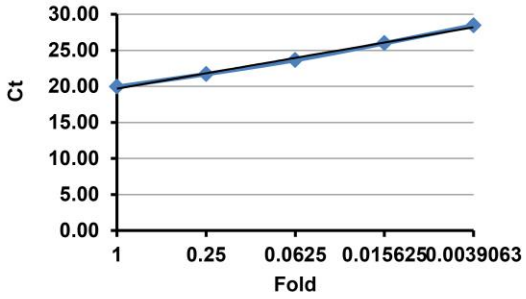


Standard curve



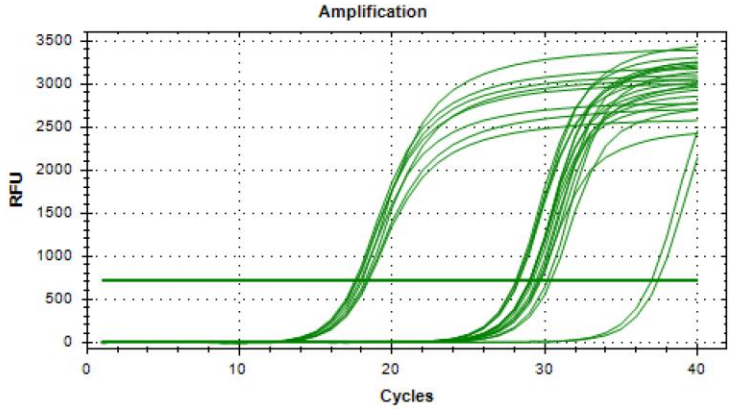
Standard curve

$y = -1.537\ln(x) + 19.688$
$R^2 = 0.9948$



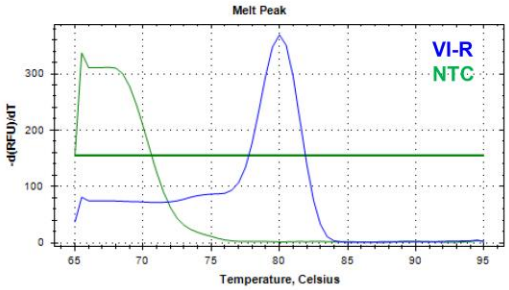
Fold	Ct
1	19.94
0.25	21.71
0.0625	23.65
0.015625	25.98
0.00390625	28.46
NTC	35.27

