Shelterin and subtelomeric DNA sequences control nucleosome maintenance and genome stability

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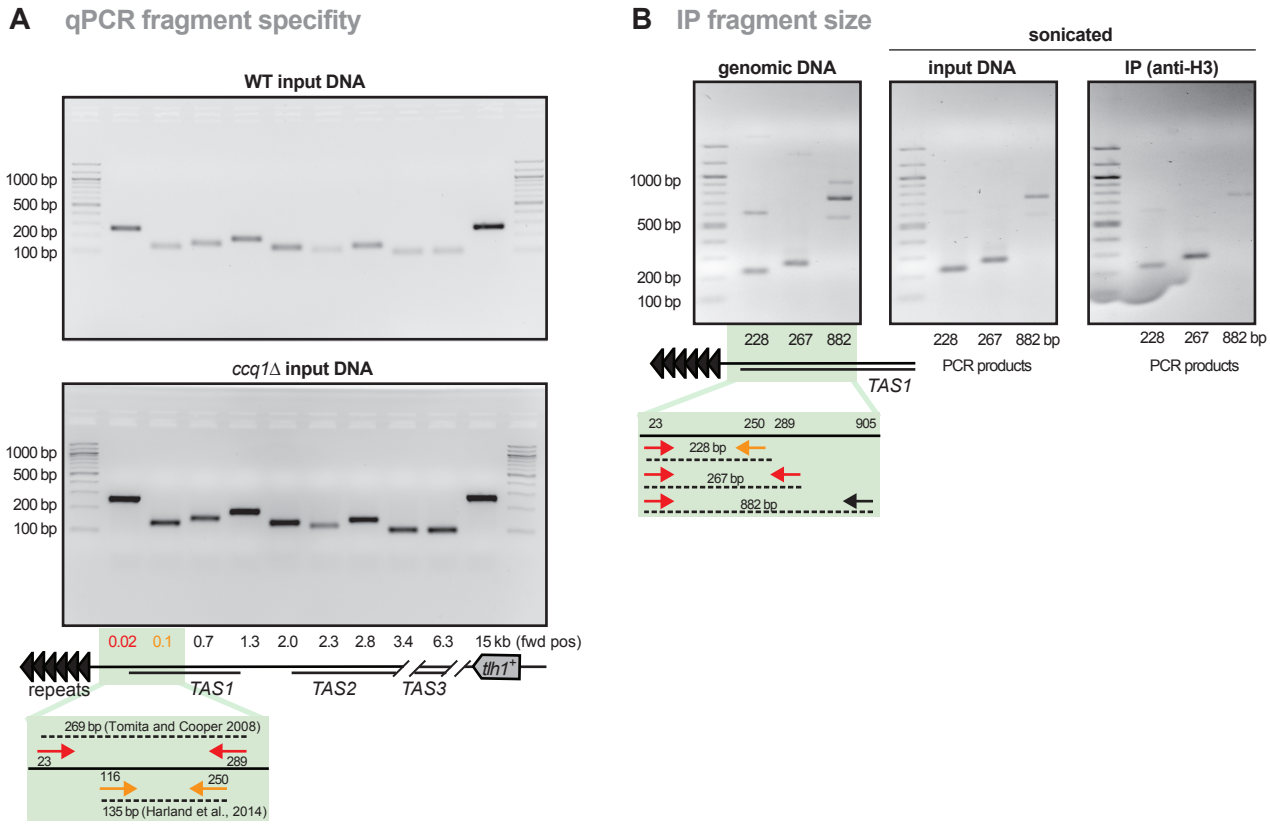
Appendix items



Appendix Figure S1 - qPCR primer specificity and DNA fragment size from ChIP samples.

