TOR complex 2 is required for chromatin-mediated gene silencing and assembly of heterochromatic domains at subtelomeres

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Supporting information

Figure S1

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Table S1

Table S2

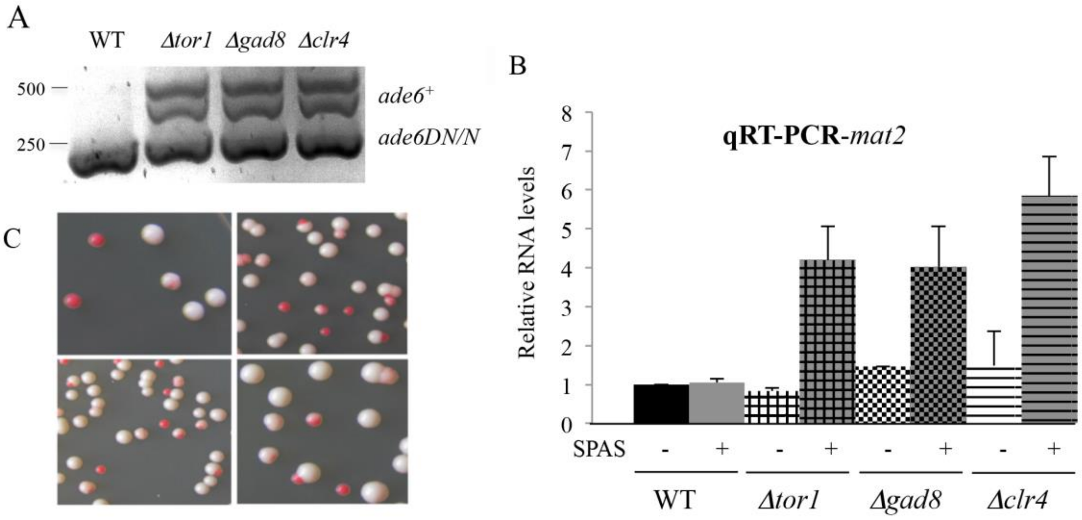

Figure S1: ***The TORC2-Gad8 pathway promotes silencing at the mating-type region*****.** **(A)** Semi-quantities RT-PCR analysis *of the ade6*$^{+}$ *gene inserted at the mating-type locus* (*mat2:BamHI::ade6*$^{+}$). A mini- *ade6*$^{+}$ gene at the *ade6*$^{+}$ locus (*ade6-DN/N*) is used as internal controls. Δ*clr4* cells are used for comparison **(B)** *Loss of TORC2-Gad8 only weekly increases the endogenous mat2 expression.* Expression levels of *mat2*$^{+}$ were examined in wild type (WT) or cells carrying mutations in *Δtor1, Δgad8* or *Δclr4* cells with or without a five-hour shift to SPAS, a starvation medium that is used to induce cells into the sexual development pathway. **(C)** *Δgad8 cells display a highly variegated phenotype with respect to expression of the mat2:BamHI::ade6*$^{+}$ reporter gene. *Δgad8* cells carrying *mat2:BamHI::ade6*$^{+}$ were spread on YE-Ade plates.

