Supplementary Information

Defects in RNA quality control factors reveal RNAi-independent nucleation of heterochromatin

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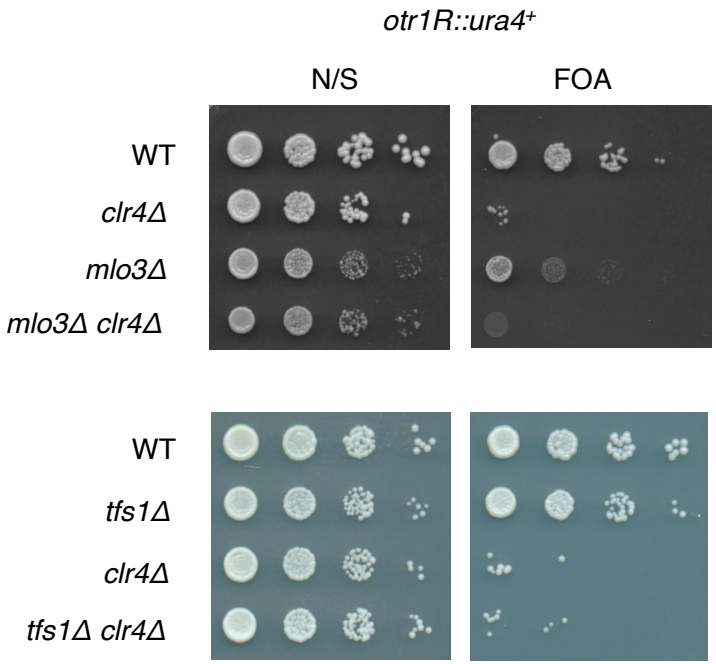