A qPCR products of indicated primer pairs using WT and *ccq1*Δ genomic DNA (input material from ChIP reactions) were analyzed on a 2% agarose gel. Each primer pair results in a single product and reproducible product size for both WT and *ccq1*Δ samples, ruling out the possibility that potential genetic rearrangements upon deletion of *ccq1*$^{+}$ affect primer specificity. The most telomere-proximal primers were used in previous studies (Tomita & Cooper, 2008; Harland et al, 2014). Note that while the 'forward' primers of these primer pairs anneal to different regions (i.e. 23 bp and 116 bp relative to the telomeric repeats, here denotated as 0.02 kb and 0.1 kb), the resulting PCR products overlap due to the position of the corresponding 'reverse' primers (i.e. 289 bp and 250 bp, respectively). Nonetheless, ChIP experiments performed with Shelterin, CLRC and H3K9me2 (see Figure 1C-E, S2A-B, 2B, respectively) suggest that these primer pairs appear to discriminate between different regions.

B DNA fragment sizes of telomere-proximal fragments (TAS1) DNA analyzed with different primer combinations, as indicated in the scheme. Shown are results for genomic DNA (not-sonicated, left), input DNA (sonicated, middle) and H3 ChIP DNA (sonicated, right). Note that despite multiple primer binding sites (due to the repetitive nature of the TAS) sonication suppresses the appearance of additional (unspecific) PCR products and that products longer than 800 bp are hardly detected in the ChIP material.

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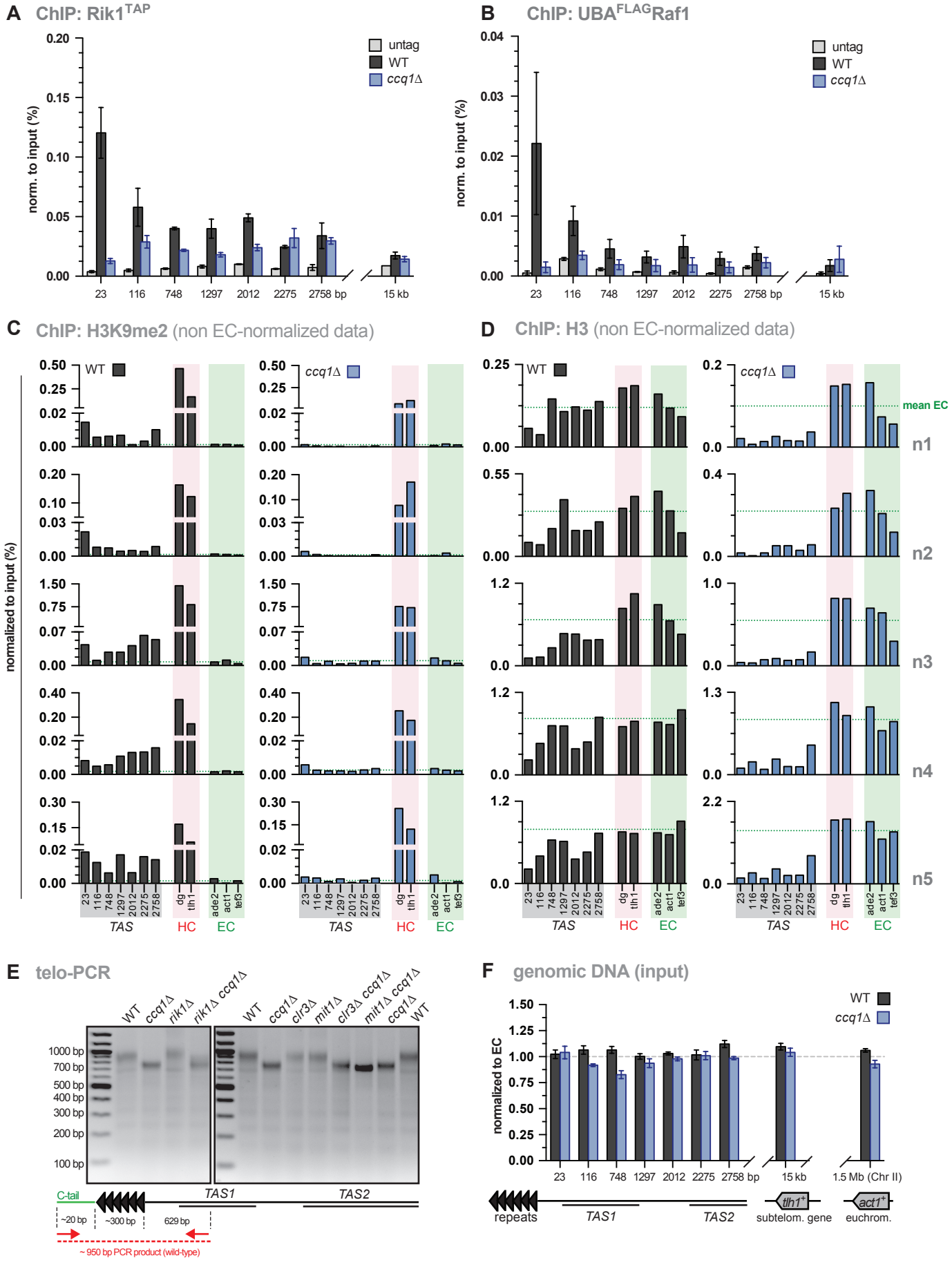