Raw data

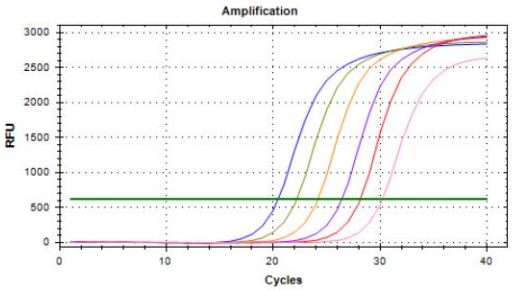


B

Melt Analysis for VI-R-ChIP primer

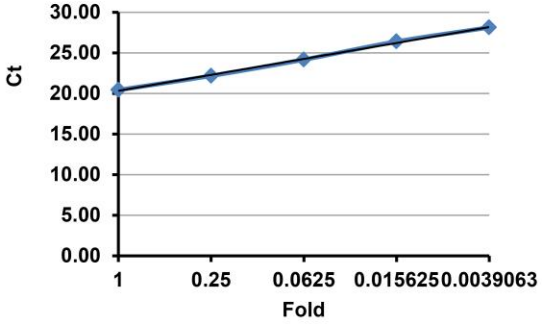


Standard curve



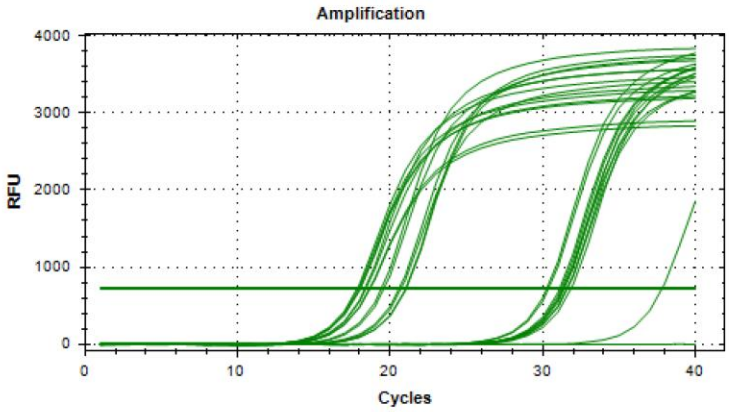
Standard curve

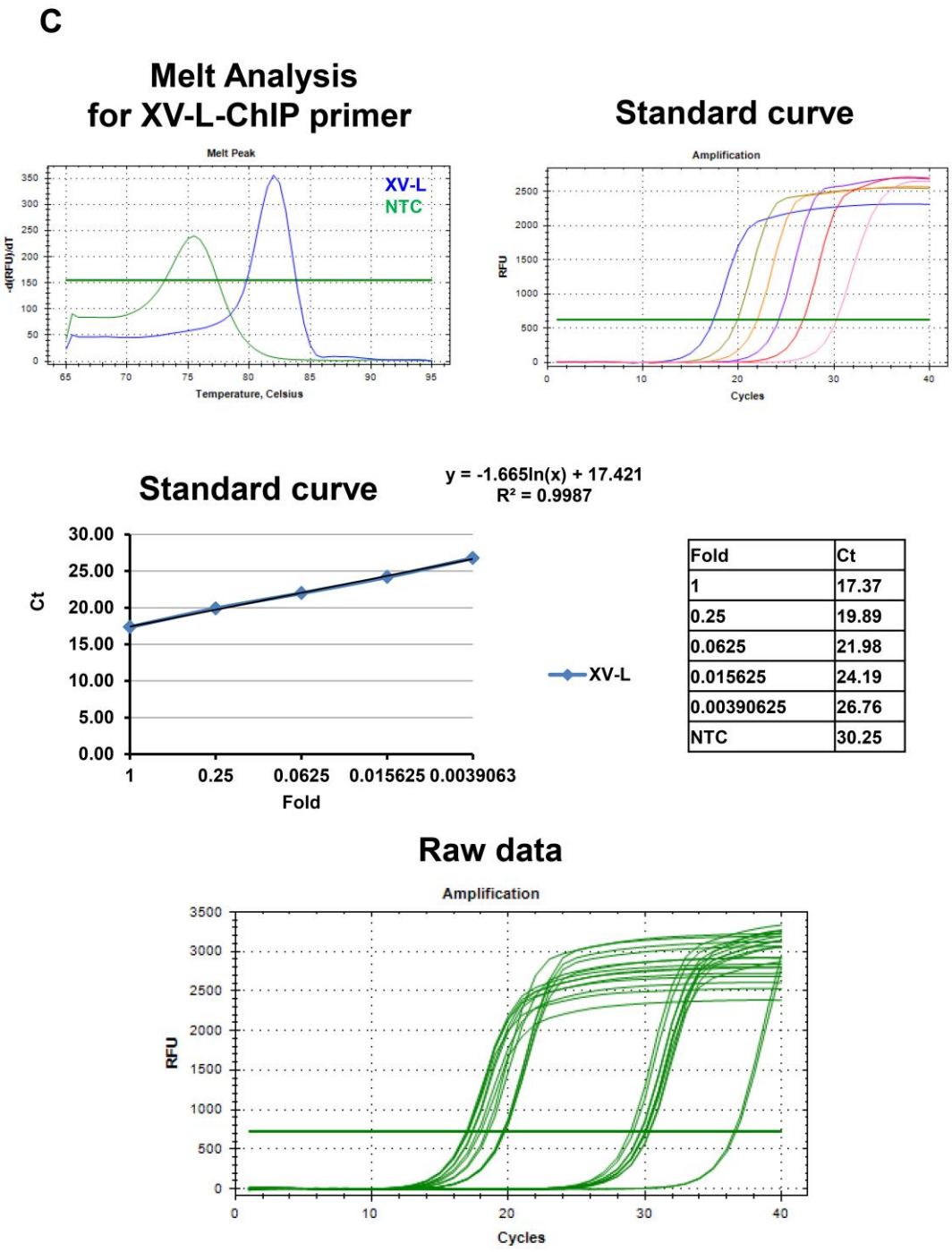
y = -1.417ln(x) + 20.338
R² = 0.9984



Fold	Ct
1	20.43
0.25	22.18
0.0625	24.18
0.015625	26.40
0.00390625	28.14
NTC	30.20

Raw data





Supplementary Figure S14. RT-qPCR assay performance in accordance with MIQE guidelines.

