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Supplementary Materials for

Epigenetic inheritance uncoupled from sequence-specific recruitment

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Materials and Methods

Plasmids

Plasmids containing *10XtetO* binding sites upstream of *ade6*$^{+}$ and *ura4-GFP* reporter genes were constructed by first synthesizing a plasmid containing 10 *tetO* sites flanked by 200bp homology sequences to facilitate reporter insertion at the *ura4* locus. The reporter genes were cloned downstream of the *tetO* binding sites using *PacI* and *AscI* restriction sites that were incorporated during the initial synthesis of the plasmid. The ade6^{+} reporter construct consists of the full-length wild type *ade6*$^{+}$ gene with endogenous upstream promoter and downstream terminator sequences. The *ura4-GFP* reporter consists of the full length *ura4*$^{+}$ gene fused at the C terminus with a monomeric yeast codon optimized GFP using Gibson assembly (*54*). This construct was subsequently cloned downstream of the 10 *tetO* sites and appended with a 114bp *ura4* promoter element and the corresponding endogenous *ura4* downstream terminator sequence. The plasmid containing *TetR-clr4-I* was constructed by modifying a pFA6a-natMX6-P$_{nmt1}$ plasmid. The promoter elements in the original plasmid were replaced with the endogenous *clr4*$^{+}$ promoter (using *BglII* and *PacI* restriction sites). The TetR construct consists of an N-terminal SV40 nuclear localization sequence followed immediately by a 2X-FLAG tag. The *clr4*$^{+}$ chromodomain deletion construct consists of a *clr4* allele lacking amino acids 7-59. The synthesis of the *TetR-clr4-I* fusion with the upstream endogenous clr4 promoter elements was achieved by Gibson assembly. The deletion of the TetR DNA binding element was achieved after modifying a pFA6a-hphMX6-P$_{nmt1}$ plasmid by insertion of the endogenous *clr4*$^{+}$ promoter and a *clr4* allele lacking the chromodomain.

Strains

A strain containing the 10 *tetO* sites was first made by insertion of the reporter gene at the *ura4*$^+$ locus. The subsequent introduction of the TetR-Clr4-I fusion protein was achieved using a PCR-based gene targeting approach (*55*). Strains with the designation TetR-Clr4-I are those in which the endogenous copy of *clr4* is replaced with the TetR-Clr4-I fusion making it the only source of Clr4 expression in the cells. In strains where the wild type copy of *clr4*$^+$ is intact, i.e. *TetR-clr4-I,clr4*$^+$, the fusion protein is inserted at the *trp1*$^+$ locus. The deletions of the various RNAi and chromatin components was achieved either by PCR-based gene targeting approaches or by a cross followed by random spore analysis and PCR based screening to select for colonies that harbored the reporter gene, the TetR fusion protein and the appropriate deletion. Strains containing deletions of the TetR DNA binding domain (*clr4-IΔ*) were constructed both by PCR based targeting approaches and crosses followed by random spore analysis. The resulting colonies were tested using allele specific primers. To isolate red colonies that harbor a deletion of the TetR DNA binding domain, sectored colonies, which tested positive for the deletion in the allele specific PCR screen, were replated to isolate single red colonies on plates containing limiting adenine. All strains used in this study are listed in Table S1. Crosses were performed between red isolates of haploid cells of opposite mating type which harbored a deletion of the TetR DNA binding module. The resulting diploid, which lacks any sequence-specific establishment factors, was then allowed to sporulate. Following tetrad dissection, spores were plated on low-adenine medium and allowed to grow at 32°C for 3 days.

Chromatin immunoprecipitation (ChIP)

Cells were grown to a density of $2.5x10^7$ cells/mL at 32°C in YEA or YEA containing tetracycline (2.5 μg/mL). Cells were crosslinked with 1% formaldehyde for 30 min at room temperature prior to quenching with 125mM glycine for 5 minutes. The subsequent steps for sample processing were performed as previously described (*56*). Immunoprecipitation was performed using the following antibodies: 2.5uL α-H3K9me2 (ab1220, Abcam) for quantifying H3K9me2 levels, 2μg α-H3K9me3 (*57*) for quantifying H3K9me3 levels, and 2.5uL α-FLAG (M2, sigma) for quantifying TetR-Clr4-I occupancy at the ectopic locus before and after addition of tetracycline. DNA purified from the ChIP experiments were analyzed by quantitative PCR using an Applied Biosystems 7900HT Fast Real-Time PCR systems. See Supplemental Table S2 for primer sequences. ChIP-seq libraries were constructed, sequenced using an Illumina High-seq platform, and processed as described previously (*49*).

Silencing assays

Strains containing the *ade6*$^+$ reporter construct were grown overnight after which 5-fold dilutions of each culture were spotted on plates containing only yeast extract (YE) and glucose without any additional adenine supplements with (+tet) or without (-tet) tetracycline (2.5 μg/mL). Each silencing assay also included centromeric silencing reporter strains which are unresponsive to tetracycline (*otr1R:ade6*$^+$ and *ura4::10XtetO-ade6*$^+$) as controls to ensure that the addition of tetracycline does not induce any changes in reporter gene expression.

FACS analysis

Cells containing *TetR-clr4-I* and *10XtetO- ura4-GFP* reporter were maintained in log phase (~2.5x10^7 cells/mL) through the course of sample preparation at various time points after addition of tetracycline (2.5μg/mL). Approximately 2.5 x10^7 cells were harvested and fixed by addition of 70% ethanol for 20 minutes. The cells were then washed twice with 1X TBS (200mM Tris pH 7.5, 150mM NaCl) and resuspended in 1mL of 1X TBS in a FACS tube (BD Falcon). GFP fluorescence was then measured using a FACScalibur instrument (Becton Dickinson) and excitation was achieved by using an argon laser emission of 488 nm. Data collection was performed using Cellquest software (Becton Dickinson) and a primary gate based on physical parameters (forward and side light scatter, FSC and SSC, respectively) was set to exclude dead cells or debris. Typically, 20000 cells were analyzed for each sample and time point. The resulting GFP fluorescence profiles were fit using Gaussian curves (Origin 8.0) assuming a model where cells exhibit two expression states i.e. either GFP-ON or GFP-OFF. The fraction of cells in each state was calculated by measuring the area under the curve for each Gaussian fit.

