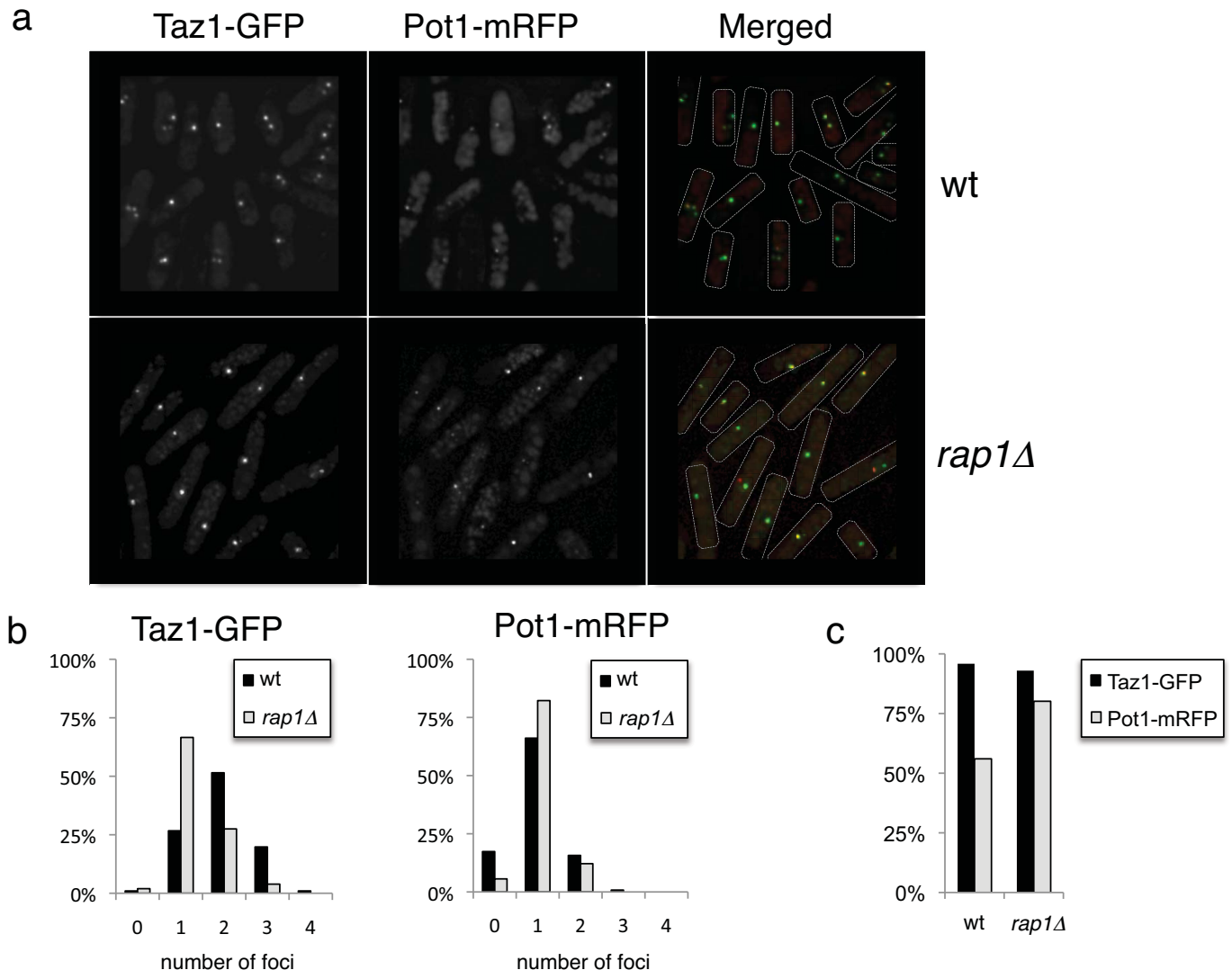
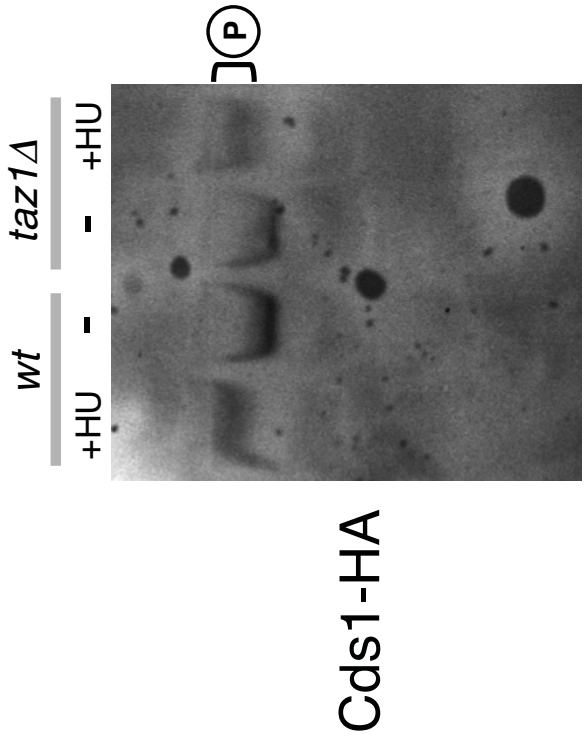
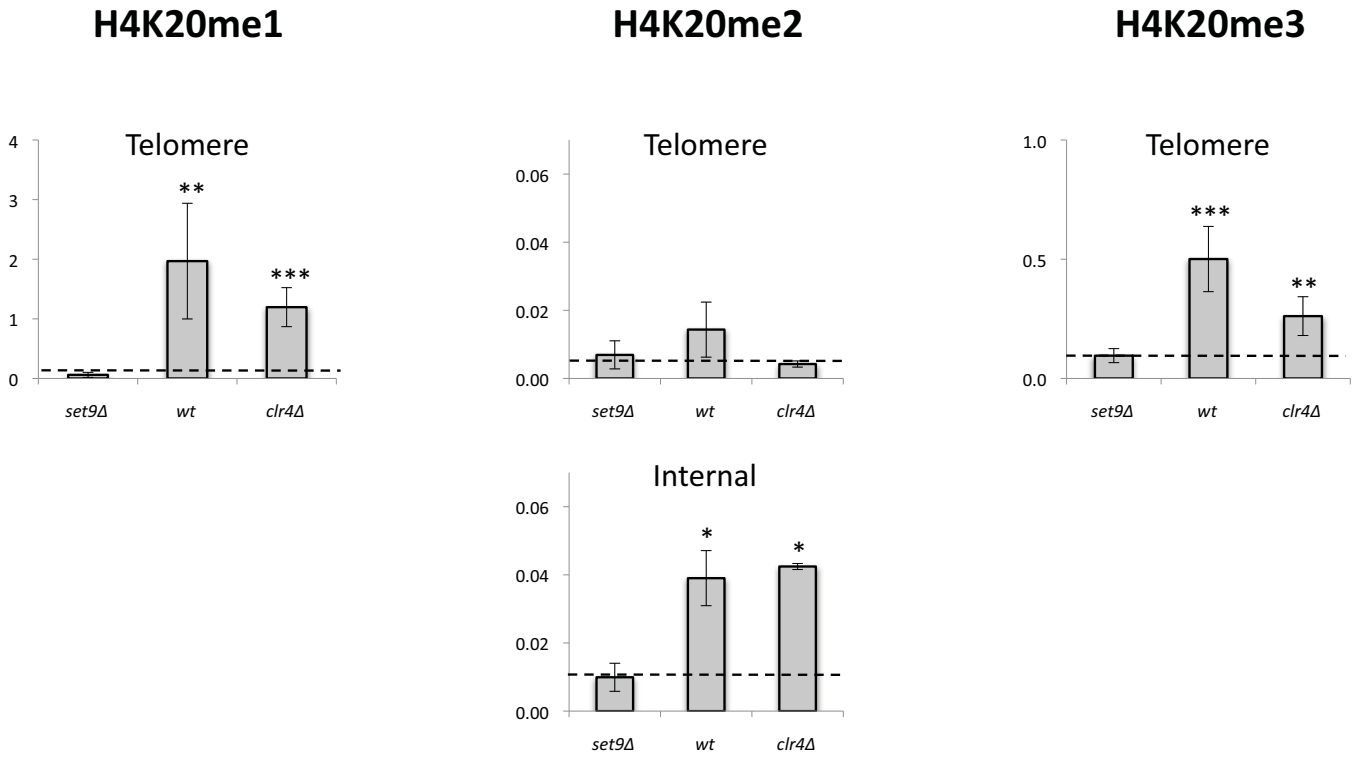


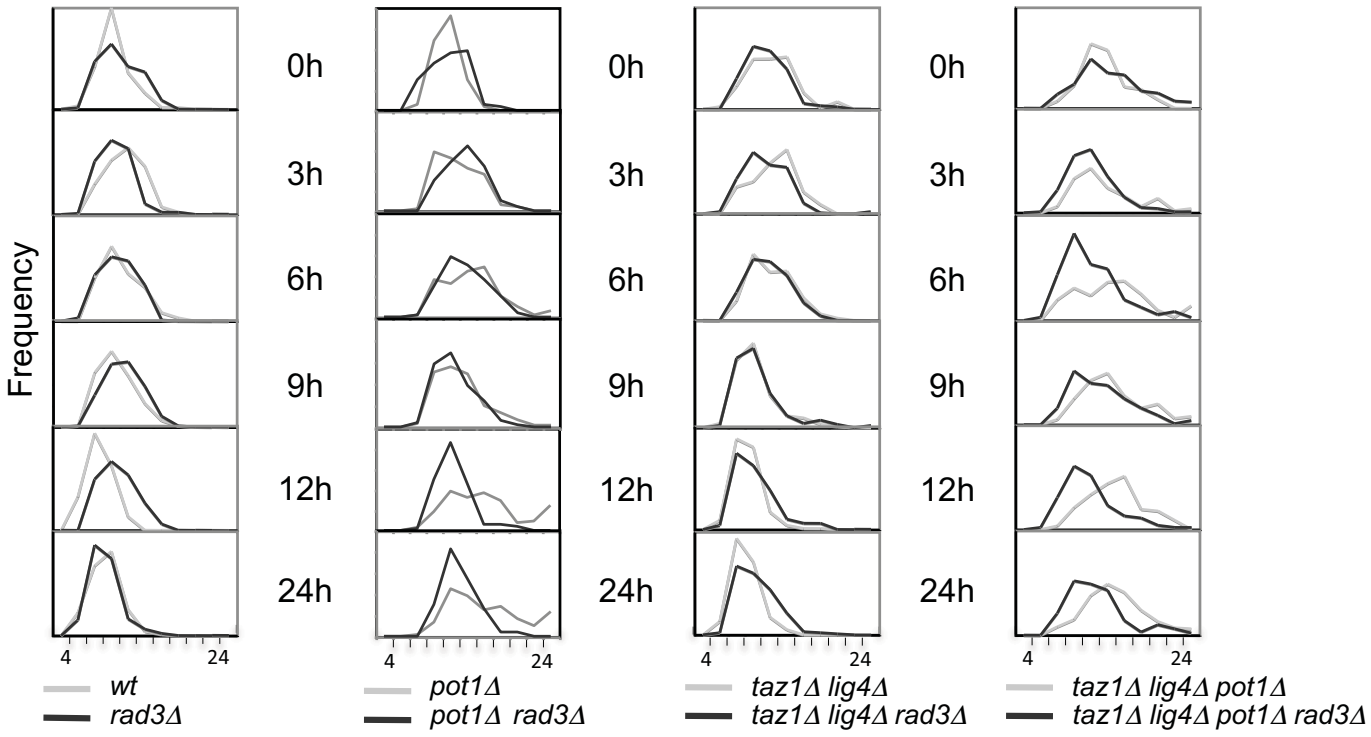
Supplementary Figure S1 – Validation of Pot1-mRFP as a marker of wt and *rap1*Δ dysfunctional telomeres. (a) Images showing Taz1-GFP and Pot1-mRFP localization in wt and *rap1*Δ cells. (b) Distribution of Taz1-GFP and Pot1-mRFP foci per cell. In wt cells, Taz1-GFP foci appear in a distribution of 1-3 foci per cells. In contrast, telomeres appear to coalesce in *rap1*Δ cells since most