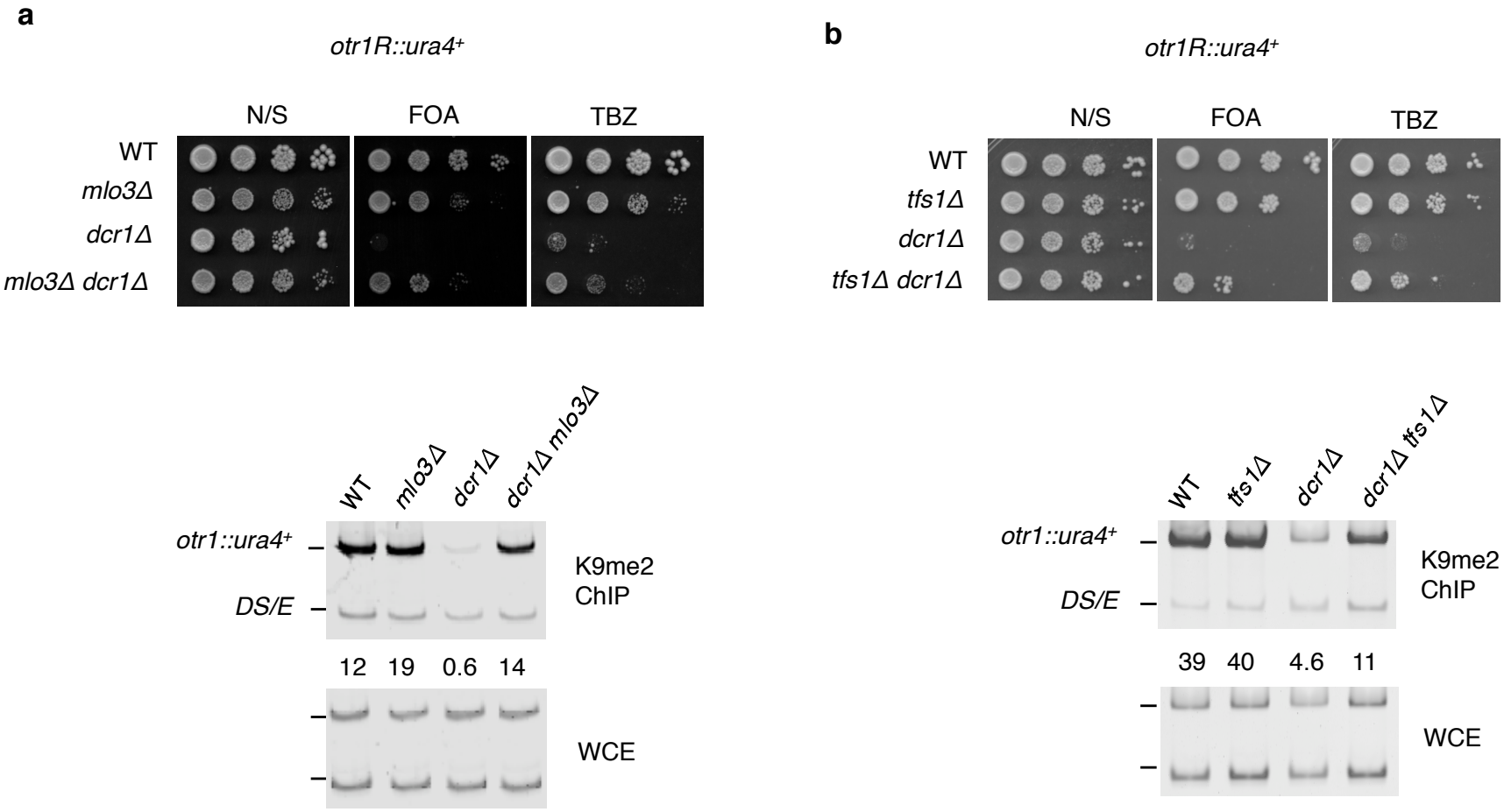
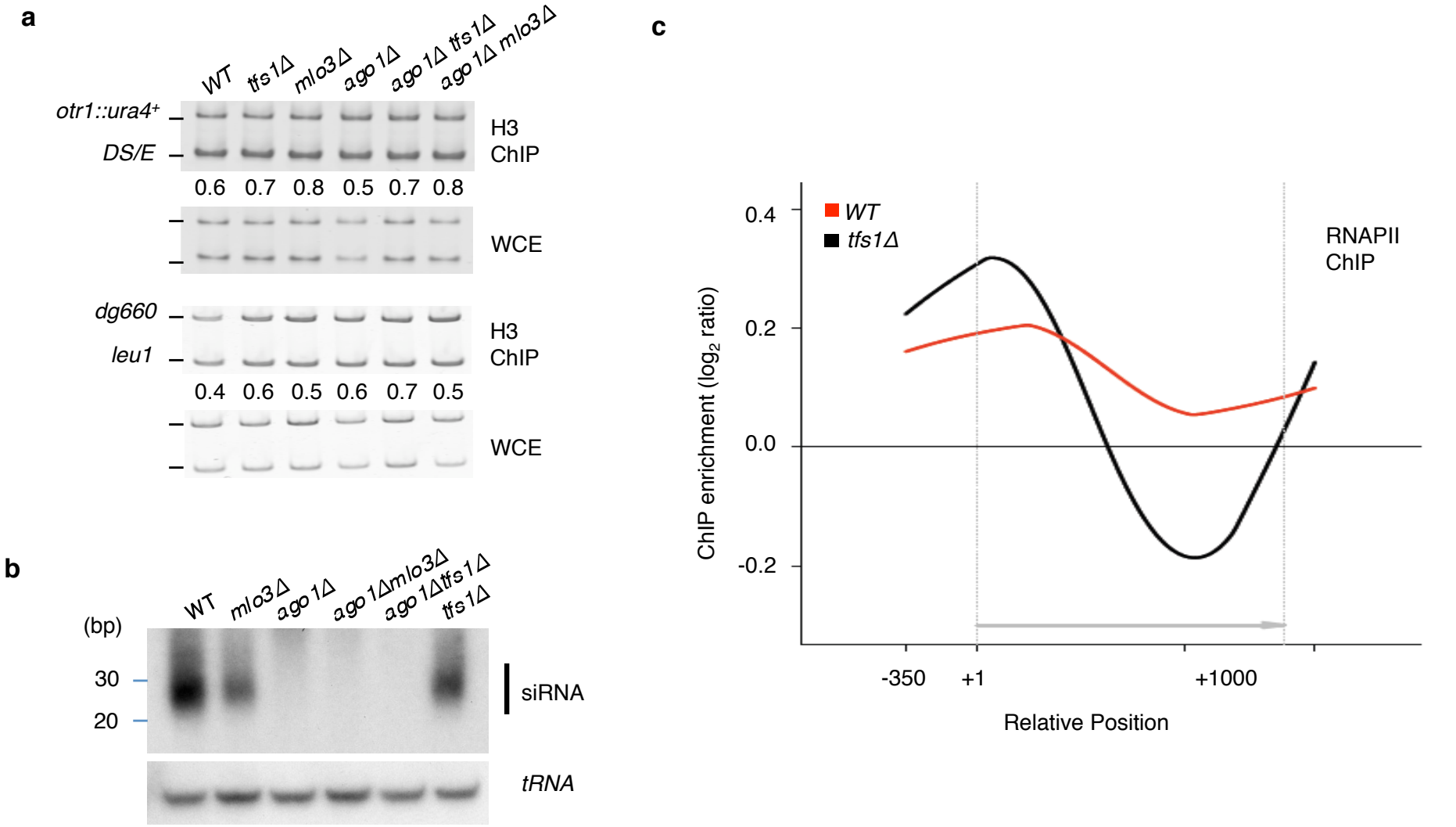
Supplementary Figures 1-8
Supplementary methods



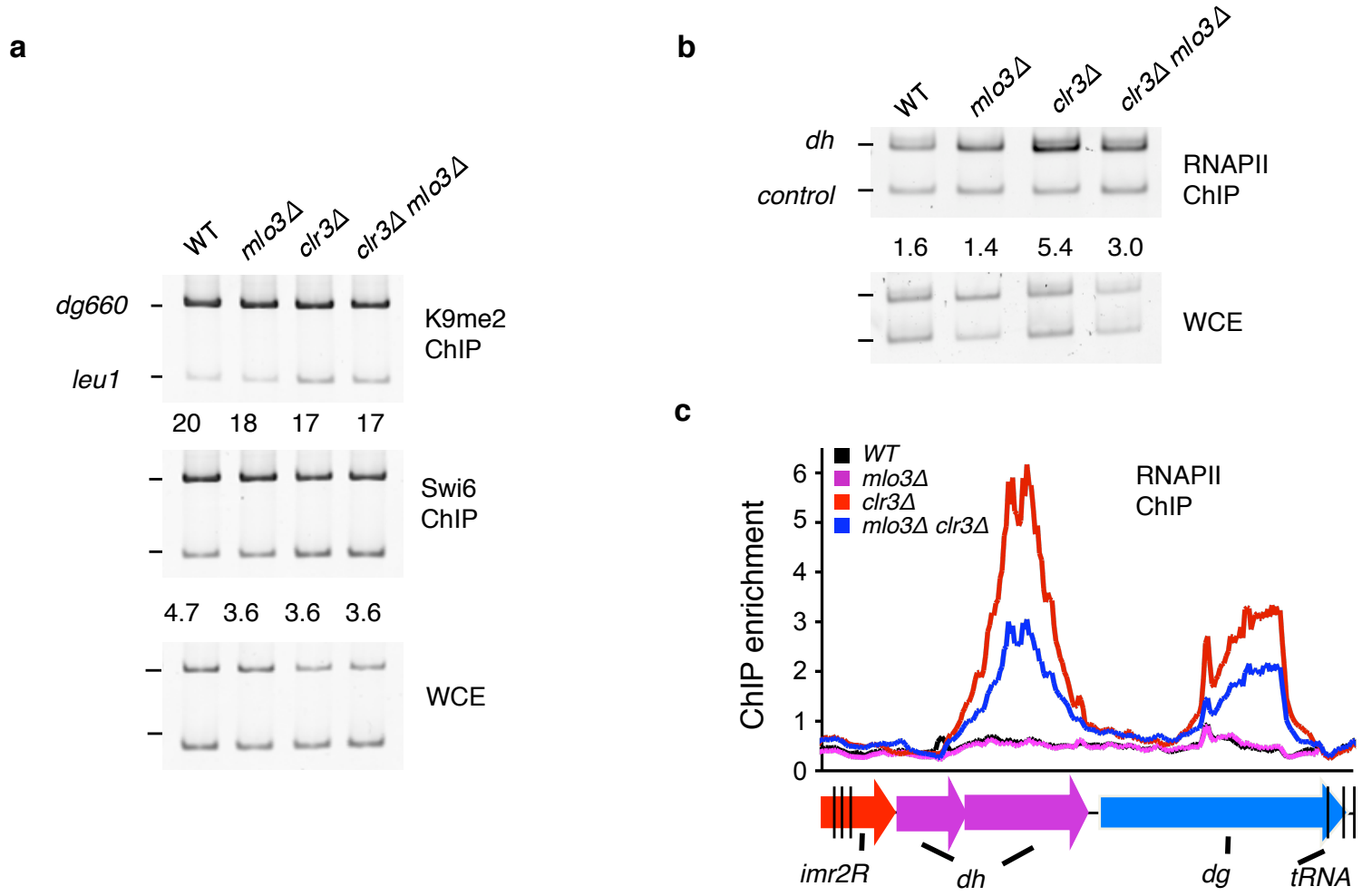
Supplementary Figure 1 Deletion of *mlo3* or *tfs1* does not suppress the silencing defect caused by *clr4Δ*. Serial dilution of wild type and mutant strains were spotted onto media with or without FOA to assay silencing at *otr1R::ura4+*.



