

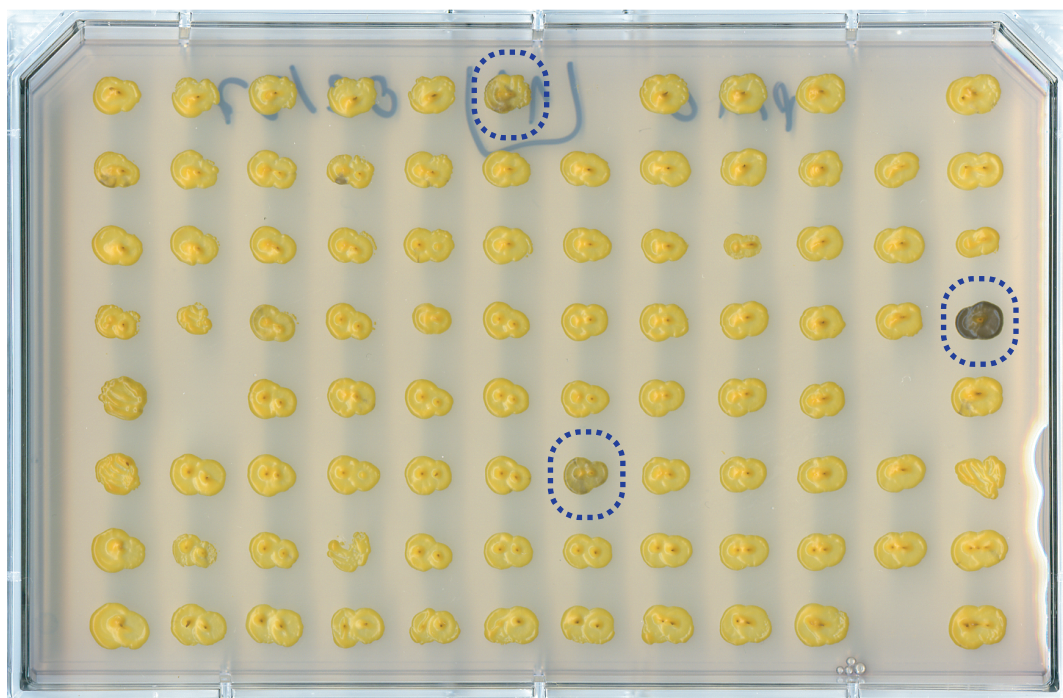
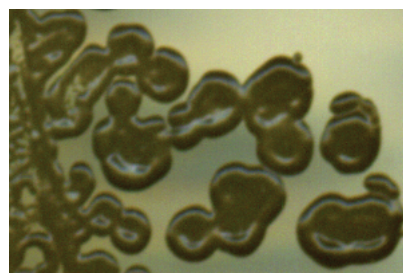
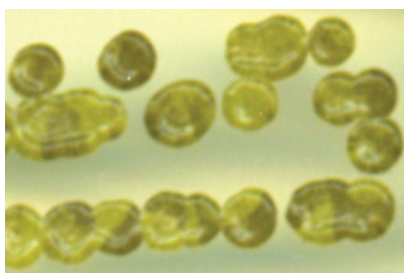
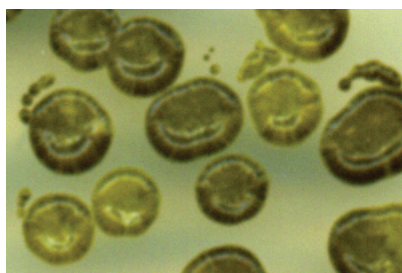
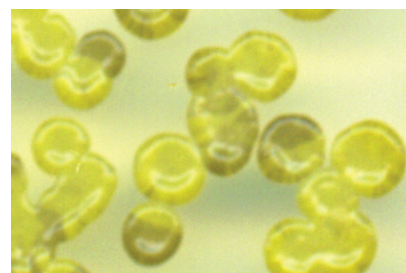
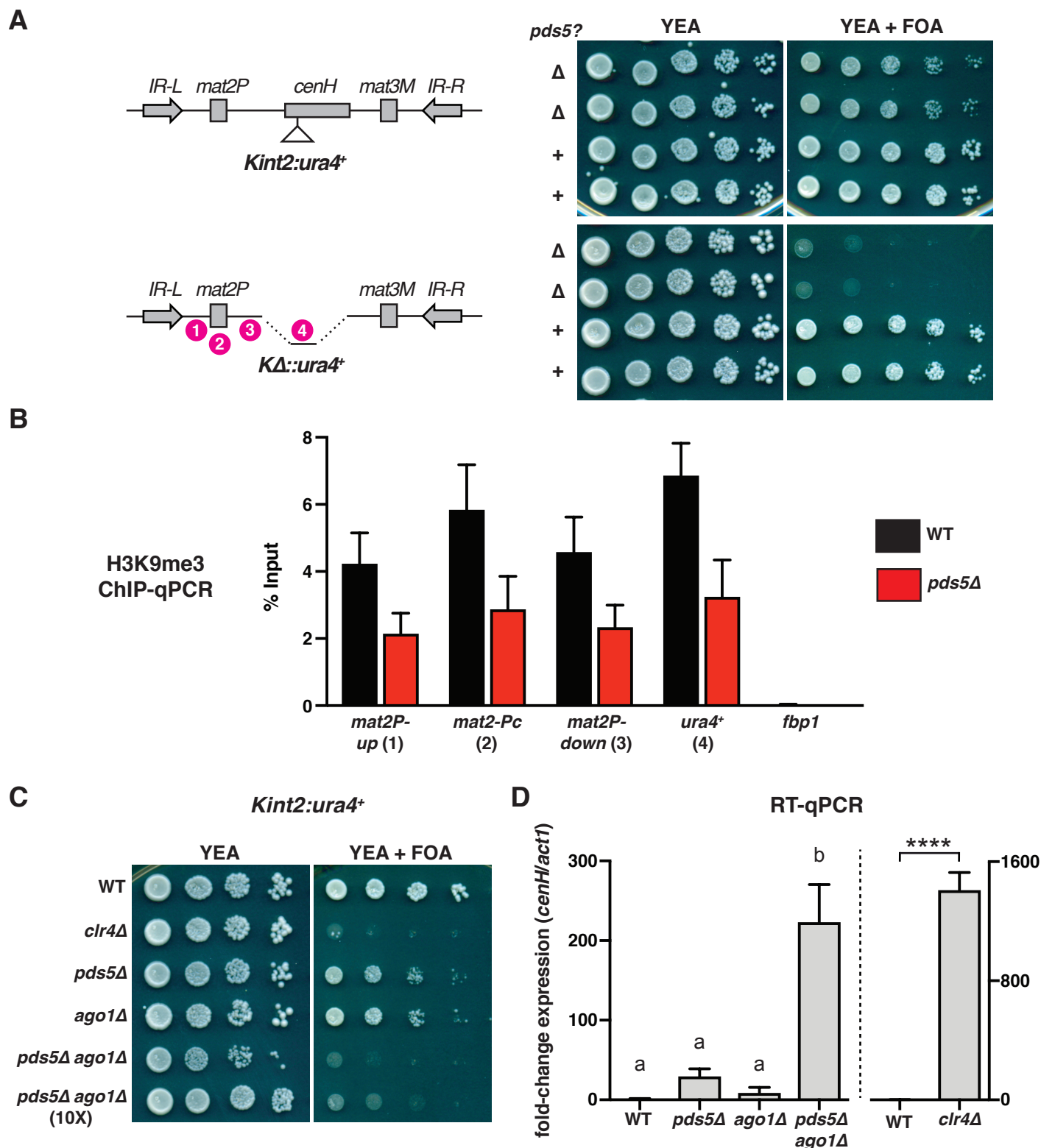
A**B****WT*****clr4Δ******dhm1Δ******rpl2102Δ******rpl39Δ***