cells possess either 1 or 2 Taz1-GFP foci. Both wt and *rap1*Δ cells exhibit 1 or 2 Pot1-mRFP foci per cell. (c) Quantification of cells exhibiting Taz1-GFP and Pot1-mRFP foci. Whereas Taz1-GFP foci are visible in the majority of cells, Pot1-mRFP is only detected in 56% of wt cells. However, Pot1-mRFP foci are visible in 80% of *rap1*Δ cells. As all Pot1-mRFP colocalize with Taz1-GFP in *rap1*Δ cells, Pot1-mRFP presents as a good telomeric marker in cells with dysfunctional telomeres.



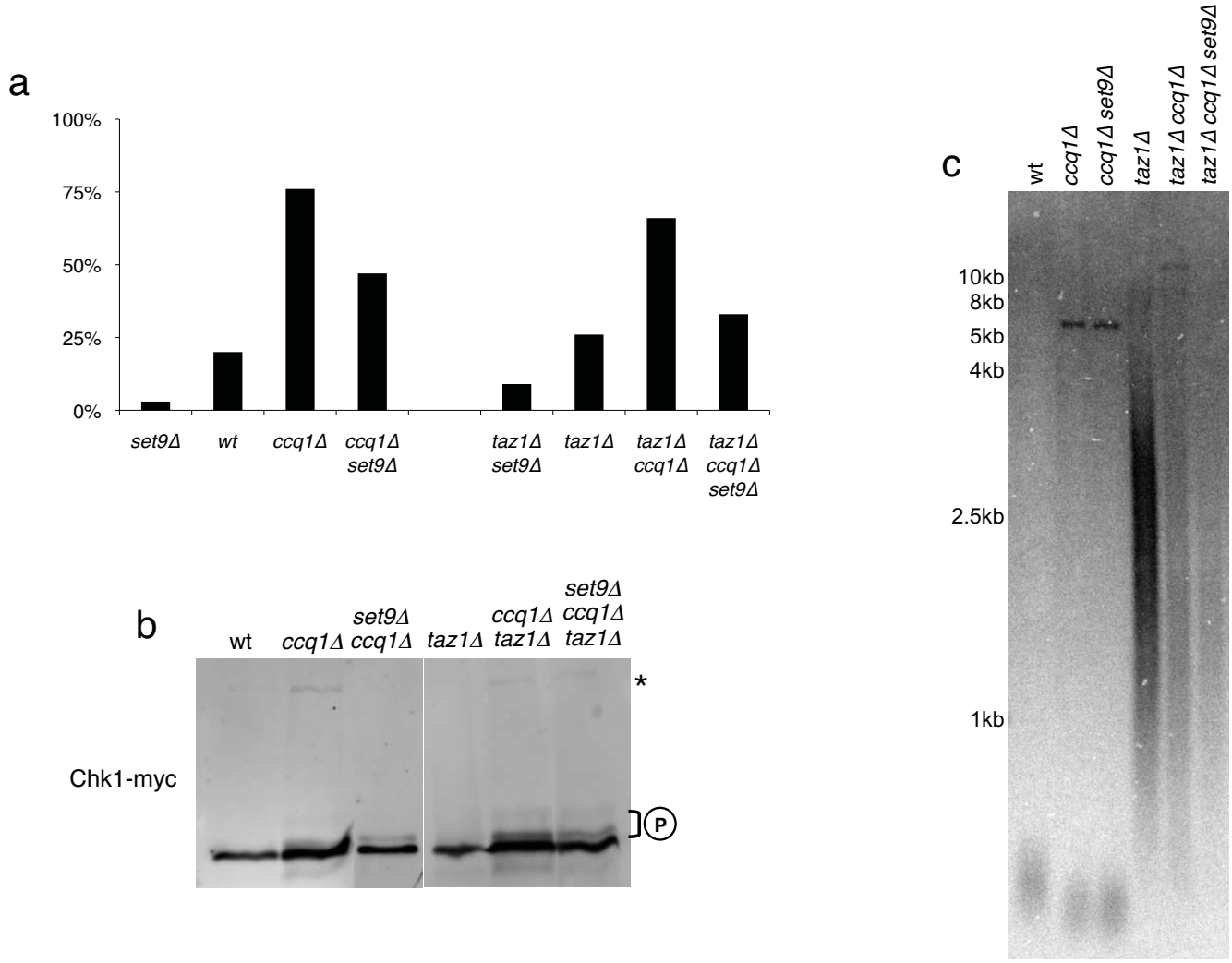