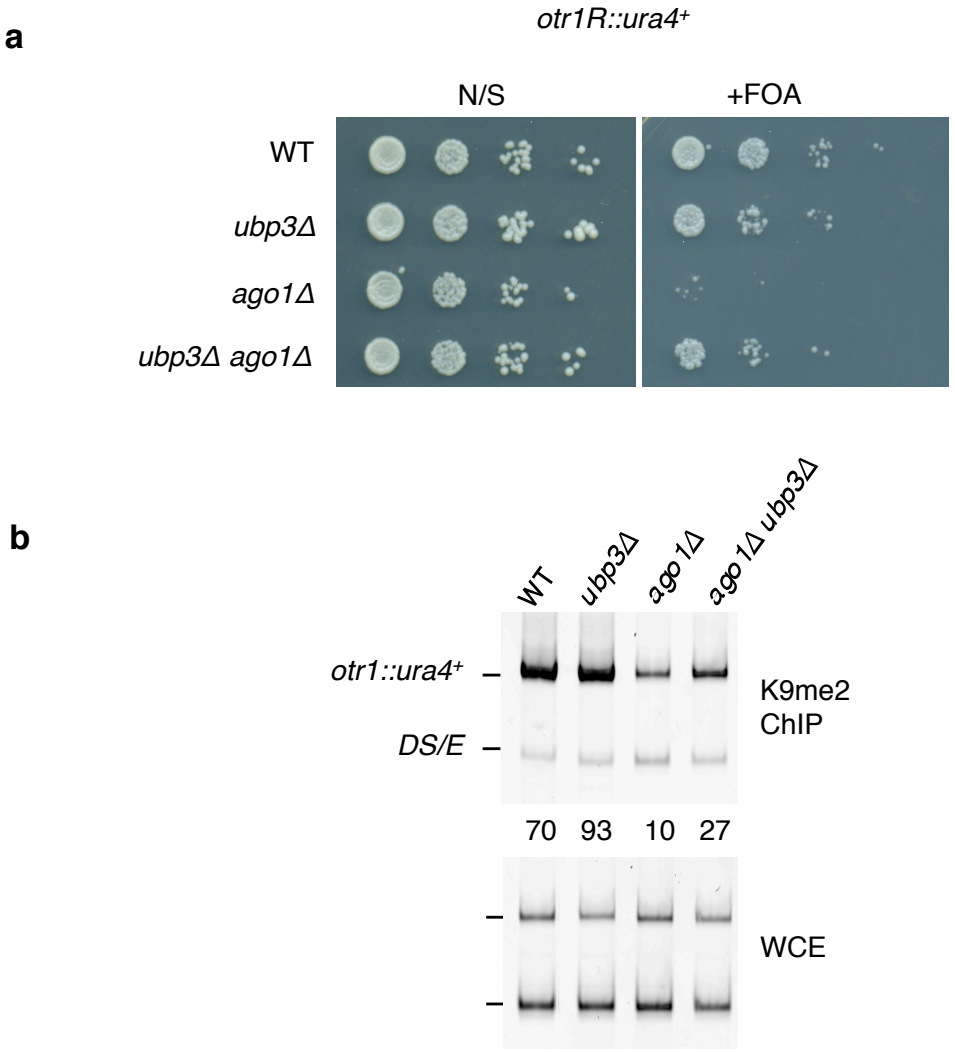
Supplementary Figure 2 Loss of Mlo3 or Tfs1 restores functional heterochromatin in *dcr1Δ* cells. (a-b) Deletion of *mlo3* **(a)** or *tfs1* (**b**) suppresses the silencing defect at *otr1R::ura4+*in *dcr1Δ* cells. Serial dilution of wild type and mutant strains were spotted onto media with or without FOA to assay silencing at *otr1R::ura4+*, and with TBZ to assay chromosome segregation defects (upper). *mlo3Δ* or *tfs1Δ* restores heterochromatin at *otr1R::ura4+* in *dcr1Δ* cells. Relative fold enrichments of H3K9me at *otr1R::ura4+* were determined by ChIP (lower).