Fig. S1_Ragunathan et al.

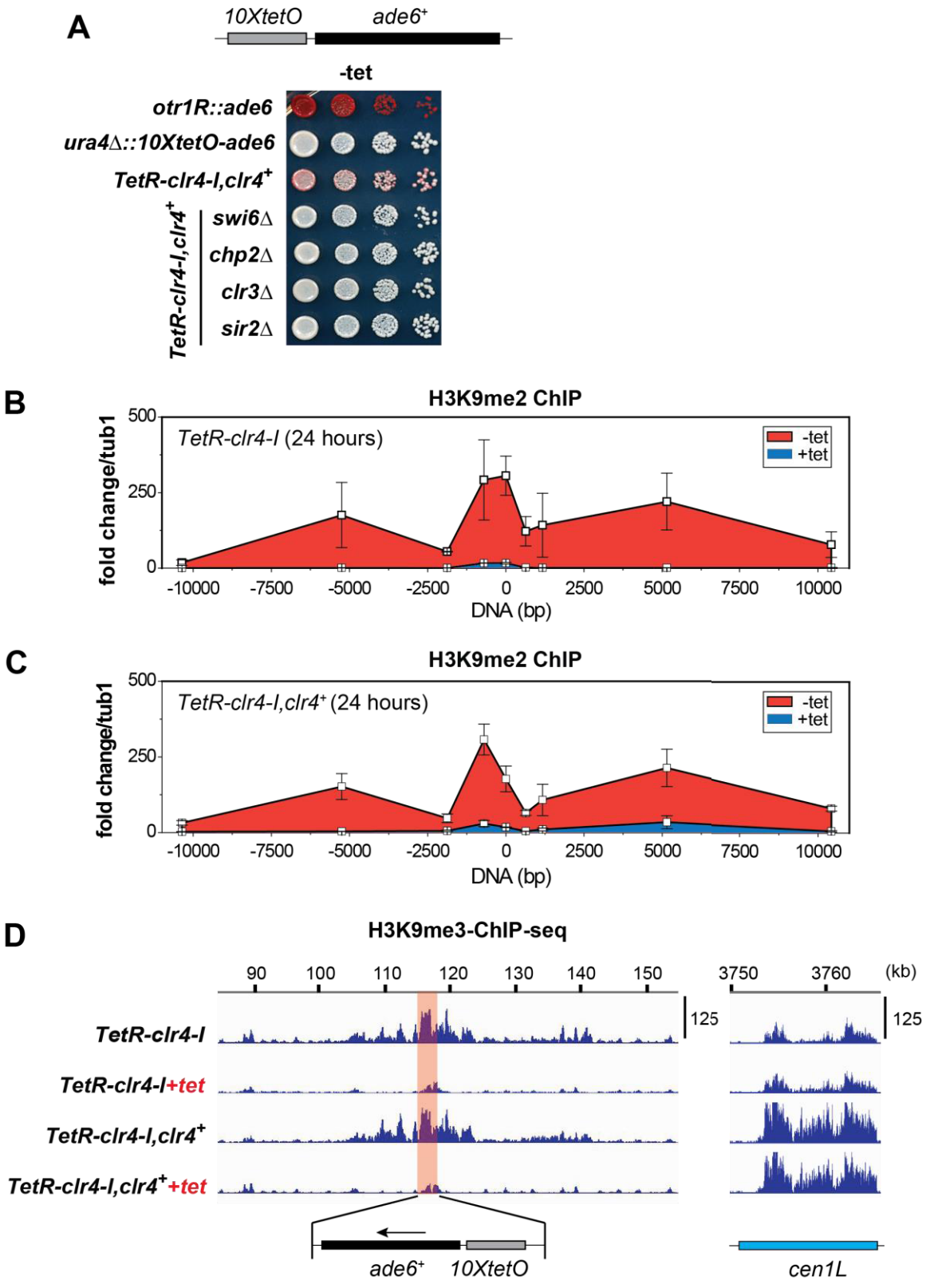

Fig. S1.

Genetic requirements for TetR-clr4-I-mediated silencing and its loss after release by tetracycline.

(A) Color silencing assays show that TetR-clr4-I-mediated silencing in *clr4*$^{+}$ cells requires HP1 proteins (lost in *swi6Δ* and *chp2Δ*) and histone decetylases (lost in *clr3Δ* and *sir2Δ*). (B, C) H3K9me2 ChIP-qPCR and (D) H3K9me3 ChIP-seq experiments show that TetR-clr4-I induces the formation of a large domain of H3K9me2 that flanks the *10XtetO-ade6*$^{+}$ locus for about 20 kb on either side. Both H3K9me2 and H3K9me3 is lost 24 hours after the addition of tetracycline. Reads for the left centromere of chromosome 1 (*cen1*) are shown for comparison. Normalized reads per million are presented (y axis) and chromosome coordinates are indicated on the top.

Fig. S2_Ragunathan et al.