Supplementary Figure S2 – Replication checkpoints are inactive in *taz1*Δ cells. Immunoblot analysis of Cds1-HA using Phos-tag™ polyacrylamide gels. Extracts derived from wt and *taz1*Δ cells exhibit hyperphosphorylation of Cds1 upon incubation with 20mM HU. Similar to wt cells, untreated *taz1*Δ cells lack Cds1 hyperphosphorylation.



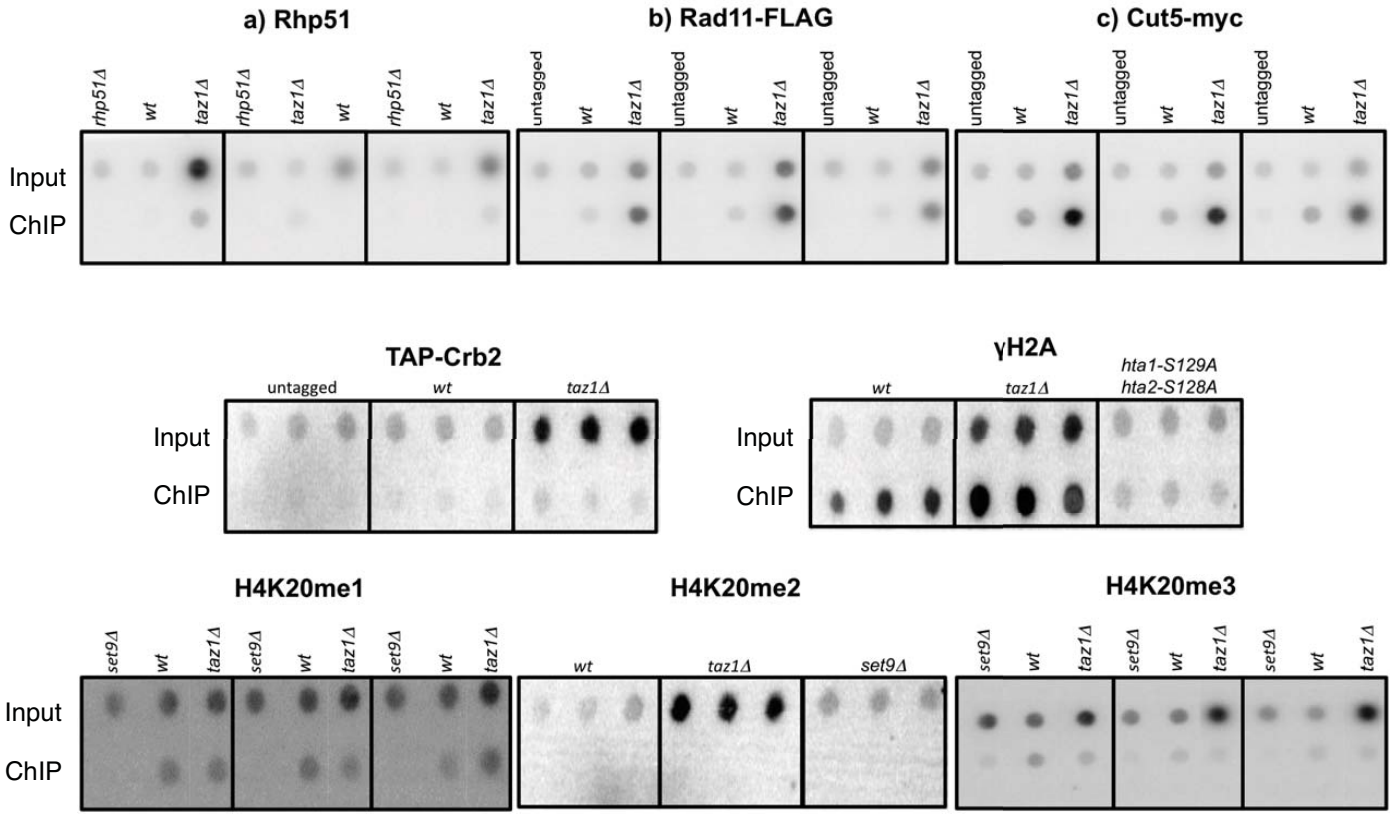
Supplementary Figure S3 – Clr4-dependent heterochromatin is not responsible for H4K20me2 telomere exclusion. Telomeres are rich in H4K20me1 and H4K20me3 but lack H4K20me2 modifications, in contrast to internal chromosomal loci. Absence of Clr4 methyltransferase (*clr4*Δ) does not greatly affect H4K20 methylation pattern at telomeres, although a reduction in H4K20me3 modification can be observed. Cells lacking Set9/Kmt5 methylase (*set9*Δ) were used as control. qPCR based ChIP analysis of H4K20me1, H4K20me2 and H4K20me3 at telomeres (Telomere) and *ade6*$^{+}$ locus (Internal) on the indicated strains shown as percentage precipitated DNA. Error bars, s.d.; n≥3; * p<0.05, ** p<0.01 and *** p<0.001 based on a two-tailed Student's t-test to controls.