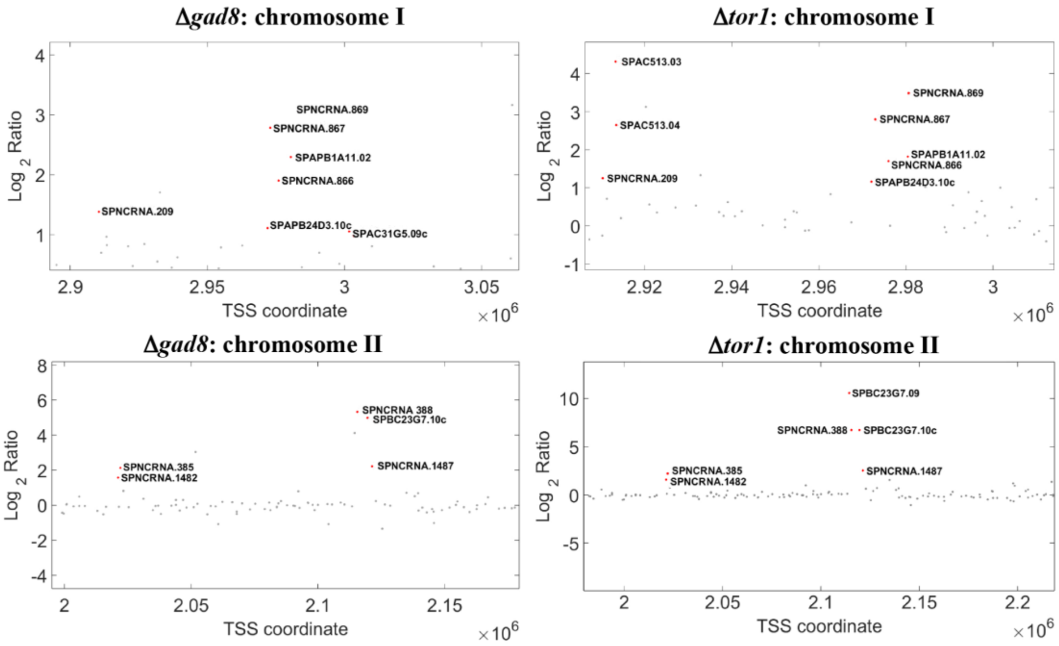

Figure S2: ***TORC2-Gad8 affects gene expression at localized chromosomal regions outside the subtelomeric regions***. Magnification of the RNA-seq data for the regions indicated by blue arrows in Fig. 2 on the arms on chromosomes I and II that show clusters of upregulated genes in Δ*tor1* and Δ*gad8* mutant cells.