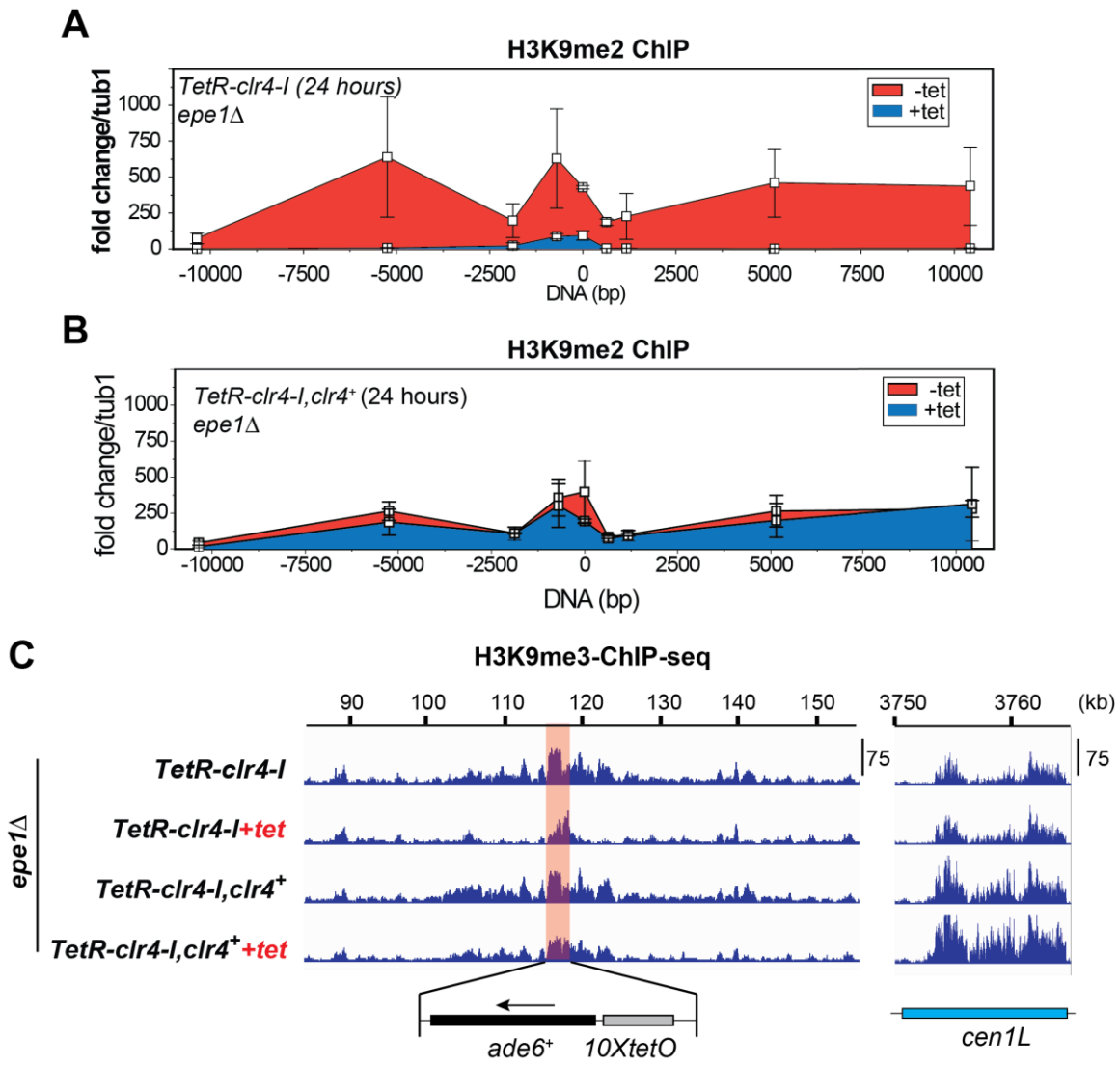

Fig. S2

Maintenance of silencing and H3K9 methylation in *epe1Δ* cells.

(A, B) H3K9me2 ChIP-qPCR and (C) H3K9me3 ChIP-seq experiments show that in *epe1Δ* cells, the H3K9me2 and H3K9me3 domain surrounding the *10XtetO-ade6*$^{+}$ locus is maintained 24 hours after the addition of tetracycline (+tet). Reads for the left centromere of chromosome 1 (*cen1*) are shown for comparison and are presented as described in Fig. S1 legend.

Fig. S3_Ragunathan et al.