Supplementary Figure S4 – Pot1 prevents Rad3/ATR checkpoints at telomeres.
Delay in cell cycle progression activated by depleting Pot1 is dependent on *rad3*$^{+}$. Histograms depicting the distribution of cell sizes in μm at different time points in haploid cells of the indicated genotype. Meiosis was induced in *rad3*$^{+/\Delta}$; *pot1*$^{+/\Delta}$*rad3*$^{+/\Delta}$; *taz1*$^{\Delta/\Delta}$*lig4*$^{\Delta/\Delta}$*rad3*$^{+/\Delta}$ and *taz1*$^{\Delta/\Delta}$*lig4*$^{\Delta/\Delta}$*pot1*$^{+/\Delta}$*rad3*$^{+/\Delta}$ diploids and the resulting spores were allowed to germinate in rich media for 7h. Subsequently, the resulting haploid cells were incubated in selective media for the desired genotype and samples were taken at different time points.



Supplementary Figure S5 – Preventing H4K20 methylation in *ccq1*Δ cells partially rescues telomere deprotection. In *ccq1Δ* and *ccq1Δ taz1Δ* cells, a reduction in the number of YFP-Crb2 foci is observed upon deletion of Set9. (a) Quantification of the number of YFP foci in cells expressing YFP-Crb2 from a pREP81 multicopy plasmid. (b Immunoblot analysis of Chk1-myc in *ccq1Δ* and *ccq1Δ taz1Δ* cells upon deletion of Set9 (c) Telomere lengh analysis by southern blotting using a telomere probe in the indicated strains.



Supplementary Figure S6 – Telomeric dot-blots used for ChIP quantification. Upper rows depict input samples whereas lower rows represent ChIP samples for three independent experiments. Input samples exhibit 40% of total DNA.

Supplementary Table 1. Strains used in this study.

Strain	*Genotype*	Creator
MGF10	*h- ade6-M210 his3-D1 leu1-32 ura4-D18*	J Cooper
MGF11	*h+ ade6-M216 his3-D1 leu1-32 ura4-D18*	J Cooper
MGF21	*h+ taz1::KanMX6*	J Cooper
MGF224	*h- ade6-M216 leu1-32 ura4-D18 pot1::pot1-mRFP-KanMX6 rad11::rad11-GFP-KanMX6*	This study
MGF225	*h- ade6-M210 leu1-32 ura4-D18 pot1::pot1-mRFP-KanMX6 rad11::rad11-GFP-KanMX6 taz1::ura4$^+$*	This study
MGF226	*h+ ade6-M210 leu1-32 ura4-D18 pot1::pot1-mRFP-KanMX6 rad22::rad22-GFP-KanMX6*	This study
MGF228	*h+ leu1-32 ura4-D18 rad26::rad26-GFP-KanMX6*	T Wolkow
MGF232	*h+ ade6-M216 ura4-D18 pot1::pot1-mRFP-KanMX6 rad26::rad26-GFP-KanMX6*	This study
MGF233	*h+ ade6-M216 leu1-32 ura4-D18 pot1::pot1-mRFP-KanMX6 rad26::rad26-GFP-KanMX6 taz1::ura4$^+$*	This study
MGF298	*h+ ade6-M210 leu1-32 ura4-D18 his3-D3 rad26::rad26-3HA*	AM Carr
MGF304	*h-/h+ ade6-M210/ade6-M216 his3-D1/his3-D1 leu1-32/leu1-32 ura4-D18/ura4-D18*	J Cooper
MGF309	*h-/h+ ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18 lig4::KanMX6/lig4::KanMX6 taz1::ura4$^+$/taz1::ura4$^+$*	J Cooper
MGF318	*h- rad4::rad4-GFP-ura4$^+$*	AM Carr
MGF320	*h- leu1-32::2xYFP-crb2-leu1$^+$ ura4-D18 crb2-D2::ura4$^+$*	P Russell
MGF320	*h- leu1-32::2xYFP-crb2-leu1$^+$ ura4-D18 crb2-D2::ura4$^+$*	P Russell
MGF321	*h+ ade6-M210 leu1-32 ura4-D18 rad9::Rad9-YFP-ura4$^+$*	AM Carr