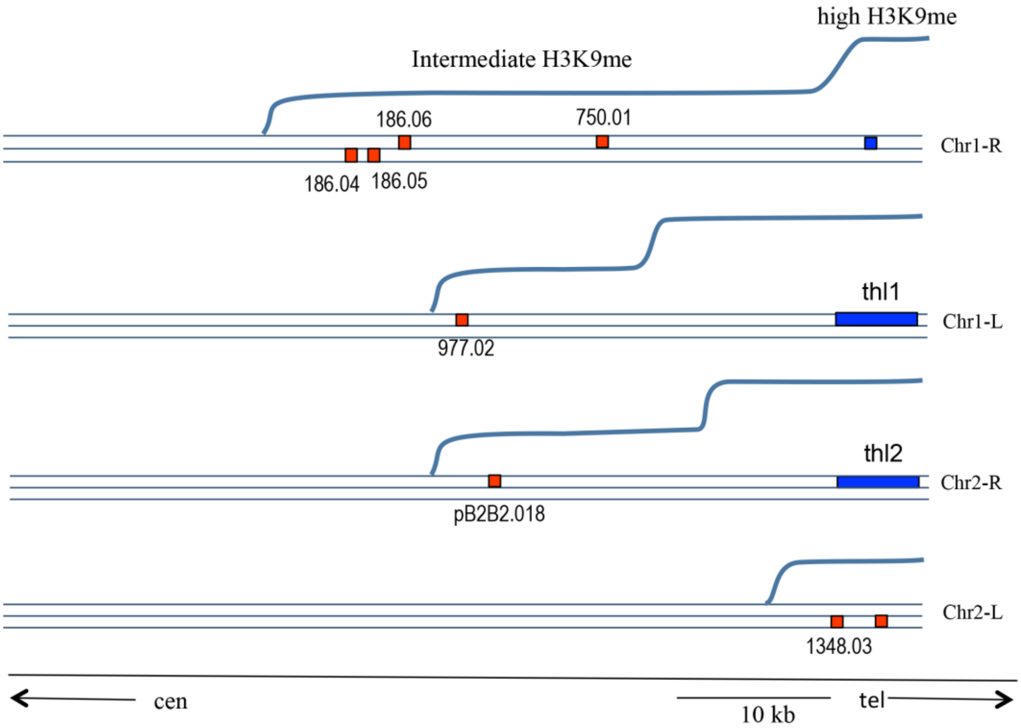

Figure S3: ***Schematic representation of open reading frames within the subtelomeric regions of chromosome I and II.*** Red boxes represent genes that are upregulated in Δ*tor1* or Δ*gad8* while blue boxes indicate genes that are unchanged or slightly reduced in gene expression according to RNA-seq analysis and qRT-PCR. Low and high H3K9me2 regions are represented according to (1) and following the scheme presented in (2).