Specificity of primers and the amplified PCR products were examined by melt analysis, standard curves and raw data for **A.** *ARO1*-ChIP primers, **B.** VI-R-ChIP primers, and **C.** XV-L-ChIP primers.

Supplementary Table S1. Distribution of survivor types.

Genotype	No. of survivors (% of total)		
	Total studied	Type I	Type II
tlc1	150	140 (93)	10 (7)
tlc1 rap1-S731A	150	127 (85)	23 (15)
tlc1 rap1-S731D	150	142 (95)	8 (5)

Supplementary Table S2. Strains Used in This Study.

Strain	Genotype	Source
YPH501	*MAT a/α ura3-52/ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 leu2-deltaΔ1/leu2-deltaΔ1*	(1)
YPH499	*MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2-deltaΔ1*	(1)
YPH500	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2-deltaΔ1*	(1)
STY2289	YPH499 *RAP1-3HA-KanMX6*	This study
CWY154	YPH499 *rap1-S142A*	This study
CWY156	YPH499 *rap1-S142D*	This study
CWY161	YPH499 *rap1-Linker 6S/TA*	This study
CWY163	YPH499 *rap1-Linker 6SD/TE*	This study
CWY187	YPH499 *rap1-T364A*	This study
CWY188	YPH499 *rap1-T364E*	This study
CWY165	YPH499 *rap1-S479/T486/S594A*	This study
CWY168	YPH499 *rap1-S479D/T486E/S594D*	This study
CWY157	YPH499 *rap1-S658/S660A*	This study
CWY159	YPH499 *rap1-S658/S660D*	This study
CWY34	YPH499 *rap1-S731A*	This study
CWY40	YPH499 *rap1-S731D*	This study
CWY274	YPH499 *rap1-ΔC-TRP1*	This study
CWY59	YPH499 *RAP1-13MYC-KanMX6*	This study
CWY60	YPH499 *rap1-S731A-13MYC-KanMX6*	This study
CWY109	YPH499 *yku80::HIS3MX6*	This study

CWY111	YPH499 *yku80::HIS3MX6 rap1-S731A*	This study
CWY113	YPH499 *yku80::HIS3MX6 rap1-S731D*	This study
CWY221	YPH501 *tlc1::LEU2/TLC1 RAP1/RAP1-13MYC-KanMX6* derived from STY95	This study
CWY230	YPH499 *yku80::HIS3MX6 RAP1-13MYC-TRP1*	This study
CWY241	YPH500 *pif1-m2 RAP1-13MYC-TRP1*	This study
CWY239	YPH499 *cdc13-S314A RAP1-13MYC-TRP1*	This study
CWY232	YPH500 *tel1::HIS3MX6 RAP1-13MYC-TRP1*	This study
CWY233	YPH499 *mec1::HIS3MX6 sml1::KanMX4 RAP1-13MYC-TRP1*	This study
CWY235	YPH501 *tel1::HIS3MX6/TEL1 mec1::HIS3MX6/MEC1 sml1::KanMX4/SML1 RAP1-13MYC-TRP1/RAP1-13MYC-TRP1*	This study
CWY237	YPH499 *tel1::HIS3MX6 mec1::HIS3MX6 sml1::KanMX4 RAP1-13MYC-TRP1* derived from CWY235	This study
CWY229	YPH501 *rif1::TRP1/RIF1 rif2::HIS3/RIF2*	This study
CWY243	YPH501 *rif1::TRP1/RIF1 rif2::HIS3/RIF2 rap1-S731A/RAP1*	This study
CWY244	YPH501 *rif1::TRP1/RIF1 rif2::HIS3/RIF2 rap1-S731D/RAP1*	This study
CWY39	YPH500 *rap1-S731A*	This study
CWY44	YPH500 *rap1-S731D*	This study
CWY250	YPH499 *rif1::TRP1 rap1-S731A*	This study
CWY251	YPH499 *rif1::TRP1 rap1-S731D*	This study
ZJY259	YPH501 *cdc13-S249/S255A/CDC13*	(2,3)
CWY213	YPH501 *cdc13-S249/S255A/CDC13 rap1-S731A/RAP1*	This study
CWY215	YPH501 *cdc13-S249/S255A/CDC13 rap1-S731D/RAP1*	This study