MGF346	*h- ade6-M210 his3-D1 leu1-32 ura4-D18 cds1::cds1-3HA-KanMX6*	This study
MGF347	*h- ade6-M210 his3-D1 ura4-D18 taz1::ura4 cds1::cds1-3HA-KanMX6*	This study
MGF348	*h- ade6-M210 his3-D1 leu1-32 ura4-D18 chk1::chk1-13myc-KanMX6*	This study
MGF381	*h-/h+ ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18 lig4::KanMX6/lig4::KanMX6 taz1::ura4$^+$/taz1::ura4$^+$ pot1$^+$/pot1::hphMX6*	This study
MGF424	*h- ade6-M210 his3-D1 leu1-32 ura4-D18 chk1::chk1-13myc-KanMX6 taz1::ura4$^+$*	This study
MGF449	*h-/h+ ade6-M210/ade6-M216 his3-D1/his3-D1 leu1-32/ leu1-32 ura4-D18/ura4-D18 pot1$^+$/pot1::ura4$^+$*	This study
MGF643	*h- ade6-M210 ura4-D18 leu1-32::2xYFP-crb2-leu1$^+$ crb2-D2::ura4$^+$ pot1::pot1-mRFP-KanMX6*	This study
MGF645	*h- ade6-M210 ura4-D18 leu1-32::2xYFP-crb2-leu1$^+$ crb2-D2::ura4$^+$ pot1::pot1-mRFP-KanMX6 taz1::Kan-MX6*	This study
MGF646	*h+ ade6-M210 ura4-D18 rad4::rad4-GFP-ura4$^+$ pot1::pot1-mRFP-KanMX6*	This study
MGF647	*h- ade6-M210 ura4-D18 rad4::rad4-GFP-ura4$^+$ pot1::pot1-mRFP-KanMX6 taz1::ura4$^+$*	This study
MGF649	*h- leu1-32::2xYFP-crb2-leu1$^+$ ura4-D18 crb2-D2::ura4$^+$ taz1::KanMX6*	This study
MGF674	*h+ ade6-M216 leu1-32 ura4-D18 rad9::rad9-YFP-ura4$^+$ pot1::pot1-mRFP-KanMX6 taz1::KanMX6*	This study
MGF675	*h+ ade6-M216 leu1-32 ura4-D18 rad9::rad9-YFP-ura4$^+$ pot1::pot1-mRFP-KanMX6*	This study
MGF726	*h- ade6-M210 leu1-32 ura4-D18 his3-D1 rad3::natMX6*	This study
MGF728	*h+ ade6-M216 leu1-32 ura4-D18 taz1::taz1-GFP-KanMX6 pot1-mRFP-KanMX6*	This study
MGF801	*h-/h+ ade6-M216/ade6-M210 his3-D1/his3-D1 ura4-D18/ura4-D18 leu1-*	This study

	32::2YFP-crb2-leu1$^+$/leu1-32::2YFP-crb2-leu1$^+$ crb2-D2::ura4$^+$/crb2-D2::ura4$^+$ taz1::KanMX6/taz1::kanMX6 lig4::kanMX6/lig4::KanMX6	
MGF802	*h-/h- ade6-M216/ade6-M210 his3-D1/his3-D1 ura4-D18/ura4-D18 leu1-32::2YFP-crb2-leu1$^+$/leu1-32::2YFP-crb2-leu1$^+$ crb2-D2::ura4$^+$/crb2-D2::ura4$^+$ taz1::KanMX6/taz1::kanMX6 lig4::kanMX6/lig4::KanMX6 pot1$^+$/pot1::hphMX6*	This study
MGF804	*h-/h+ ade6-M210/ade6-M216 his3-D1/his3-D1 leu1-32/leu1-32 ura4-D18/ura4-D18 taz1::KanMX6/taz1::KanMX6*	This study
MGF821	*h- ade6-M210 leu1-32 ura4-D18 his3-D1 rad3::natMX6 taz1::KanMX6*	This study
MGF873	*h- ade6-M210 his3-D1 leu1-32 ura4-D18 chk1::chk1-13myc-KanMX6 + pJR81XL(LEU2)*	This study
MGF876	*h- ade6-M210 his3-D1leu1-32 ura4-D18 taz1::ura4$^+$ chk1::chk1-13myc-KanMX6 + pJR81XL(LEU2)*	This study
MGF882	*h- ade6-M210 his3-D1 leu1-32 ura4-D18 chk1::chk1-13myc-KanMX6 + pJR81XL-myb-2YFP-crb2(LEU2)*	This study
MGF885	*h- ade6-M210 his3-D1leu1-32 ura4-D18 taz1::ura4 chk1::chk1-13myc-KanMX6 + pJR81XL-myb-2YFP-crb2(LEU2)*	This study
MGF888	*h+ ade6-M210 leu1-32 ura4-D18 pot1::pot1-mRFP-KanMX6 + pJR81XL-myb-2YFP-crb2(LEU2)*	This study
MGF894	*h+ ade6-M216 leu1-32 ura4-D18 pot1::pot1-mRFP-KanMX6 taz1::ura4$^+$+ pJR81XL-myb-2YFP-crb2(LEU2)*	This study