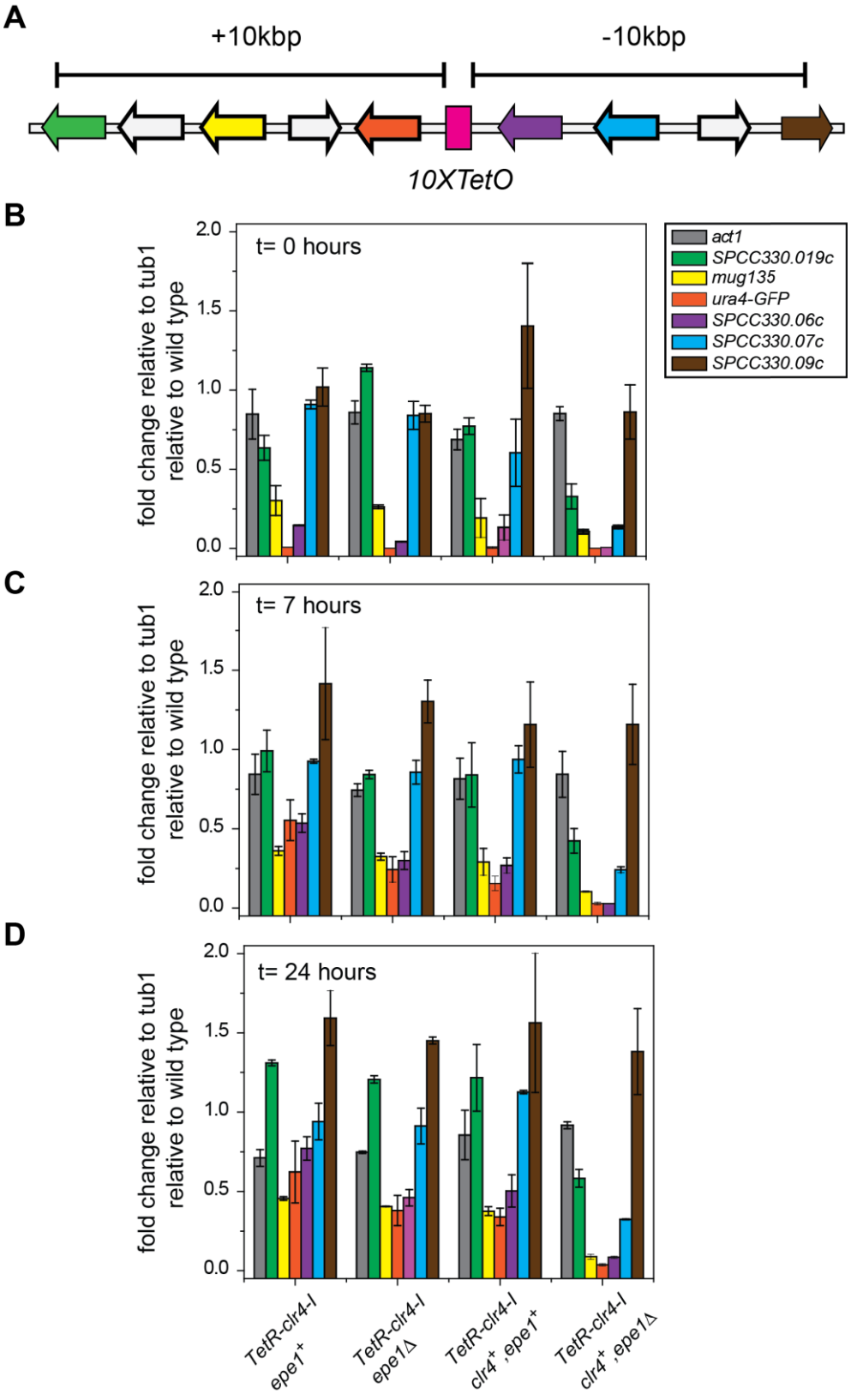

Fig. S3

TetR-Clr4-I tethering induces heritable silencing of flanking genes.

(A) Schematic diagram of the annotated open reading frames surrounding the *10XtetO-ura4-GFP* locus. (B-C) qRT-PCR quantification of RNA levels for the indicated loci at 0, 7, and 24 hours after the addition tetracycline.

Fig. S4_Ragunathan et al.

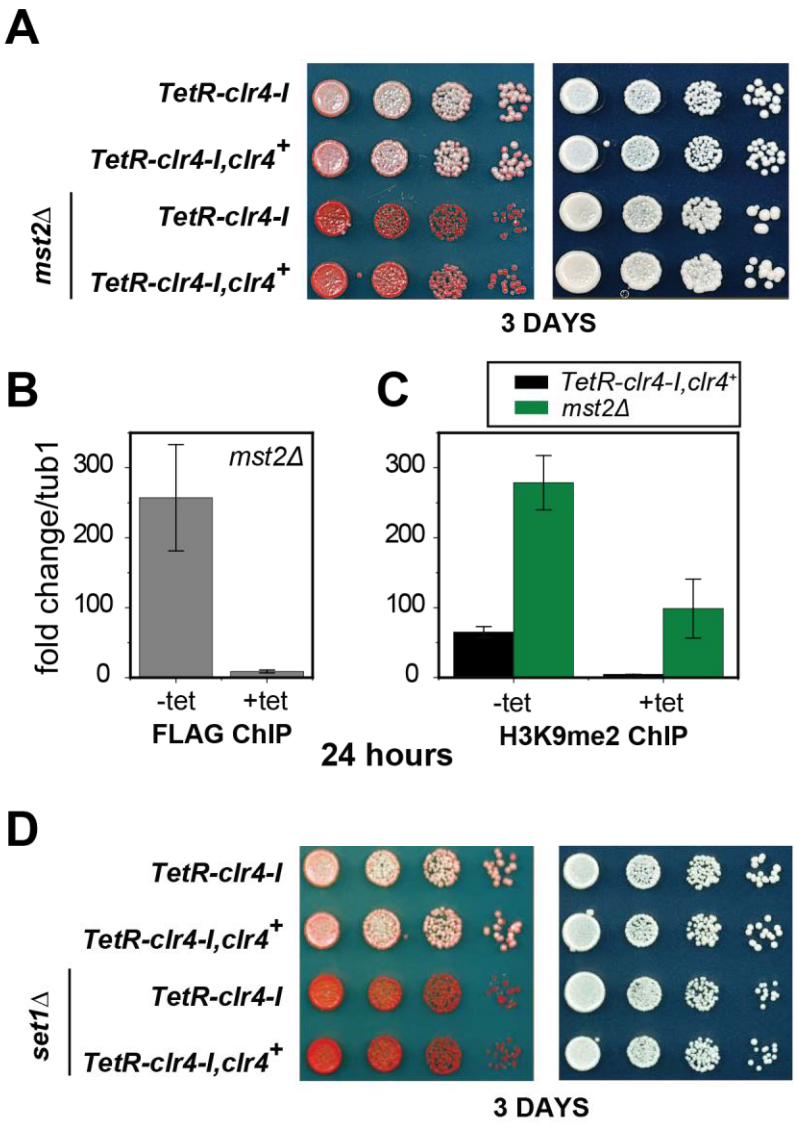

Fig. S4.

The effect of deleting enzymes associated with transcriptional activation, *mst2*$^{+}$ and *set1*$^{+}$, on the establishment and maintenance of silencing.

(A and D) Color silencing assays show that *mst2Δ* and *set1Δ* cells cause enhanced silencing under conditions of establishment on –tet, which is not maintained on +tet medium. (B) ChIP-qPCR experiments show that tetracycline promotes the release of

FLAG-tagged TetR-Clr4-I from *tetO* sites 24 hours after the addition of tetracycline. (C) H3K9me2 is enhanced in *mst2Δ* cells on the absence of tetracycline (-tet) and maintained 24 hours after tetracycline addition (+tet). Error bars represent standard deviations.