STY95	YPH501 *tlc1::LEU2/TLC1*	(4)
CWY1	YPH501 *tlc1::LEU2/TLC1 rap1-S731A/RAP1*	This study
CWY4	YPH501 *tlc1::LEU2/TLC1 rap1-S731D/RAP1*	This study
CWY148	YPH500 *mec1::HIS3MX6 tel1::HIS3MX6 sml1::KanMX4* + pKR5 (*TEL1-HA*)	This study
CWY150	YPH500 *tel1::HIS3MX6* + pJM8 (*tel1-HA*(*KD*))	This study
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	(5)
LSS90	BY4741 *18MYC-MEC1::LEU2 5HA-tel1-KD::kanMXURA3 pep4::AUR1^{r}*	(6)
LSS93	BY4741 *18MYC-mec1-KD::URA3 5HA-TEL1 sml1::His3MX pep4::AUR1^{r}*	(6)
L40	*MATa his3-delta200 trp1-901 leu2-3, 112 ade2 lys2-801am LYS2::(lexAop)4-HIS3URA3::(lexAop)8-lacZ GAL4*	Invitrogen
W303-1A	*MATa leu2-3, 112 ura3-1 trp1-1 his3-11, 15 ade2-1 can1-100*	(7)
CWY88	W303-1A *rap1-S731A*	This study
CWY89	W303-1A *rap1-S731D*	This study
MS179	W303-1A Lev220 *bar1::Kan RIF1*-Gly_8-Myc_9 *adh4::FRT-TEL*	(8)
STY2305	MS179 *rap1-S731A*	This study
CWY14	MS179 *rap1-S731D*	This study
MS206	W303-1A Lev220 *bar1::Kan RIF2*- Gly_8-Myc_9 *adh4::FRT-TEL*	(8)
STY2307	MS206 *rap1-S731A*	This study
CWY22	MS206 *rap1-S731D*	This study
CWY98	W303-1A *SIR3-3HA-HIS3MX6*	This study

CWY99	W303-1A *SIR3-3HA-HIS3MX6 rap1-S731A*	This study
CWY100	W303-1A *SIR3-3HA-HIS3MX6 rap1-S731D*	This study
CWY121	W303-1A *sir3::HIS3MX6*	This study
CWY123	W303-1A *sir3::HIS3MX6 rap1-S731A*	This study
CWY125	W303-1A *sir3::HIS3MX6 rap1-S731D*	This study
210-3d	W303-1A *hml-Δ::NAT*	(9)
209-1c	W303-1A *hml-Δ::NAT pACE1-UBR1 pACE1-ROX1 rap1-(Δ)::KANr*	(9)
CWY270	210-3d *hml-Δ::NAT rap1-S731A*	This study
CWY272	210-3d *hml-Δ::NAT rap1-S731D*	This study
UCC3505	*MATa ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1Δ63 ura3-52 ppr1::HIS3 adh4::URA3-TEL-VIIL DIA5-1 VR-ADE-TEL*	(10)
STY2144	UCC3505 *RAP1-TRP1*	This study
STY2145	UCC3505 *rap1-S731A-TRP1*	This study
STY2146	UCC3505 *rap1-S731D-TRP1*	This study
CWY209	UCC3505 *RAP1-TRP1 sir3::KanMX6*	This study
CWY275	UCC3505 *rap1-ΔC-TRP1*	This study
UCC3515	*MATα ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1Δ63 ura3-52 hml::URA3*	(10)
STY2147	UCC3515 *RAP1-TRP1*	This study
STY2148	UCC3515 *rap1-S731A-TRP1*	This study
STY2149	UCC3515 *rap1-S731D-TRP1*	This study
CWY210	UCC3515 *RAP1-TRP1 sir3::KanMX6*	This study
CWY276	UCC3515 *rap1-ΔC-TRP1*	This study

UCC4564	*MATα ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1Δ63 ura3-52 ppr1::LYS2 hmr::URA3*	(10)
STY2150	UCC4564 *RAP1-TRP1*	This study
STY2151	UCC4564 *rap1-S731A-TRP1*	This study
STY2152	UCC4564 *rap1-S731D-TRP1*	This study
CWY211	UCC4564 *RAP1-TRP1 sir3::KanMX6*	This study
CWY277	UCC4564 *rap1-ΔC-TRP1*	This study

Supplementary Table S3. Constructs Used in This Study.