Appendix Figure S2 - Normalization of histone qPCR-ChIP data and telomere length detection in WT and (early) *ccq1*Δ cells.

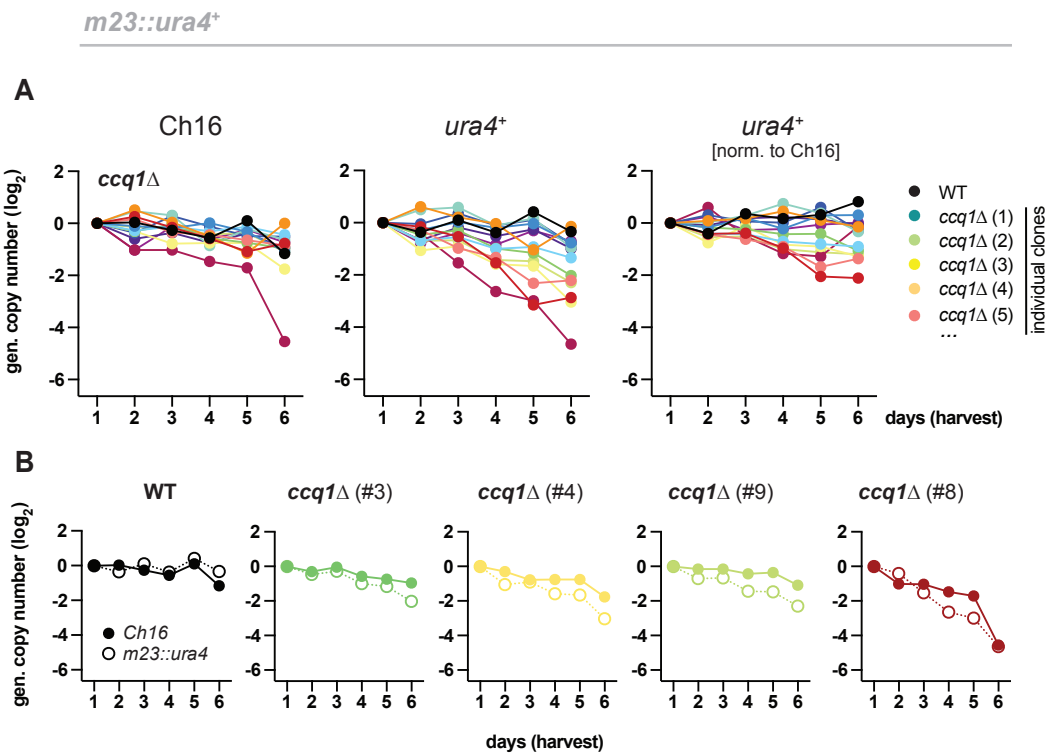
A,B ChIP-qPCR analysis of Rik1^{TAP} (A) and UBA^{FLAG}Raf1 levels (B) in WT and *ccq1 Δ* cells (negative control: untagged strain) (n = 2 and 3 independent biological experiments for Rik1^{TAP} and UBA^{FLAG}Raf1, respectively).