Fig. S5_Ragunathan et al.

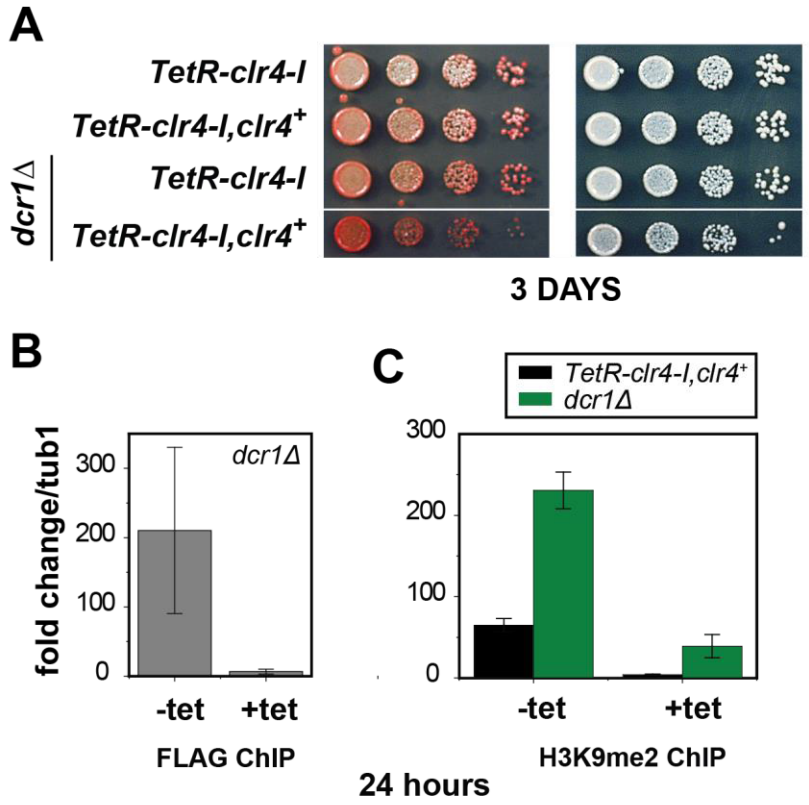

Fig. S5.

The effect of mutations in *dcr1*$^+$ and *poz1*$^+$ on the establishment and maintenance of H3K9 methylation and silencing.

(A and D) Color silencing assays show that silencing of *10XtetO-ade6*$^+$ is not maintained on +tet medium in *dcr1Δ* cells (A) and is very weakly maintained in *poz1Δ* cells (D). ChIP-qPCR experiments show that 24 hours after the addition of tetracycline (+tet) TetR-Clr4-I is released from the *tetO* sites in *dcr1Δ* (B) and *poz1Δ* (E) cells while H3K9me2 is maintained (C and F). Error bars represent standard deviations.

Fig. S6_Ragunathan et al.

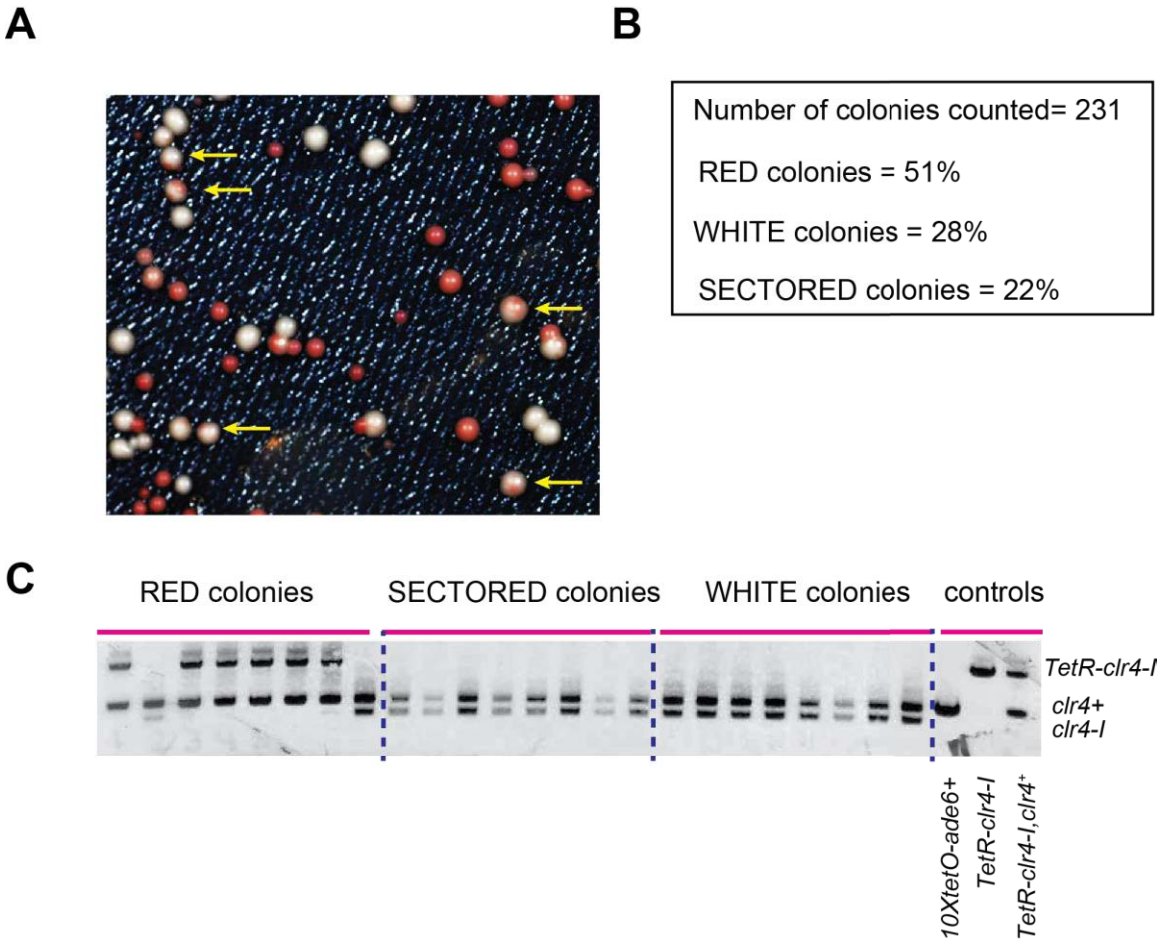

Fig. S6.