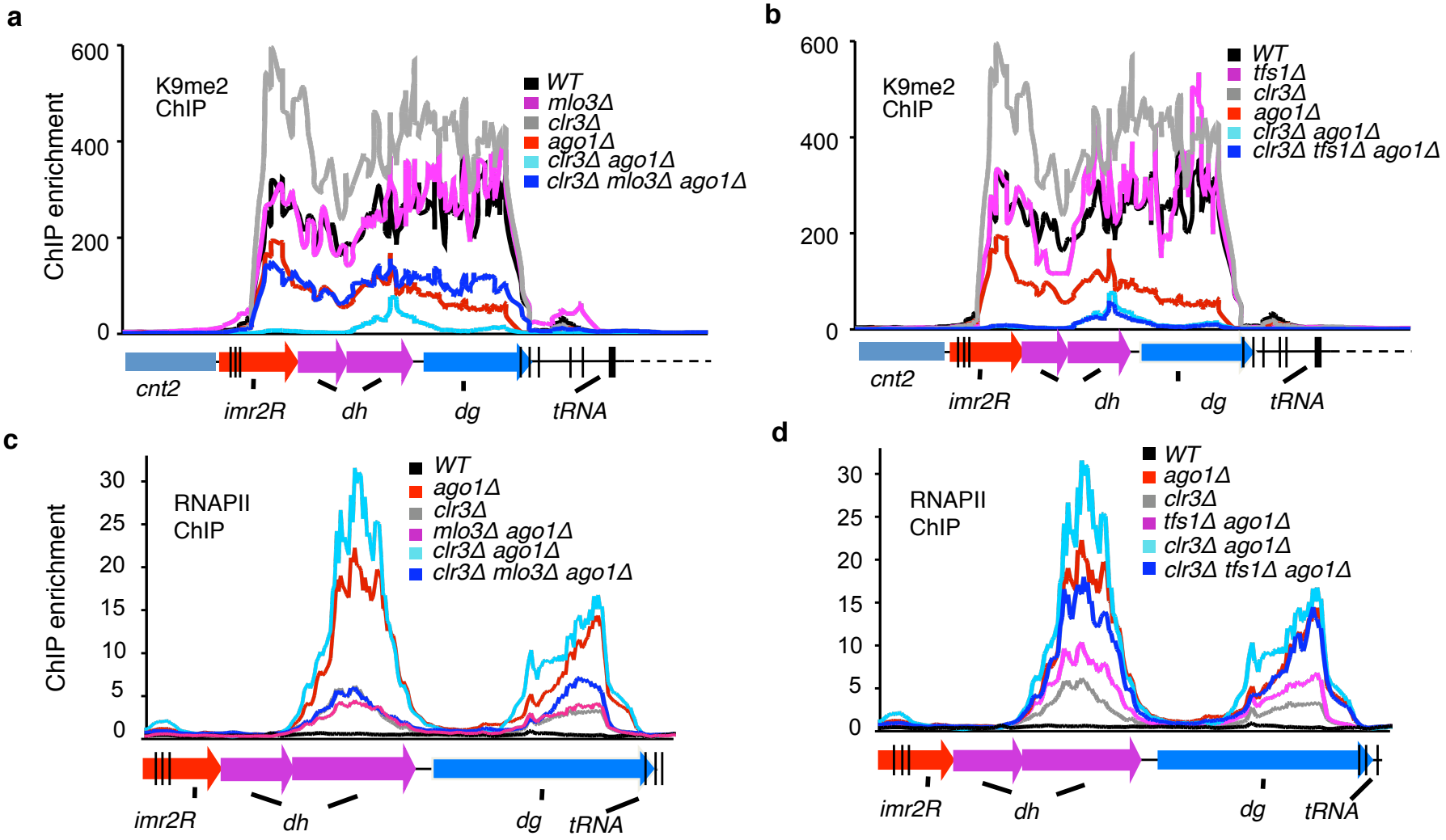
Supplementary Figure 3 Loss of Mlo3, or RNAPII processivity factor Tfs1, does not alter steady state levels of histones at centromeric loci or restore centromeric siRNA. (**a**) Enrichment of histone H3 at *otr1R::ura4*$^{+}$ (upper panel) and *dg660* (lower panel) was measured by ChIP. (**b**) Northern blot analysis of centromeric siRNAs in wild type and mutant cells. tRNA was used as a loading control. (**c**) *tfs1*Δ affects RNAPII distribution at transcribed loci. ChIP-chip analysis of RNAPII was determined in WT and *tfs1Δ* cells using the 8WG16 antibody against RNAPII. The average gene profile shown was prepared by dividing the ORFs into 30 equality-sized sections. The grey arrow represents the average ORF.