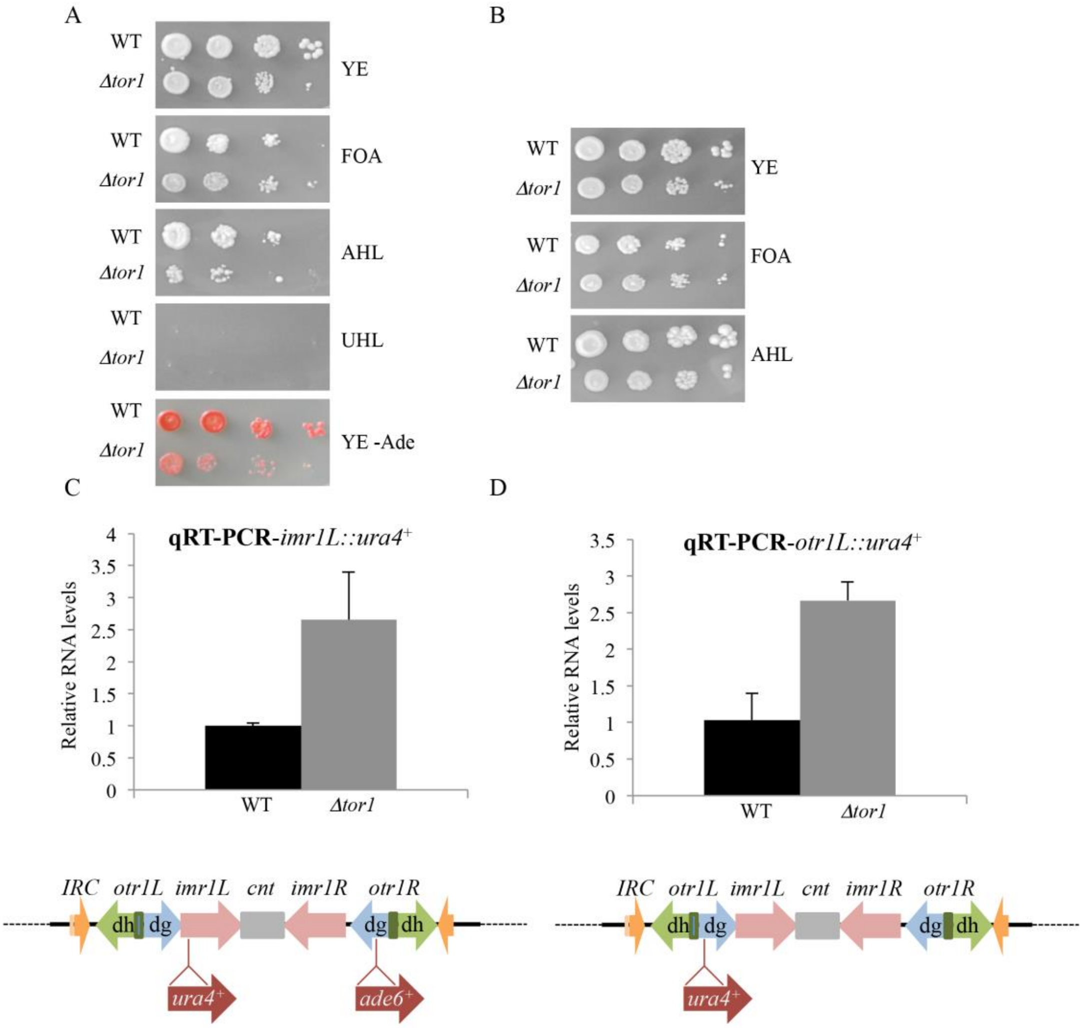

Figure S4: ***TORC2-Gad8 is not required for heterochromatic gene silencing at the centromeric region.*** **(A-B)** Silencing assays showing that Tor1 is not required for heterochromatic *ura4*$^{+}$ or *ade6*$^{+}$ gene silencing. **(C-D)** qRT-PCR analysis showing that the level of *imr1L*::*ura4*$^{+}$ or *otr1L*::*ura4*$^{+}$ is only slightly induced in Δ*tor1* mutant cells.