Maintenance of *10XtetO-ade6*$^{+}$ silencing after deletion of the TetR DNA binding domain. (A) Growth of *10XtetO-ade6*$^{+}$, *nat*^{R}*-TetR-clr4-I, clr4*$^{+}$*, epe1Δ* cells, transformed with a PCR DNA fragment to replace *nat*^{R}*-TetR-clr4-I* with *hph*^{R}*-clr4-IΔ*, on hygromycin-containing low-adenine medium. The resulting transformants produced red, white, and sectored colonies. Importantly white and sectored colonies constituted nearly half of all transformants, about 22% of which were sectored (B). Allele-specific PCR confirmed that both the white (representing loss of the silent state) and sectored (representing

maintenance of the silent state) colonies contained the expected replacement of the *TetR-clr4-I* allele with *clr4-I* at a very high frequency (C). The resulting *clr4-I* is designated *clr-IΔ* to reflect the deletion of the TetR DNA-binding domain.

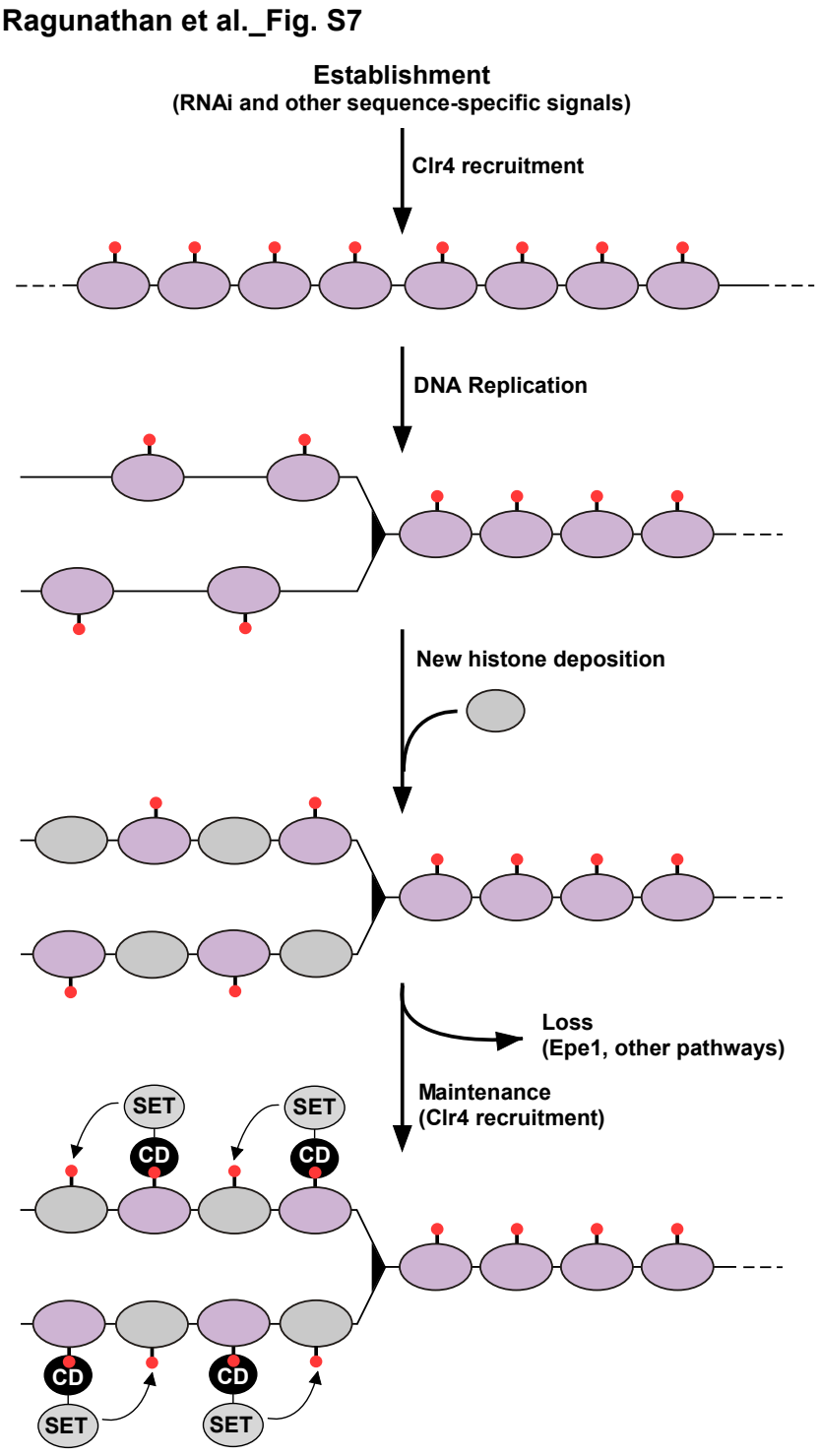

Fig. S7.

Establishment and epigenetic maintenance of H3K9 methylation.