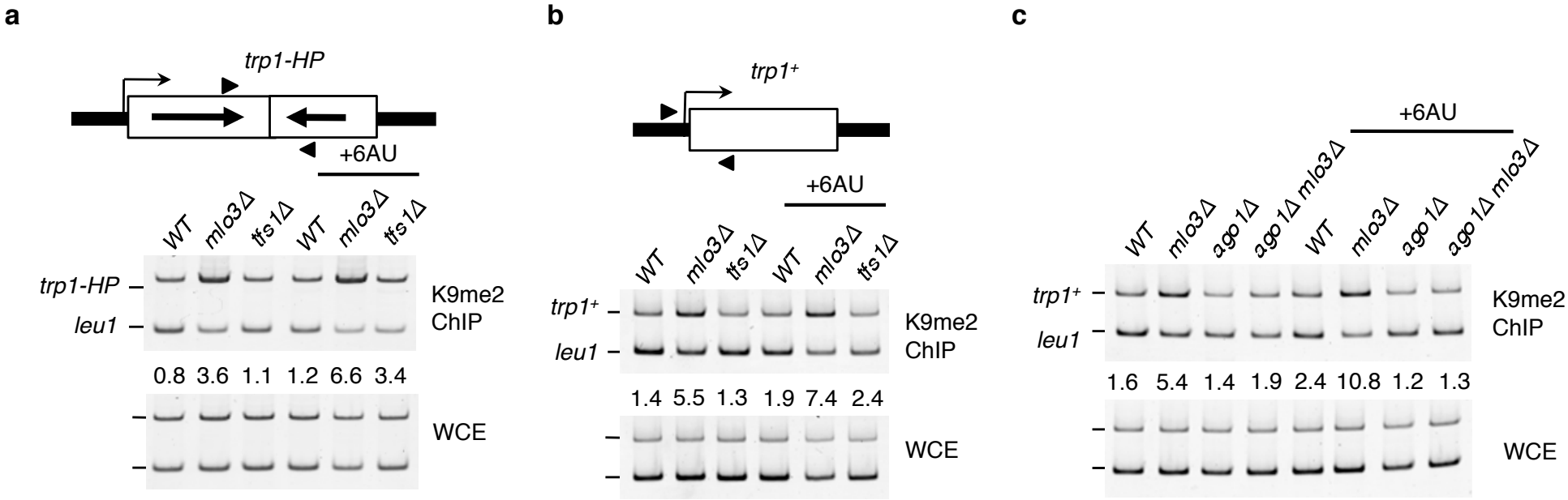
Supplementary Figure 4 *mlo3Δ* decreases RNAPII occupancy at *dh* repeats in *clr3Δ* cells. (**a**) *mlo3Δ* does not affect H3K9me2 or Swi6 localization in *clr3Δ* cells. Levels of H3K9me2 and Swi6 at *dg* repeats relative to *leu1*$^{+}$ were determined by ChIP. (**b**) Enrichment of RNAPII at *dh* repeats relative to a non-transcribed control DNA at the mating type locus (indicated by control) was measured by ChIP using anti Ser2 phospho RNAPII antibody. (**c**) *mlo3Δ* decreases RNAPII occupancy at centromeric repeats in *clr3Δ* cells. RNAPII distribution at *dh* and *dg* repeats was determined by ChIP using anti Ser2 phospho RNAPII antibody.