MGF927	*h+ ade6-M216 leu1-32 ura4-D18 taz1::taz1-GFP-KanMX6 pot1::pot1-mRFP-KanMX6 rap1::hphMX6*	This study
MGF977	*h- ade6-M210 leu1-32 ura4-D18 his3-D1 rad3::natMX6 taz1::KanMX6 + pJR81XL(LEU2)*	This study
MGF978	*h- ade6-M210 leu1-32 ura4-D18 his3-D1 rad3::natMX6 taz1::KanMX6 + pJR41XL-myb-2YFP-Crb2(LEU2)*	This study
MGF998	*h- ade6-M210 his3-D1 leu1-32 ura4-D18 rad9::rad9-3HA-hphMX6*	This study
MGF999	*h+ taz1::kanMX6 rad9::rad9-3HA-hphMX6*	This study
MGF1000	*h-/h+ ade6-M210/ade6-M216 his3-D1/his3-D1 leu1-32/leu1-32 ura4-D18/ura4-D18 chk1::ckh1-13myc-natMX6/chk1$^+$*	This study
MGF1001	*h-/h+ ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18 lig4::KanMX6/lig4::KanMX6 taz1::ura4$^+$/taz1::ura4$^+$ chk1::chk1-13myc-natMX6/chk1$^+$*	This study
MGF1002	*h-/h+ ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18 lig4::KanMX6/lig4::KanMX6 taz1::ura4$^+$/taz1::ura4$^+$pot1::hphMX6/pot1$^+$ chk1::ckh1-13myc-natMX6/chk1$^+$*	This study
MGF1003	*h-/h+ ade6-M210/ade6-M216 his3-D1/his3-D1 leu1-32/leu1-32 ura4-D18/ura4-D18 chk1::ckh1-13myc-natMX6/chk1$^+$ pot1::ura4$^+$/pot1$^+$*	This study
MGF1004	*h-/h+ ade6-M210/ade6-M216 his3-D1/his3-D1 leu1-32/leu1-32 ura4-D18/ura4-D18 rad3::natMX6/rad3$^+$*	This study
MGF1005	*h-/h+ ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18 lig4::KanMX6/lig4::KanMX6 taz1::ura4$^+$/taz1::ura4$^+$ rad3::natMX6/rad3$^+$*	This study
MGF1030	*h- ade6-M210 leu1-32 ura4-D18 his3-D1 rad3::natMX6 taz1::KanMX6 chk1::chk1-13myc-hphMX6 + pJR81XL(LEU2)*	This study
MGF1031	*h- ade6-M210 leu1-32 ura4-D18 his3-D1 rad3::natMX6 taz1::KanMX6 chk1::chk1-13myc-hphMX6 taz1-::KanR + pJR81XL-myb-2YFP-crb2(LEU2)*	This study
MGF1044	*h+ ade6-M210 leu1-32 ura4-D18 his3-D3 rad26::rad26-3HA taz1::kanMX6*	This study
MGF1045	*h-/h+ ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18 lig4::KanMX6/lig4::KanMX6 taz1::ura4$^+$/taz1::ura4$^+$ pot1**::**hphMX6/pot1$^+$ rad3::natMX6/rad3$^+$*	This study
MGF1046	*h-/h+ ade6-M210/ade6-M216 his3-D1/his3-D1 leu1-32/leu1-32 ura4-D18/ura4-D18 pot1::ura4$^+$/pot1$^+$ rad3::natMX6/rad3$^+$*	This study
MGF1054	*h-/h+ ade6-M210/ade6-M216 taz1::ura4$^+$/taz1$^+$*	J Cooper
MGF1055	*h+ ade6-M216 his3-D1 leu1-32 ura4-D18 pot1::pot1-mRFP-KanMX6*	This study

	rad22::rad22-GFP-KanMX6 taz1::ura4$^+$	
MGF1263	*h- ade6-M210 his3-D1 leu1-32 ura4-D18 chk1::chk1-13myc-KanMX6 ccq1::hphMX6*	This Study
MGF1264	*h- ade6-M210 his3-D1 leu1-32 ura4-D18 chk1::chk1-13myc-KanMX6 trt1::hphMX6*	This Study
MGF1265	*h- ade6-M210 his3-D1 leu1-32 ura4-D18 chk1::chk1-13myc-KanMX6 taz1::ura4$^+$ ccq1::hphMX6*	This Study
MGF1344	*h- ade6-M210 his3-D1 leu1-32 ura4-D18 chk1::chk1-13myc-KanMX6 + pJR81XL-2YFP-crb2(LEU2)*	This Study
MGF1345	*h- ade6-M210 his3-D1 leu1-32 ura4-D18 chk1::chk1-13myc-KanMX6 taz1::ura4$^+$ + pJR81XL-2YFP-crb2(LEU2)*	This Study
MGF1366	*h- ade6-M210 his3-D1 leu1-32 ura4-D18 chk1::chk1-13myc-KanMX6 ccq1::hphMX6 + pJR81XL-2YFP-crb2(LEU2)*	This Study