C,D ChIP-qPCR analyses of H3K9me2 (A) and H3 (B) in WT and *ccq1Δ* cells for TAS regions, heterochromatic (HC, shaded in red) and euchromatic loci (EC, shaded in green). The dotted line indicates average EC level based on levels of three euchromatic genes (*ade2*$^+$, *act1*$^+$ and *tef3*$^+$) (n = 5 independent biological experiments). Note that relative differences among various loci are highly reproducible within an individual replicate despite some variability in ChIP efficiency among different replicates, underscoring the significance of using internal references for ChIP normalization.

E Telomere length analysis of different mutants using telo-PCR, which amplifies the entire repeats and 629 bp of the adjacent TAS1 region (Moravec et al, 2016). PCR products are analyzed on a 0.8% agarose gel. Note that telomeres are shortened in early *ccq1*Δ (~200 nt) but still retain telomeric repeats. Telomeric shortening is not seen in mutants deficient in CLRC or SHREC despite the similar nucleosome-loss phenotype as seen in *ccq1*Δ cells (see Fig. 3).

F qPCR analysis of genomic input DNA in WT and *ccq1Δ* cells normalized to EC which is set to 1 (dotted line). Only minor differences (i.e. < 20%) were detected between WT and ccq1Δ, which have been taken into account by normalizing ChIP data (n = 3 independent experiments).

Data information: In (A, B), data are normalized to input (error bars: range and SEM). In (F), data are input normalized (see methods) and represented as mean ± SEM.



Appendix Figure S3 - Normalization for minichromosome loss.

A qPCR analysis as in (Fig. 7A) but with strains harboring the minichromosomesome *Ch16 m23::ura4*$^{+}$. Right panel shows same data as Fig. 7B (note different scale); left and middle panels show analyses for minichromosome (Ch16) and *ura4*$^{+}$ (not normalized for minichromosome loss).

B Selected mutants from A, displayed individually (matching color code) for direct comparison of Ch16 (closed dots) and *ura4*$^{+}$ (open dots) levels.

Appendix Table S1 - Strains used in this study, Related to Experimental Procedures

Strain	*Genotype*	Source	Figure
PSB1615	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ ccq1-HA:kanMX rik1::hygMX*	*1*	1b-c EV1f
PSB1444	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ ccq1-HA:kanMX*	*1*	1b-c, e 3B EV1e-f
PSB0017 (FY1193)	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+*	*2*	1b-e 2b-c 3b-d 4a 5a, d 6a-b EV1b-c S1b S2c-d EV2a-c EV3b EV4b-c
PSB1622	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ ccq1-HA:kanMX clr4::hygMX*	*1*	1c 3b
PSB1099	*P (h+) leu1-32 ade6-MS/E ura4-DS/E taz1-GFP:kanMX cut11-mCherry:hygMX*	*1*	1d
PSB1614	*P (h+) leu1-32 ade6-MS/E ura4-DS/E taz1-GFP:kanMX cut11-mCherry:hygMX rik1::natMX*	*1*	1d
PSB1450	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ rap1-HA:kanMX*	*1*	1d EV1e-f
PSB1447	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ tpz1-HA:kanMX*	*1*	1d EV1f
PSB1548	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ poz1-HA:kanMX*	*1*	1d EV1f
PSB1571	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ pot1-HA:kanMX*	*1*	1d EV1f
PSB1619	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ tpz1-HA:kanMX rik1::hygMX*	*1*	1d EV1f
PSB1744	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ rap1-HA:kanMX rik1::hygMX*	*1*	1d EV1f
PSB1745	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ poz1-HA:kanMX rik1::hygMX*	*1*	1d EV1f