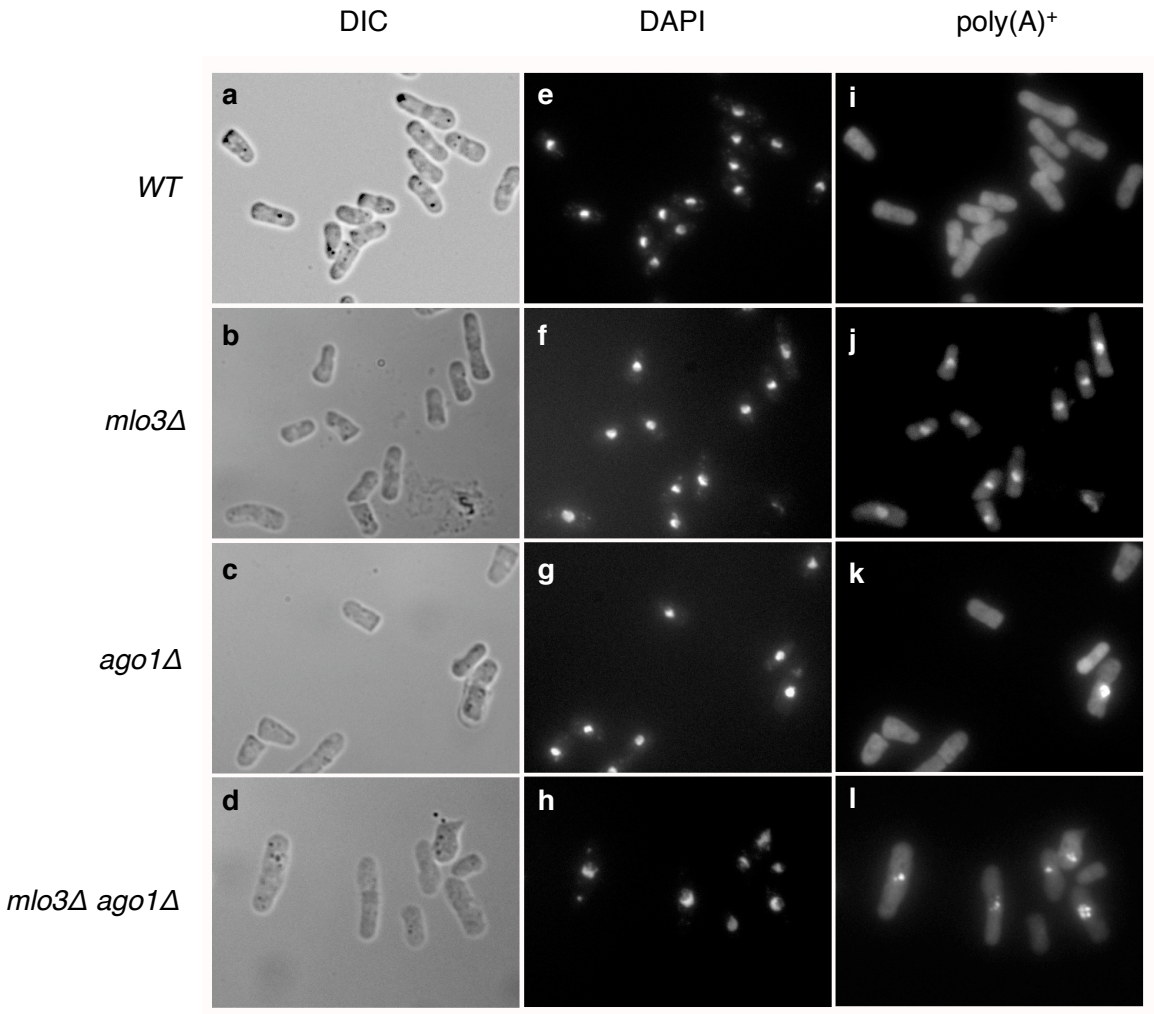
Supplementary Figure 5 Loss of Ubp3 partially rescues centromeric heterochromatin defects in *ago1Δ* cells. (**a**) Deletion of *ubp3* suppresses the silencing defect at *otr1R::ura4+* in *ago1Δ* cells. Serial dilution of wild type and mutant strains were spotted onto media with or without FOA to assay silencing at *otr1R::ura4+*. (**b**) *ubp3Δ* restores heterochromatin at *otr1R::ura4+* in *ago1Δ* cells. Relative fold enrichments of H3K9me at *otr1R::ura4+* were determined by ChIP.



Supplementary Figure 6 *mlo3Δ*, but not *tfs1Δ*, restores H3K9me and dimnishes RNAPII occupancy at the pericentromeric domains in *clr3Δ ago1Δ* cells. Relative fold enrichment of H3K9me2 (**a-b**) or RNAPII (**c-d**) across the pericentromeric domain of cen2 as determined by ChIP-chip analysis in the indicated strains.