H3K9 methylated domains (denoted by filled red circles on oval nucleosomes) and silent chromatin are established by RNAi or other sequence-specific signals that recruit the Clr4 methyltransferase. Following DNA replication, H3K9 methylation is copied from parental nucleosomes to newly deposited nucleosomes by a direct "read-write" mechanism that involves the recognition of methylated nucleosomes by the chromodomain of Clr4 (Maintenance). H3K9 methylation can be reversed due to the activity of Epe1 or other pathways that promote nucleosome exchange (Loss).

Table S1.

Strains used in this study

SPY77	*h- leu1-32 ade6-M210*	Lab stock
SPY1964	*h- leu1-32 ura4-D18*	Lab stock
SPY5081	*h$^-$ leu1-32 ade6-M210 ura4Δ::10XTetO-ade6$^+$*	This study
SPY5288	*h$^-$ leu1-32 ade6-M210 ura4Δ::10XTetO-ade6$^+$ epe1Δ::kanMX6*	This study
SPY5285	*h$^-$ leu1-32 ade6-M210 ura4Δ::ade6$^+$ clr4Δ::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I*	This study
SPY5286	*h$^-$ leu1-32 ade6-M210 ura4Δ::ade6$^+$ clr4$^+$ trp1::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I*	This study
SPY5073	*h$^-$ leu1-32 ade6-M210 ura4Δ::10XTetO-ade6$^+$ clr4Δ::natMX6-P$_{clr4}$-TetR-2XFLAG- clr4-I*	This study
SPY5075	*h$^-$ leu1-32 ade6+-M210 ura4Δ::10XTetO-ade6$^+$ clr4$^+$ trp1::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I*	This study
SPY5079	*h$^-$ leu1-32 ade6+-M210 ura4Δ::10XTetO-ade6$^+$ clr4$^+$ trp1::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I dcr1Δ::hphMX6*	This study
SPY5289	*h$^-$ leu1-32 ade6+-M210 ura4Δ::10XTetO-ade6$^+$ clr4$^+$ trp1::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I swi6Δ::hphMX6*	This study
SPY5290	*h$^-$ leu1-32 ade6+-M210 ura4Δ::10XTetO-ade6$^+$ clr4$^+$ trp1::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I chp2Δ::hphMX6*	This study
SPY5293	*h$^-$ leu1-32 ade6+-M210 ura4Δ::10XTetO-ade6$^+$ clr4$^+$ trp1::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I clr3Δ::hphMX6*	This study
SPY5291	*h$^-$ leu1-32 ade6+-M210 ura4Δ::10XTetO-ade6$^+$ clr4$^+$ trp1::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I sir2Δ::hphMX6*	This study
SPY5320	*h$^-$ leu1-32 ade6+-M210 ura4Δ::10XTetO-ade6$^+$ clr4Δ::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I poz1Δ::kanMX6*	This study
SPY5321	*h$^+$ leu1-32 ade6+-M210 ura4Δ::10XTetO-ade6$^+$ clr4$^+$ trp1::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I poz1Δ::kanMX6*	This study
SPY5083	*h$^-$ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6$^+$ clr4Δ::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I mst2Δ::ura4$^+$*	This study
SPY5084	*h$^-$ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6$^+$ clr4$^+$ trp1::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I mst2Δ::ura4$^+$*	This study
SPY5305	*h$^+$ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6$^+$ clr4Δ::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I set1Δ::kanMX6*	This study
SPY5306	*h$^-$ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6$^+$ clr4$^+$ trp1::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I set1Δ::kanMX6*	This study
SPY5086	*h$^-$ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6$^+$ clr4Δ::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I epe1Δ::kanMX6*	This study
SPY5090	*h$^-$ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6$^+$ clr4$^+$ trp1::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I epe1Δ::kanMX6*	This study
SPY5093	*h$^-$ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6$^+$ clr4$^+$ trp1::natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I epe1Δ::kanMX6*	This study

	dcr1Δ::hphMX6	
SPY5295	*h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6⁺ clr4⁺ trp1∷natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I epe1Δ::kanMX6 swi6Δ::hphMX6*	This study
SPY5297	*h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6⁺ clr4⁺ trp1∷natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I epe1Δ::kanMX6 chp2Δ::hphMX6*	This study
SPY5300	*h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6⁺ clr4⁺ trp1∷natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I epe1Δ::kanMX6 clr3Δ::hphMX6*	This study
SPY5298	*h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6⁺ clr4⁺ trp1∷natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I epe1Δ::kanMX6 sir2Δ::hphMX6*	This study
SPY5309	*h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6⁺ clr4 Δ::hphMX6- P$_{clr4}$-clr4IΔ trp1∷natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I*	This study
SPY5307	*h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6⁺ clr4 Δ::hphMX6- P$_{clr4}$-clr4IΔ trp1∷natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I epe1 Δ::kanMX6*	This study
SPY5099	*h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO-ura4-GFP*	This study
SPY5101	*h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO-ura4-GFP clr4Δ∷natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I*	This study
SPY5103	*SPY5103 h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO-ura4-GFP clr4⁺ trp1∷natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I*	This study
SPY5314	*SPY5314 h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO-ura4-GFP clr4Δ∷natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I epe1Δ::kanMX6*	This study
SPY5317	*h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO-ura4-GFP clr4⁺ trp1∷natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I epe1Δ::kanMX6*	This study
SPY5355	*h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6⁺ clr4⁺ trp1∷natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I ago1Δ::hphMX6*	This study
SPY5361	*h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6⁺ clr4⁺ trp1∷natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I epe1Δ::kanMX6 ago1Δ::hphMX6*	This study
SPY5410	*h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6⁺ clr4⁺ trp1∷natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I epe1-K314A-3XFLAG-hphMX6*	This study
SPY5412	*h⁻ leu1-32 ade6+-M210 ura4Δ::10XTetO- ade6⁺ clr4⁺ trp1∷natMX6-P$_{clr4}$-TetR-2XFLAG-clr4-I epe1-H297A-3XFLAG-hphMX6*	This study
SPY28	*h⁺ leu1-32 ura4-D18 imr1R(Ncol)::ura4⁺ oriI ade6-M216*	Lab stock
SPY825	*h⁺ leu1-32 ura4-D18 imr1R(Ncol)::ura4⁺ oriI ade6-*	Lab stock