PSB1746	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ pot1-HA:kanMX rik1::hygMX*	*1*	1d EV1f
PSB1677	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ ccq1-HA:kanMX swi6::natMX chp2::hygMX*	*1*	1e 3b
PSB1801 (A2730)	*P (h+) ura4::[4xTetOade6+] leu1+:nmt181xTetRoff-2xFLAG-clr4-cdd ade6-DN/N*	*3*	1f
PSB1861	*P (h+) ura4::[4xTetOade6+] leu1+:nmt181xTetRoff-2xFLAG-clr4-cdd ade6-DN/N ccq1-HA:kanMX*	*1*	1f
PSB1728	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ ccq1::natMX*	*1*	2b-c 3b-d 5a 6a-b EV1c, e-f S1a EV2a EV3b EV4b-c
PSB2127 (FY520)	*Mat1Msmt0 leu1-32 his2- ura4 DS/E ade6-210 m23::ura4-Tel72*	*8*	2b-c 5b 7b EV5b S3
PSB0074 (PM0304)	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ rik1::natMX*	*5*	3b,d 6a-b S1e EV2a EV4b-c
PSB1741	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ rik1::natMX ccq1::kanMX*	*1*	3b,d 6a-b S1e EV4b-c
PSB0067 (SPT812)	*P (h+) leu1-32 ade6-210 otr1::ura4^{+} mit1::kanMX*	*6*	3c
PSB0025 (SBP007)	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ mit1::kanMX*	*5*	3c 6a-b 5b S1e EV2a EV3b EV4c
PSB1730	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ clr3::kanMX*	*1*	3c 6a-b S1e EV4c
PSB2028	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ mit1::kanMX ccq1::natMX*	*1*	3c 6a-b S1e EV4c
PSB2029	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ clr3::kanMX ccq1::natMX*	*1*	3c 6a-b S1e EV4c
PSB0068 (SPT981)	*P (h+) leu1-32 ade6-210 otr1::ura4^{+} mit1-K587A*	*6*	3c-d
PSB0069 (SPT981)	*P (h+) leu1-32 ade6-210 otr1::ura4^{+}*	*6*	3c-d

PSB2338	*M (h-) ade6-M216 his7-366 leu1-32 ura4-D18 SH1L::ura4+ SH1R::his7+ SH2L::his7+ SH2R::his7+ SH3L::ura4+ Leu1::Leu1-TAS1(800bp)*	*1*	5a
PSB2369	*M (h-) ade6-M216 his7-366 leu1-32 ura4-D18 SH1L::ura4+ SH1R::his7+ SH2L::his7+ SH2R::his7+ SH3L::ura4+ Leu1::Leu1-TAS1(800bp) ccq1::natMX*	*1*	5a
PSB0072 (FY1862)	*P (h90) leu1-32 his3D1 ade6-210 ura4-D18 otr1R(Sph1)::ade6+ tel(1L)::his3+ tel(2L)::ura4+*	*9*	5b 7a EV3a EV5a
PSB0023 (SBP005)	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ clr1::kanMX*	*5*	6a-b EV4c
PSB0952	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ natMX:DSK2-FLAG-raf1*	*1*	EV1a-c S2b Table 1
PSB1036	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ natMX:DSK2-FLAG-raf1 rik1::hygMX*	*1*	EV1a-c Table 1
PSB1446	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ natMX:DSK2-FLAG-raf1 ccq1-HA:kanMX rik1::hygMX*	*1*	EV1d
PSB1448	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ natMX:DSK2-FLAG-raf1 tpz1-HA:kanMX*	*1*	EV1d
PSB1449	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ natMX:DSK2-FLAG-raf1 tpz1-HA:kanMX rik1::hygMX*	*1*	EV1d
PSB1445	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ natMX:DSK2-FLAG-raf1 ccq1-HA:kanMX*	*1*	EV1d S1b
PSB0192 (SBP105)	*P (h-) smt0 leu1-32 ade6-210 ura4-D18 epe1:CBP-2x-FLAG:kanMX*	*5*	EV1e
PSB0269 (SBP157)	*P (h-) smt0 leu1-32 ade6-210 ura4-D18 epe1:CBP-2x-FLAG:kanMX pREP-nmt1p-6His-ubi-LEU2*	*5*	EV1e
PSB1519	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ ccq1-HA:kanMX pREP-nmt1p-6His-ubi-LEU2*	*1*	EV1e
PSB1552	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ rap1-HA:kanMX pREP-nmt1p-6His-ubi-LEU2*	*1*	EV1e
PSB1661	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ ccq1-HA:kanMX rik1::hygMX pREP-nmt1p-6His-ubi-LEU2*	*1*	EV1e