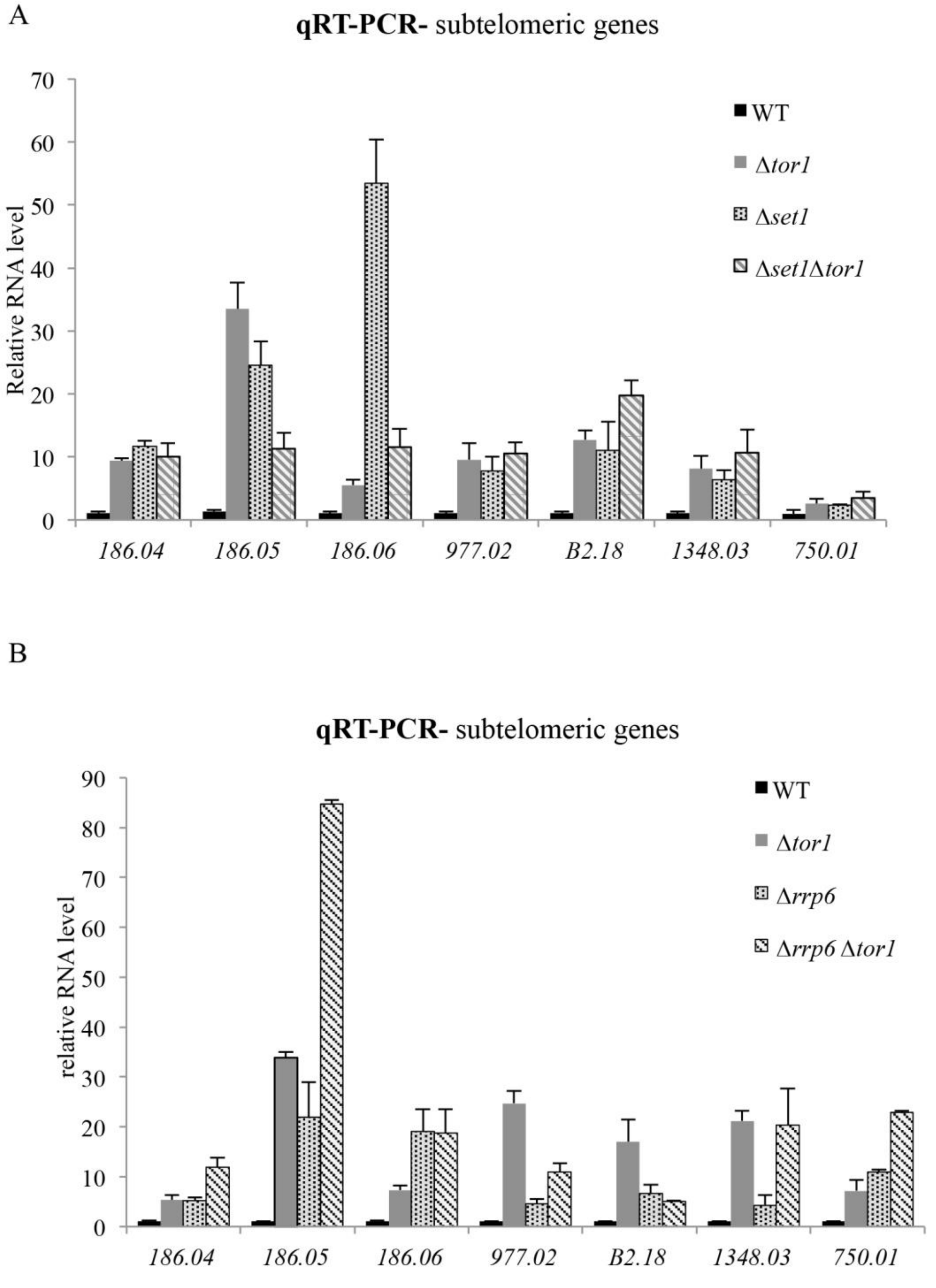

Figure S5: ***The silencing defect in Δtor1 is epistatic with disruption of set1$^+$ but not with disruption of rrp6$^+$.*** Expression levels of the indicated genes were determined by qRT-PCR as described in Fig. 3.

Table S1: Strains used in this study.

Strain	**Genotype**	**Source**
TA1	*975 h^{+}*	Laboratory stock
TA2	*h^{-} leu1-32 ura4-D18 ade6-M210*	Laboratory stock
TA16	*h^{90} leu1-32 ura4-D18 ade6-M216*	Laboratory stock
TA101	*h^{-} tor1::ura4^{+} leu1-32 ura4-D18 ade6-216*	Laboratory stock
TA390	*h^{-} tor1::ura4^{+} ura4-D18*	Laboratory stock
TA910	*h^{+} set1::kanMX6 leu1-32 ura4-D18*	YGRC
TA914	*h^{-} sin1::kanMX6 leu1-32 ura4-D18*	K. Shiozaki (3)
TA956	*mat1Msmt0 (BamHI)::ade6^{+} leu1-32 ura-D18 ade6-210 his2*	A. Cohen (4)
TA972	*mat1Msmt0 (BamHI)::ade6^{+} tor1::ura4^{+} leu1-32 ura4D-18 ade6-M210 his2*	This study
TA1029	*h^{-} gad8::ura4^{+} leu1-32 ura4-D18 ade6-M216*	M. Yamamoto (5)
TA1091	*h^{+} tor1:: ura4^{+} set1::kanMX6 leu1-32 ura-D18*	Laboratory stock
TA1114	*h^{-} ste20::kanMX6 leu1-32 ura4-D18*	K. Shiozaki (3)
TA1128	*mat1Msmt0 (BamHI)::ade6^{+} sin1::kanMX6 leu1-32 ura4-D18 ade6-M210 his2*	This study
TA1132	*h^{+} gad8::ura4^{+} ura4-D18*	Laboratory stock
TA1146	*h^{-} rrp6::kanMX6 leu-32 ura4-D18 ade6-M210*	S. Whitehall (6)
TA1164	*mat1Msmt0 (BamHI)::ade6^{+} ste20::kanMX6 leu1-32 ura4-D18 ade6-M210 his2*	This study
TA1168	*mat1Msmt0 (BamHI)::ade6^{+} gad8::ura4^{+} leu1 ura4-D18 ade6-M210 his2*	This study
TA1231	*h^{-} clr4:Nat leu1-32 ura4-D18 ade6-210 his3D arg3-D*	R. Allshire (7)
TA1258	*mat1Msmt0 (BamHI)::ade6^{+} epe1::KanMX6 leu1-32 ura4D-18 ade6-210*	This study
TA1263	*mat1-Msmt0 (BamHI)::ade6+ otr1 (dh/BglII)::ura4^{+} leu1-32 ura4-DS/E ade6-210 his1989*	A. Cohen
TA2008	*h^{90} ryh1::KanMX leu1-32 ura4-D18 ade6*	Laboratory stock
TA2127	*Mat1Msmt0 (BamHI)::ade6^{+} clr4:: KanMX leu1-32 ura4-D18 Ade6DN/N*	This study
TA2959	*h^{+} tor1::ura4^{+} rrp6:: KanMX leu1-32 ura4-D18 ade6-216*	This study