Supplementary Figure 7 Expression of an exogenous hairpin RNA in *mlo3Δ* and *tfs1Δ* cells induces H3K9me *in cis* and *trans* at its cognate site. (**a**) Effects of *mlo3Δ* or *tfs1Δ* on hairpin-induced heterochromatin modifications *in cis*. A plasmid carrying a hairpin construct corresponding to the *trp1*$^{+}$ gene (*trp1-HP*) was transformed into *mlo3Δ* or *tfs1Δ* cells. The hairpin was expressed under the control of the *nmt1* promoter. Wild-type and mutant cells carrying the hairpin plasmid were grown in presence or absence of 6-AU. H3K9me2 enrichment at the *trp1-HP* locus relative to *leu1*$^{+}$ was assessed by ChIP. The black triangles in the schematic diagram represent the locations of the primers used to PCR amplify *trp1-HP*. (**b**) Effects of *mlo3Δ* or *tfs1Δ* on hairpin-induced heterochromatin modifications *in trans*. Wild type and mutant cells expressing *trp1-HP* were grown in the presence or absence of 6-AU. H3K9me2 enrichment at the endogenous *trp1*$^{+}$ locus relative to *leu1*$^{+}$ was assessed by ChIP. The black triangles represent the location of the primers used to PCR amplify *trp1*$^{+}$. (**c**) Ago1 is required for H3K9me2 enrichment at the endogenous *trp1*$^{+}$ gene *in trans* in *mlo3Δ* cells. Wild type and mutant cells expressing *trp1-HP* were grown in presence or absence of 6-AU. H3K9me2 enrichments at the endogenous *trp1*$^{+}$ gene relative to *leu1*$^{+}$ were assessed by ChIP.



Supplementary Figure 8 *mlo3Δ ago1Δ* cells exhibit nuclear accumulation of poly(A)⁺ RNA. In situ hybridization of poly(A)⁺ RNA was carried out in WT and indicated strains. (**a-d**) DIC image of WT and mutant *S. pombe* cells. (**e-h**) DAPI stained nuclei of WT and mutant strains. (**i-l**) In situ hybridization of poly(A)⁺ RNA in WT and indicated strains.

SUPPLEMENTARY METHODS

Hairpin construction and expression. A hairpin corresponding to *trp1*$^+$ locus (*trp1-HP*) was constructed by PCR amplifying two overlapping fragments of *trp1*$^+$. The first fragment was amplified using the following primers: 5'-TCCGCTCGAGTGTCTGAAAAAGTTGACGTTGG TG-3' and 5'-CAGGATCCGAAGCTCTCATAAGAGATTCACC-3', while the second fragment was amplified with the following primer set: 5'-CGGGATCCTATACATGGAGGTGC TATGTTAAGG-3' and 5-TCCCCCGGGTGTCTGAAAAAGTTGACGTTGGTG-3'. The first fragment product was subcloned into the SmaI and BamHI sites of pREP3X. The second fragment was sequentially subcloned into the BamHI and XhoI sites of pREP3X to produce the pESC2 plasmid. The pESC2 plasmid was transformed into the wild type and mutant strains. Expression of the *trp1-HP* was achieved by growing 100 ml of cells in EMM-ura-leu media to OD_{595}= 0.4. The cells were split into two 50-milliliter cultures, and 600 μg/ml of 6-AU was added to one of the cultures. The cells were grown at 30°C for two more hours before crosslinking with pFA.

RNA in-situ hybridization. *In situ* hybridization against poly(A)$^+$ RNA was done as previously described with some modifications[1]. Briefly, cells were grown in rich medium to a density of $1X10^7$/ml at 30^{o}C. Cells were immediately fixed with 3% formaldehyde for 1 hour, washed with 1X PBS after fixation, and spheroplasted with Zymolase 100T. After adhering spheroplasts to poly-L-lysine-coated slide, the cells were dehydrated in methyl alcohol overnight. Cells were rehydrated by incubation in 2XSSC for 10 seconds. Cells were hybridized overnight in 2 X SSC, 10% Dextran sulfate, 2 mg/ml bovine serum albumin, 20mM ribonucleoside vanadyl complex, 0.2 mg/ml tRNA, and 10μg/ml Cy3-labeled oligo-dT probe. After the hybridization, slides were washed three times with 2 X SSC and mounted with buffer containing 4',6-diamidino-2-phenylindole for microscopy.

1 Santos-Rosa, H. *et al.* Nuclear mRNA export requires complex formation between Mex67p and Mtr2p at the nuclear pores. *Mol. Cell. Biol.* 18, 6826-6838 (1998).