	M216 clr4Δ::kanMX6	
SPY86	*h^+ leu1-32 ura4-D18 imr1R(Ncol)::ura4$^+$ oriI ade6-M216 dcr1Δ::TAP-kanMX6*	Lab stock
SPY120	*h^+ leu1-32 ura4-D18 imr1R(Ncol)::ura4$^+$ oriI ade6-M216 ago1Δ::TAP-kanMX6*	Lab stock
SPY2765	*h^+ leu1-32 ura4-D18 imr1R(Ncol)::ura4$^+$ oriI ade6-M216 ago1Δ::TAP-kanMX6 clr4Δ::nat → hph-5'UTR(1kb)-clr4*	This study
SPY2768	*h^+ leu1-32 ura4-D18 imr1R(Ncol)::ura4+ oriI ade6-M216 dcr1Δ::TAP-kanMX6 clr4Δ::nat → hph-5'UTR(1kb)-clr4*	This study
SPY4453	*h^+ leu1-32 ura4-D18 imr1R(Ncol)::ura4+ oriI ade6-M216 ago1Δ::kanMX6 trp1::natMX6-P_{clr4}-3XFLAG-clr4$^+$*	This study
SPY4485	*h^+ leu1-32 ura4-D18 imr1R(Ncol)::ura4+ oriI ade6-M216 ago1Δ::kanMX6 trp1::natMX6-P_{clr4}-3XFLAG-clr4 (W31G)*	This study

Table S2.
Primers used in this study

10XtetO_binding-fwd	GCAGTGGTGCTTCTGTCCTAACAGC	FLAG, K9me2 qChIP
10XtetO_binding-rev	GTCCGTGTAGCTCGAGCTCGACTT	FLAG, K9me2 qChIP
10XtetO-600bp-fwd	GCGGTACGAAGATGATTTTGCACA	qChIP (-600bp ura4)
10XtetO-600bp-rev	TGCTGCAATTATTGCTGCTTCTTCC	qChIP (-600bp ura4)
10XtetO-1900bp-fwd	TAGCAACAGTGGCGACAGGGAC	qChIP (-1900bp ura4)
10XtetO-1900bo-rev	CCGCAGCTTCAAGAGGGTTGC	qChIP (-1900bp ura4)
10XtetO-5000bp-fwd	CTTTGGGGGCAGTTTGTTGGC	qChIP (-500bp ura4)
10XtetO-5000bp-rev	CGTCGCC GATCAAGGGTTGA	qChIP (-5000bp ura4)
10XtetO-10000bp- fwd	GTTGAATTCAACGGAATGTC	qChIP (-10000bp ura4)
10XtetO-10000bp-rev	CGTCTCGAGTGCATTTGATGCG	qChIP (-10000bp ura4)
10XtetO+100bp-fwd	AAGTCGAGCTCGAGCTACACGGAC	qChIP (+100bp ura4)
10XtetO+100bp-rev	CTGGATTTCGTTTACCTCACCACCA	qChIP (+100bp ura4)
10XtetO+700bp-fwd	TTGCAGGAGAGGGTTCAACAGCA	qChIP (+700bp ura4)
10XtetO+700bp-fwd	AATGCATCATCTTGGATGCAGCAA	qChIP (+700bp ura4)
10XtetO+1200bp-fwd	TGTGAGGCCGAGGAGCAATTTC	qChIP (+1200bp ura4)
10XtetO+1200bp-rev	TATGCCCCTGCTCGTCTTCCCT	qChIP (+1200bp ura4)
10XtetO+5000bp-fwd	GAGCCTCATGTCCATACGATCAACCT	qChIP (+5000bp ura4)
10XtetO+5000bp-rev	AATCGATGGATGAGTGGAGAAAGTCG	qChIP (+5000bp ura4)
10XtetO+10000bp-fwd	CCTGATGCCCTCAAATCGGTTACTT	qChIP (+10000bp ura4)
10XtetO+10000bp-rev	TGAGGCTGTTCAAGGAACTTAGTGTCC	qChIP (+10000bp ura4)
GJ195	GGTTAAAGCGGTTGTTTGGCACTG	qChIP
GJ196	TGACGAGGCACATTCCTTATACGC	qChIP
tub1_ref-fwd	AACGCTTGGCCATGGAATACACG	qChIP

tub1_ref-rev	GAGAGGCGGTGATGGAAGAAACAAC	qChIP
+10kb SPCC330.19c-rev	CGCCCGACAAGTTGGGCAT	qRT-PCR
+10kb SPCC330.19c-fwd	TCATACCGCTTGTCTTGCTTTTTGC	qRT-PCR
-10kb SPCC330.09-fwd	AAGGCGTAACCCAACGCTTACCTAA	qRT-PCR
-10kb SPCC330.09-rev	TGAGGTTTGTACACACCAGTTGCCA	qRT-PCR
-2kb SPCC330.06c-fwd	GCCGTAAATGACGTTTTCGTCACC	qRT-PCR
-2kb SPCC330.06c-rev	ACCTTGACAACCTTGCCATTCTCG	qRT-PCR
act1Fwd2	CAACCCTCAGCTTTGGGTCTTG	qRT-PCR
act1Rev2	TCCTTTTGCATACGATCGGCAATAC	qRT-PCR
ura4set5F	TACAGTGCCAGGCGAGGGTATTAT	qRT-PCR
ura4set5R	TAGGAACCAGTAGCCAAAGAGCCT	qRT-PCR
Clr4promoter-fwd	CGCGGTCGTCTATTATTTTAGTCC	allele specific PCR
Clr4internal-rev	TCGAGATTGTCTCTTGGTAGTTTGAG	allele specific PCR

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