TA2997	*mat1Msmt0 (BamHI)::ade6$^+$ leo1:: KanMX leu1-32 ura4 ade6-210*	This study
TA3017	*mat1Msmt0 (BamHI)::ade6$^+$ leo1:: KanMX tor1::ura4$^+$ leu1-32 ura4 ade6-210*	This study
TA3018	*mat1Msmt0 (BamHI)::ade6$^+$ leo1:: KanMX gad8::ura4$^+$ leu1-32 ura4-DS/E or D18 ade6-210 his2*	This study
TA3022	*mat1Msmt0 (BamHI)::ade6$^+$ epe1:: KanMX tor1::ura4$^+$ leu1-32 ura4-D18 ade6-210*	This study
TA3033	*h$^+$ paf1::kanMX4 leu1-32 ura4-DS/E ade6 his?*	This study
TA3073	*h^{90} tor1::ura4$^+$ paf1::KanMX6 leu1-32 ura4 ade6 his2*	This study
TA3194	*mat1Msmt0 (BamHI)::ade6$^+$ sir2:: KanMX6 leu1-32 ura4-D18 ade6-210*	This study
TA3201	*h^{90} Swi6-5XFlag::hphMX6 leu1-32 ura4-D18 ade6-M216*	This study
TA3222	*h^{90} tor1::ura4$^+$ Swi6-5XFlag::hphMX6 leu1-32 ura4-D18 ade6*	This study
TA3224	*h^{90} gad8::ura Swi6-5XFlag::hphMX6* leu1-32 ura4-D18 ade6	This study
TA3227	*h^{90} clr4:Nat Swi6-5XFlag::hphMX6 leu1-32 ura4-D18 ade6*	This study
TA3234	*h^{90} epe1::kanMX6 leu1-32 ura4-D18 ade6-M216*	This study
TA3238	*h^{90} tor1::ura4 epe1:: KanMX leu1-32 ura4-D18 ade6-M216*	This study
TA3240	*h^{90} tor1::ura4$^+$ leu1-32 ura4-D18 ade6-216*	Laboratory stock
TA3275	*h$^+$ imr1L(NcoI)::ura4$^+$ otr1R (SphI)::ade6$^+$ leu1-32 ura4-DS/E ade6-M210*	S. Braun
TA3287	*h$^+$ imr1L(NcoI)::ura4$^+$ otr1R (SphI)::ade6 tor1:: kanMX leu1-32 ade6-M210 ura4-DS/E*	This study
TA3293	*mat1-Msmt0 (BamHI)::ade6$^+$ otr1 (dh/BglII)::ura4$^+$ tor1:: KanMX6 leu1-32 his2 ura4-DS/E ade6-210*	This study

*YGRC Yeast Genetic Resource Center, Japan

Table S2: Oligonucleotides used for qRT-PCR analyses

Name	**Target**	**Sequence**
#481 mat2-Pc R	*mat2-Pc*	TGTTAGACTTGCCTGGTCACAATT
#480 mat2-Pc F	*mat2-Pc*	TTGAATATAGTATGCGCTCTAACTTGG
#916 ADE6 F	*ade6*	GCAGTTTAGACGGAAAAGTTTATGC
#917 ADE6 R	*ade6*	ATTGAGAAGGGAAGCGAGCAGG
#994 ACT1 F	*act1*	GGTTTCGCTGGAGATGATG
#995 ACT1R	*act1*	ATACCACGCTTGCTTTGAG
#1220 TLH1/2 F	*tlh1/2*	ATGGTCGTCGCTTCAGAAATTGC
#1221 TLH1/2 R	*tlh1/2*	CTCCTTGGAAGAATTGCAAGCCTC
#1238 MEI4 F	*mei4*	TCAGATCCGTGGAATCCTTC
#1239 MEI4 R	*mei4*	CGCACTTGAGTAGCCACTTG
#1242 SPAC186.05 F	*SPAC186.05*	AAATTTTCCCGGGCTTTCAT
#1243 SPAC186.05 R	*SPAC186.05*	TCCGACAATCACCGCTACC
#1260 SPBC1348.03 F	*SPBC1348.03*	ACCAAGACTAAGCCTCACAGTGAAATATTGT
#1261 SPBC1348.03 R	*SPBC1348.03*	CTACGACGCATCCAAATGTAAAGGATC
#1262 SPAC977.02 F	*SPAC977.02*	ACCAAGACTAAGCCTCACAGTGAAATATTGT
#1263 SPAC977.02 R	*SPAC977.02*	ACTACGACGCATCCAAATGTAAAGGATC
#1264 SPAC750.01 F	*SPAC750.01*	TATTGGGAAGACTGGGTGCTTGAAGA
#1265 SPAC750.01 R	*SPAC750.01*	CCAACCAATTCTTCTGACACCCCA
#1266 SPBPB2B2.18 F	*SPBPB2B2.18*	GTTGTTCTCAGTGTGACTGGCACGA