PSB1751	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ rap1-HA:kanMX rik1::hygMX pREP-nmt1p-6His-ubi-LEU2*	*1*	EV1e
PSB0064 (SPY44)	*P (h+) leu1-32ade6-M210 ura4-DS/E imr(Nco1)::ura4+ ori1 rik1-TAP:kanMX*	*4*	S1A
PSB1755	*P (h+) leu1-32ade6-M210 ura4-DS/E imr(Nco1)::ura4+ ori1 rik1-TAP:kanMX ccq1::natMX*	*1*	S1a
PSB2056	*P (h90) leu1-32 his3D1 ade6-210 ura4-D18 otr1R(Sph1)::ade6+ tel(1L)::his3+ tel(2L)::ura4+ ccq1::natMX*	*1*	EV3a
PSB1134	*P (h+) leu1-32 ade6-210 Ura4-DS/E imr1L(NcoI)::ura4^{+}otr1R(SphI)::ade6^{+} taz1::hygMX*	*1*	EV3b
PSB2082	*P (h+) leu1-32 ade6-210 ura4-DS/E imr1L(NcoI)::ura4+ otr1R(SphI)::ade6+ mit1::kanMX taz1::hygMX*	*1*	EV3b
PSB2083	*P (h+) leu1-32 ade6-210 Ura4-DS/E imr1L(NcoI)::ura4^{+}otr1R(SphI)::ade6^{+} taz1::hygMX ccq1::kanMX*	*1*	EV3b
PSB2102 (K240)	*M (h-) leu1 -32*	*7*	EV3c
PSB2103 (KYP176)	*M (h-) leu1 -32 snf21 -36(ts)*	*7*	EV3c
PSB2125	*M (h-) leu1 -32 mit1::kanMX*	*1*	EV3c
PSB2126	*M (h-) leu1 -32 snf21 -36(ts) mit1::kanMX*	*1*	EV3c
PSB0078	*P (h90) leu1-32 his3D1 ade6-210 ura4-D18 otr1R(Sph1)::ade6+ tel(1L)::his3+ tel(2L)::ura4+ rik1::natMX*	*1*	EV5a
PSB2336	*P (h90) leu1-32 his3D1 ade6-210 ura4-D18 otr1R(Sph1)::ade6+ tel(1L)::his3+ tel(2L)::ura4+ clr3::natMX*	*1*	EV5a
PSB2371	*P (h90) leu1-32 his3D1 ade6-210 ura4-D18 otr1R(Sph1)::ade6+ tel(1L)::his3+ tel(2L)::ura4+ clr3::natMX*	*1*	EV5a

Strain numbers in brackets indicate original strain numbers.

1 = This study; 2, 3, 8 and 9 = Allshire laboratory (Ekwall *et al*, 1999); 4 = Danesh Moazed laboratory; 5 = Madhani laboratory (Braun *et al*, 2011); 6 and 7 = Grewal laboratory (Sugiyama *et al*, 2007) (Yamada *et al*, 2008).

Appendix Table S2 - Primer sets used for RT-qPCR, ChIP analysis, Telomere-PCR and strain generation, Related to Experimental Procedures