Name	Source
pRS306*RAP1*	This study
pRS304*RAP1*	This study
pRS304-Rap1-ΔC(672-827)	This study
pGEX-4T-Rif1(1709-1916)	This study
pGEX-4T-Rif2(1-395)	This study
pGEX-4T-Rap1(353-827)	This study
pGEX-4T-Rap1(716-746)	This study
pKR5 (*TEL1-HA*)	(11)
pJM8 (*tel1-HA*(*KD*))	(11)
YEpFAT7-*SIR3*-HA_3	This study
pBTM116-Rap1(679-827)	(12)
pACT2-Rif1(1614-1916)	(12)
pACT2-Rif2(14-395)	(12)
pACT2-Sir3(356-975)	(12)

Supplementary Table S4. Primers Used in This Study.

Primer	Sequence
	Site-direct mutagenesis
rap1-S142A	GCGACGCGGATGCGCATGACGCGTTAAATGATATTGATCAATTAG
rap1-S142D	GCGACGCGGATGCCCATGACGACTTAAATGATATTGATCAATTAG
rap1-S237A	GATAATAGCAATTCGAATGCCGATAACAAGGATTCTATCAGGCCC
rap1-S237D	GATAATAGCAATTCGAATGATGATAACAAGGATTCTATCAGGCCC
rap1-S261/T262A	TGGCGCTACGGAAGACGCAGCTAGCGAAAAAGTTATGGTAGACGC
rap1-S261D/T262E	TGGCGCCACGGAAGACGACGAGAGCGAAAAAGTTATGGTAGACGC
rap1-S288/S289A	CAGCTCCTTCGTCAACACGTGGCCGCCACCGCATCAATCACAAGC
rap1-S288/S289D	CAGCTCCTTCGTCAACATGTCGACGACACCGCATCAATCACAAGC
rap1-S342A	ACAGCAGATGAGGGGAATGCAGCTTTTCAAGCACAAAGGTCCATG
rap1-S342D	ACAGCAGATGAGGGGAATGCAGACTTTCAAGCACAAAGGTCCATG
rap1-T364E	TTGCCCTCCCACAATAAAGCTTCTTTCGAAGATGAGGAAGATGAG
rap1-T364A	TTGCCCTCCCACAATAAAGCATCTTTTGCAGATGAGGAAGATGAG
rap1-S479/T486A	CTGGAAGAGCGCTTATCACAGATGAGGATGCACCCACTGCTATAG
rap1-S479D/T486E	CTGGAAGAGATCTTATCACAGATGAGGATGACCCCACTGCTATAG
rap1-S594A	CCCACTCCTGGCAATTATAACGCGGCCGCCAAGAGGGCAAGAAAT
rap1-S594D	CCCACTCCTGGCAATTACAACGACGCCGCCAAGAGGGCAAGAAAT
rap1-S658/S660A	CTATCCAATATTGCAAATGCATTGCCCTTTGAGTATCCACACGAG
rap1-S658/S660D	CTATCCAATATCGATAATGACTTGCCCTTTGAGTATCCACACGAG
rap1-S731A	GAGATTATGAGCCGGCACAGGCTGAAAAACTGGTACAG
rap1-S731D	ATTTCAGGAGATTATGAGCCTGATCAGGCTGAAAAACTGGTACAG

	rap1-ΔC construct
RAP1-del2014-2481	GAGTATCCACACGAGATTGCGGAATGAGTAATTGAATTAAGTAACA
RAP1-del2014-2481 antisense	TGTTACTTAATTCAATTACTCATTCCGCAATCTCGTGTGGATACTC
	pGEX-4T-Rap1 construct
RAP1-F-716-BamHI	GGATCCTTTATGGATAAACTTCATGAAG
RAP1-R-746-XhoI	CTCGAGTATACCAGTTTCATCGCAAAG
RAP1-F-353-EcoRI	TCCCCGGAATTCGGCGCTTTGCCCTCCCACAATAAAG
RAP1-R-827-XhoI	AGCTTCTCGAGTCATAACAGGTCCTTCTC
	pGEX-4T-Rif1 (1709-1916)
RIF1-F-1709-EcoRI	CCCCGGAATTCGGAGATAAGGATGCCAATAT
RIF1-R-1916-XhoI	AGCTTCTCGAGATTCATATCATTATCCCTGTTTG
	pGEX-4T-Rif2 (1-395)
RIF2-F-1-EcoRI	CTCCCGAATTCATGGAGCATGTAGATTCCG
RIF2-R-395-XhoI	AGCTTCTCGAGTCTATCATGTACTTTTCGAG
	Rap1-HA_3 and Rap1-Myc_{13} tagging
RAP1-cF2	GGTAGAATGGAAATGAGGAAAAGATTTTTTGAGAAGGACCTGTTAC GGATCCCCGGGTTA
RAP1-cR1	AAGGAGTAAAATAAGTTAAACAATGATGTTACTTAATTCAATTACGA ATTCGAGCTCGTT
	Sir3-HA_3 and *sir3::HIS3MX6* construct
SIR3-3HA-F	TACGCCTTTTCGATGGATGAAGAATTCAAAAATATGGACTGCATTCG