#1267 SPBPB2B2.18 R	*SPBPB2B2.18*	TGAGATTCGGGACTAGCATCGGTAAT
#1271 SPAC186.04 F	*SPAC186.04*	GCGAAGAAAACCCAACAAGC
#1272 SPAC186.04 R	*SPAC186.04*	TCATCGTTTACTCTGATCCGTGA
#1273 SPAC186.06 F	*SPAC186.06*	GGGAGTGGAGCTGGATCAGT
#1274 SPAC186.06 R	*SPAC186.06*	CGCCACCAACATGAATATCG
#1279 dg1 F	*dg1*	ACGGCATCGCTTGTACTTTT
#1280 dg1 R	*dg1*	TGAGGTTCATGATGGGTTCA
#1382 URA4 F	*ura4*	GTCGAGGATTTCGACCAGGATA
#1383 URA4 R	*ura4*	GCTTGACGGTATTTCCAATGTCT

1. Cam, H. P., Sugiyama, T., Chen, E. S., Chen, X., FitzGerald, P. C., and Grewal, S. I. (2005) Comprehensive analysis of heterochromatin- and RNAi-mediated epigenetic control of the fission yeast genome. *Nature genetics* **37**, 809-819
2. Dheur, S., Saupe, S. J., Genier, S., Vazquez, S., and Javerzat, J. P. (2011) Role for cohesin in the formation of a heterochromatic domain at fission yeast subtelomeres. *Molecular and cellular biology* **31**, 1088-1097
3. Ikeda, K., Morigasaki, S., Tatebe, H., Tamanoi, F., and Shiozaki, K. (2008) Fission yeast TOR complex 2 activates the AGC-family Gad8 kinase essential for stress resistance and cell cycle control. *Cell Cycle* **7**, 358-364
4. Ayoub, N., Goldshmidt, I., and Cohen, A. (1999) Position effect variegation at the mating-type locus of fission yeast: a cis-acting element inhibits covariegated expression of genes in the silent and expressed domains. *Genetics* **152**, 495-508
5. Matsuo, T., Kubo, Y., Watanabe, Y., and Yamamoto, M. (2003) Schizosaccharomyces pombe AGC family kinase Gad8p forms a conserved signaling module with TOR and PDK1-like kinases. *The EMBO journal* **22**, 3073-3083
6. Anderson, H. E., Wardle, J., Korkut, S. V., Murton, H. E., Lopez-Maury, L., Bahler, J., and Whitehall, S. K. (2009) The fission yeast HIRA histone chaperone is required for promoter silencing and the suppression of cryptic antisense transcripts. *Molecular and cellular biology* **29**, 5158-5167
7. Ekwall, K., Nimmo, E. R., Javerzat, J. P., Borgstrom, B., Egel, R., Cranston, G., and Allshire, R. (1996) Mutations in the fission yeast silencing factors clr4+ and rik1+ disrupt the localisation of the chromo domain protein Swi6p and impair centromere function. *Journal of cell science* **109 (Pt 11)**, 2637-2648