oligo name	FOR oligo	REV oligo	locus	reference
Sg0243/0244 (P059/060)	TGCTCTGACTTGGCTTGTCTT	CCCTAACTTGGAAAGGCACA	*cen-dg*	(Braun *et al*, 2011)
Sg0272/0273 (P_F'tlh1-mb274/276)	ATGGTCGTCGCTTCAGAAATTGC	CTCCTTGGAAGAATTGCAAGCCTC	*tlh1*$^{+}$ *5'*	(Bühler *et al*, 2007)
Sg0423/0424 (P638/639)	AACCCTCAGCTTTGGGTCTT	TTTGCATACGATCGGCAATA	*act1*$^{+}$	(Braun *et al*, 2011)
Sg1026/1027	CAGCAATATCGTACTCCTGAA	ATGCTGAGAAAGTCTTTGCTG	*ura4*$^{+}$	This study
Sg1032/1033	GAGCATGGTGGTGGTTATGGA	CGACTAAACCGAAAGCCTCGA	*cen-imr*	This study
Sg1038/1039	GAAGTTCACTCAGTCATAATTAATTGGAAC	GGGCCCAATAGTGGGGGCATTGTATTTGTG	*TERRA*	(Bah *et al*, 2012)
Sg1906/1097 (Telomeric STE1)	CGGCTGACGGGTGGGGCCCAATA	GTGTGGAATTGAGTATGGTGAA	20bp from tel repeats	(Tomita & Cooper, 2008)
Sg1908/1909 (JK380/381)	TATTTCTTTATTCAACTTACCGCACTTC	CAGTAGTGCAGTGTATTATGATAATTAAAATGG	100bp from tel repeats / TAS1	(Harland *et al*, 2014)
Sg2038/2039	TTATTCACCCATACACACTACACC	GATGAATGGATTAAAAGGTGTTGG	744bp from tel repeats	This study
Sg2102/2103	ATCTACTCCAATATAGTCCTCTGC	GATAATGGATGGAGGTAAGAGAGG	1297bp from tel repeats	This study
Sg2106/2107	TTATATTCCTGCATCCCAACACAT	AAAGAAGATAAAAGCAGGGGACTA	2275bp from tel repeats	This study
Sg2139/2149	TCGTTAACAACATTTAACGATTACTCG	ACGTTTGTTGAGTGATATGTCGTCG	2758bp from tel repeats	This study
Sg2141/2142	TACTCCAACACACTCAATACATACC	AAGTAGGAGAATGAAGAAGTAATCAAAG	2012bp from tel repeats	This study

Sg2309/2310 (qura4 5'for/rev)	TCGCAGACATTGGAAATACC	ATGGCAATTTGTGATATGAGC	-0.5 Kb from *tetO*	(Audergon *et al*, 2015)
Sg2527/2585	GTAGACAGCAATCCAGGTCAAAGAC	TTTCCTACCAGCGGTCCGTCTTCC	HC island 14	(Zofall *et al*, 2016) this study
Sg2671/2672	AGGCATCTGATCCCAATGAG	ATTTTGGATGCCTTGGATGA	*ade2*$^{+}$	This study
Sg2708/2709	TGAGTGTGCTGGAGTACGTT	CGGGATCCGGGGGGGGGGGGGGGGGG	Telo-PCR	(Moravec *et al*, 2016)
Sg2736/2737	TGGCCTTCTTAGCCTTTTCA	CTGAGGAAGTTTGGGCTGTC	*tef3*$^{+}$	This study
Sg2972/2973	AGAAGAAGAACAGACGGGTGA	CCCCATAACCCTAACCTCGT	HC island 15	This study
Sg2983/2984	GGTAAGCCTAGTAACGATGCC	GTGCCAACAGTGATACGCAA	*his3*$^{+}$	This study
Sg2985/2986	CCCCGACGACATATCACTCA	ATGAACGACAAACAGCAGGC	TAS2	This study
Sg2991/2992	CCTCTGACAGATGCTCAAACC	TGGTTACGGTTATTAGGTGATGT	TAS3	This study
Sg3119/3120	CAATTGGGCCGAATGATGGT	TGCTCACGTCCTCCATCAAT	*ade6*$^{+}$	This study
Sg3121/3122	TGACCCCGATGCAATTGTTG	AGAGTTGCAGGAGAGGGTTC	*ade6*$^{+}$	This study
Sg3123/3124	TTCCAGTAATCGGCGTTCCT	CGACAGGCTAAAATACCGGC	*ade6*$^{+}$	This study
Sg3182/3183	GGGCCCCCCCTCGAGGTCGACTATCTTTATTCAACTTACCGCACTTC	CGCTCTAGAACTAGTGGATCCGATGAATGGATTAAAAGGTGTTGG	TAS1 region	This study

Names in brackets indicate original oligonucleotide names used by previous studies.

Appendix References

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