	GATCCCCGGGTTAATTAA
SIR3-3HA-R	GTACATAGGCATATCTATGGCGGAAGTGAAAATGAATGTTGGTGGG
	AATTCGAGCTCGTTTAAAC
SIR3-del-F1-F	GGGGTTTAAGAAAGTTGTTTTGTTCTAACAATTGGATTAGCTAAACG
	GATCCCCGGGTTAATTAA
	YEpFAT7-*SIR3*-HA_3 construct
SIR3-pro-F	GTACAATGTTCTTGGCGAAG
SIR3-ter-R	ACGTCAAGACTGTCAAGGAG
	yku80::HIS3MX6 construct
yKU80-del-F	ATGTCAAGTGAGTCAACAACTTTCATCGTGGATGTTTCACCATCAC
	GGATCCCCGGGTTAATTAA
yKU80-del-R	ATTATTGCTATTGTTTGGACTTCCCCTACTGTGTTGTTCACCGCGGA
	ATTCGAGCTCGTTTAAAC
	ChIP
ARO1-ChIP-F	TCGTTACAAGGTGATG
ARO1-ChIP-R	AATAGCGGCAACAAC
VI-R-ChIP-F	ATCATTGAGGATCTATAATC
VI-R-ChIP-R	CTTCACTCCATTGCG
XV-L-ChIP-F	TAACCCTGTCCAACCTGTCT
XV-L-ChIP-R	ATACTATAGCATCCGTGGGC
	NHEJ
X2	TGTGGTGGTGGGATTAGAGTGGTAG
Y2	TTAGGGCTATGTAGAAGTGCTG

	EMSA
Scer19	TGTGGTGTGTGGGTGTGTG
Scer19-Rev	CACACACCCACACACCACA

Supplementary Table S5. MIQE Guidelines Checklist.

ITEM TO CHECK	IMPORTANCE	CHECKLIST	COMMENTS/ WHERE?
EXPERIMENTAL DESIGN			
Definition of experimental and control groups	E	YES	Materials and Methods
Number within each group	E	YES	Materials and Methods
Assay carried out by core lab or investigator's lab?	D	YES	Investigator's Lab
Acknowledgement of authors' contributions	D	N/A	
SAMPLE			
Description	E	YES	Materials and Methods
Volume/mass of sample processed	D	YES	Materials and Methods
Microdissection or macrodissection	E	N/A	
Processing procedure	E	YES	Materials and Methods
If frozen - how and how quickly?	E	N/A	
If fixed - with what, how quickly?	E	YES	Materials and Methods
Sample storage conditions and duration (especially for FFPE samples)	E	N/A	
NUCLEIC ACID EXTRACTION			
Procedure and/or instrumentation	E	N/A	
Name of kit and details of any modifications	E	N/A	
Source of additional reagents used	D	N/A	
Details of DNase or RNase treatment	E	N/A	
Contamination assessment (DNA or RNA)	E	N/A	

Nucleic acid quantification	E	N/A	
Instrument and method	E	N/A	
Purity (A260/A280)	D	N/A	
Yield	D	N/A	
RNA integrity method/instrument	E	N/A	
RIN/RQI or Cq of 3' and 5' transcripts	E	N/A	
Electrophoresis traces	D	N/A	
Inhibition testing (Cq dilutions, spike or other)	E	N/A	
REVERSE TRANSCRIPTION			
Complete reaction conditions	E	N/A	
Amount of RNA and reaction volume	E	N/A	
Priming oligonucleotide and concentration	E	N/A	
Reverse transcriptase and concentration	E	N/A	
Temperature and time	E	N/A	
Manufacturer of reagents and catalogue numbers	D	N/A	
Cqs with and without RT	D	N/A	
Storage conditions of cDNA	D	N/A	
qPCR TARGET INFORMATION			
If multiplex, efficiency and LOD of each assay	E	N/A	
Sequence accession number	E	YES	*ARO1*: NC_001136.10 ChXV-L: CP020137.1 ChVI-R: CP020128.1
Location of amplicon	D	YES	Supplementary Figure S13.
Amplicon length	E	YES	Supplementary Figure S13.