MGF1369	*h- ade6-M210 his3-D1 leu1-32 ura4-D18 chk1::chk1-13myc-KanMX6 taz1::ura4$^+$ ccq1::hphMX6 + pJR81XL-2YFP-crb2(LEU2)*	This Study
MGF1371	*ade6-M210 leu1-32 his3-D1 ura4-D18 taz1::ura4$^+$ trt1::his3$^+$ chk1::chk1-13myc-KanMX6*	This Study
MGF1422	h- *his3-D1 leu1-32 ura4-D18 set9::KanMX6 chk1::chk1-13myc-hphMX6 ccq1::natMX6*	This Study
MGF1424	*h- his3-D1 leu1-32 ura4-D18 set9::KanMX6 taz1::ura4$^+$ chk1::chk1-13myc-hphMX6 ccq1::natMX6*	This Study
MGF1430	*h- his3-D1 leu1-32 ura4-D18 set9::KanMX6 chk1-13myc-hphMX6 + pJR81XL-2YFP-Crb2(LEU2)*	This Study
MGF1431	*h- his3-D1 leu1-32 ura4-D18 set9::KanMX6 taz1::ura4$^+$ chk1::chk1-13myc-hphMX6 + pJR81XL-2YFP-Crb2(LEU2)*	This Study
MGF1432	*h- his3-D1 leu1-32 ura4-D18 set9::KanMX6 taz1::ura4$^+$ chk1::chk1-13myc-hphMX6 ccq1::natMX6 + pJR81XL-2YFP-Crb2(LEU2)*	This Study
MGF1454	*his3-D1 leu1-32 ura4-D18 set9::KanMX6 chk1-13myc-hphMX6 ccq1::natMX6 + pJR81XL-2YFP-Crb2(LEU2)*	This Study
TN1583	*h- leu1-32 ura4-D18 ade6-M210 his3-D1 hta1-S129A-ura4$^+$ hta2-S128A-his3$^+$*	T. Nakamura
TN436	*h+ leu1-32 ura4-D18 ade6-M216 his3-D1 rhp51::ura4$^+$*	T. Nakamura
TN2411	*h- leu1-32 ura4-D18 his3-D1*	T. Nakamura
TN3875	*h- leu1-32 ura4-D18 TAP-crb2$^+$*	T. Nakamura
TN4422	*h- leu1-32 ura4-D18 his3-D1 cut5::cut5-13myc-kanMX6*	T. Nakamura
TN5599	*h- leu1-32 ura4-D18 his3-D1 rad11::rad11-5FLAG-KanMX6*	T. Nakamura
TN6331	*h+ leu1-32 ura4-D18 his3-D1 taz1-2::ura4$^+$ rad11-5FLAG:KanMX*	T. Nakamura
LK8667	*h- leu1-32 ura4-D18 his3-D1 ccq1::hphMX6*	This Study
LK8880	*h- leu1-32 ura4-D18 his3-D1 taz1-2::ura4$^+$ ccq1::hphMX6*	This Study
LK10138	*h+ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4$^+$ TAP-crb2$^+$*	This Study
LK10142	*h+ leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4$^+$ cut5::cut5-13myc-kanMX6*	
LK10146	*h- leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4$^+$ hta1-S129A:ura4$^+$ hta2-S128A:his3$^+$*	This Study
LK10152	*h+ leu1-32 ura4-D18 his3-D1 taz1-2::ura4$^+$ set9::kanMX6*	This Study
LK10177	*h- leu1-32 ura4-D18 his3-D1 ccq1::hphMX set9::kanMX6*	This Study
LK10180	*h- leu1-32 ura4-D18 his3-D1 ccq1::hphMX TAP-crb2$^+$*	This Study
CF213	*h- leu1-32 ura4-D18 ade6-M210 his3-D1 taz1-2::ura4$^+$*	T. Nakamura
WL5954	*h- leu1-32 ura4-D18 his3-D1 arg3::HO-KanRa TAP-crb2$^+$ ars1(Mlu1)::nmt(41)-HOwt:his3$^+$*	This Study
BAM4195	*h- leu1-32 ura4-D18 his3-D1 clr4::kanMX6*	This Study
BAM4202	*h- leu1-32 ura4-D18 his3-D1 set9::kanMX6*	This Study

Supplementary Table 2 - DNA primers used for ChIP.

Location	Primer Name	Primer Sequence (5' to 3')	References
telomere (TAS1)	Jk380	TATTTCTTTATTCAACTTACCGCACTTC	6
	Jk381	CAGTAGTGCAGTGTATTATGATAATTAAAATGG	6
ade6+ locus	TN633	TGATGGAGGACGTGAGCACATTGA	This Study
	TN634	TTGAATGCATCGCAGAGTTGCAGG	This Study
HO site (*arg3*$^{+}$ locus)	TN545	GCATACGATATATTACGGCGCCAA	This Study
	TN546	TTCGTACCCAATTCGCCCTATAGT	This Study