In silico specificity screen (BLAST, etc)	E	YES	Supplementary Figure S13.
Pseudogenes, retropseudogenes or other homologs?	D	N/A	
Sequence alignment	D	N/A	
Secondary structure analysis of amplicon	D	N/A	
Location of each primer by exon or intron (if applicable)	E	N/A	
What splice variants are targeted?	E	NO	
qPCR OLIGONUCLEOTIDES			
Primer sequences	E	YES	Supplementary Table S4.
RTPrimerDB Identification Number	D	N/A	
Probe sequences	D	N/A	
Location and identity of any modifications	E	N/A	
Manufacturer of oligonucleotides	D	YES	MDBio, Inc
Purification method	D	YES	RPC
qPCR PROTOCOL			
Complete reaction conditions	E	YES	Materials and Methods
Reaction volume and amount of cDNA/DNA	E	YES	Supplementary Table S6.
Primer, (probe), Mg++ and dNTP concentrations	E	YES	Supplementary Table S6.
Polymerase identity and concentration	E	YES	KAPA SYBR® FAST qPCR Kits
Buffer/kit identity and manufacturer	E	YES	KAPA SYBR® FAST qPCR Kits
Exact chemical constitution of the buffer	D	NO	Manufactures proprietary
Additives (SYBR Green I, DMSO, etc.)	E	YES	Materials and Methods

Manufacturer of plates/tubes and catalog number	D	YES	Supplementary Table S6.
Complete thermocycling parameters	E	YES	Materials and Methods
Reaction setup (manual/robotic)	D	YES	Manual setup
Manufacturer of qPCR instrument	E	YES	Materials and Methods
qPCR VALIDATION			
Evidence of optimization (from gradients)	D	N/A	
Specificity (gel, sequence, melt, or digest)	E	YES	Melt analysis Supplementary Figure S14.
For SYBR Green I, Cq of the NTC	E	YES	Supplementary Figure S14.
Standard curves with slope and y-intercept	E	YES	Supplementary Figure S14.
PCR efficiency calculated from slope	E	N/A	
Confidence interval for PCR efficiency or standard error	D	N/A	
r2 of standard curve	E	YES	Supplementary Figure S14.
Linear dynamic range	E	YES	Supplementary Figure S14.
Cq variation at lower limit	E	N/A	
Confidence intervals throughout range	D	N/A	
Evidence for limit of detection	E	NO	
If multiplex, efficiency and LOD of each assay	E	N/A	
DATA ANALYSIS			
qPCR analysis program (source, version)	E	YES	Materials and Methods
Cq method determination	E	YES	Materials and

			Methods
Outlier identification and disposition	E	N/A	
Results of NTCs	E	YES	Supplementary Figure S14.
Justification of number and choice of reference genes	E	YES	Materials and Methods
Description of normalization method	E	YES	Materials and Methods
Number and concordance of biological replicates	D	YES	Materials and Methods
Number and stage (RT or qPCR) of technical replicates	E	YES	Materials and Methods
Repeatability (intra-assay variation)	E	YES	Materials and Methods
Reproducibility (inter-assay variation, %CV)	D	NO	
Power analysis	D	NO	
Statistical methods for result significance	E	YES	Biological replicates
Software (source, version)	E	YES	Bio-Rad CFX Manager, version 3.1
Cq or raw data submission using RDML	D	N/A	

Supplementary Table S6. Reaction Conditions for qPCR.

Components	Amount
2x KAPA SYBR® FAST qPCR Master Mix (KAPA BIOSYSTEMS)	10 µL
Forward Primer (10 µM)	0.4 µL
Reverse Primer (10 µM)	0.4 µL
Template DNA	2 µL
PCR Grade water	7.2 µL
Total volume	20 µL
PCR tubes (96 x 0.1 mL plate, LP, LF, NON Sk, BIOplastics catalog number B50601).	

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