Figure S1. Genetic screen for factors involved in heterochromatin formation. (A) Example of a plate from the screen containing crosses grown in PMG5S minimal medium with triple antibiotic selection and subjected to iodine staining. Putative candidates are indicated by circles. (B) Examples of mutant strains exhibiting variegation patterns. WT and *clr4Δ* as shown as controls. The indicated strains were streaked to single colonies in YEA, then replica plated onto PMG5S and grown at 32°C for 3 d prior to iodine staining.



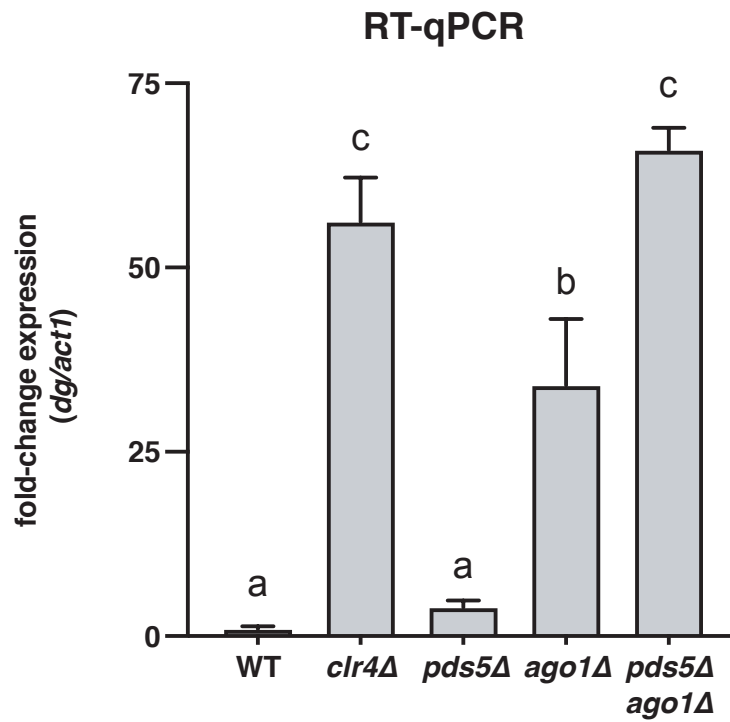


Figure S3. Pds5 and RNAi play distinct roles in promoting heterochromatic silencing. RT-qPCR amplification of centromere *dg* repeats in the indicated strains. The relative fold enrichment over *act1* is shown. Error bars denote SD; $N \geq 3$ independent experiments. Mean values marked with different letters (a, b or c) indicate results that are significantly different from each other, as established by One Way ANOVA and Holm-Sidak test for multiple comparisons ($P < 0.01$).

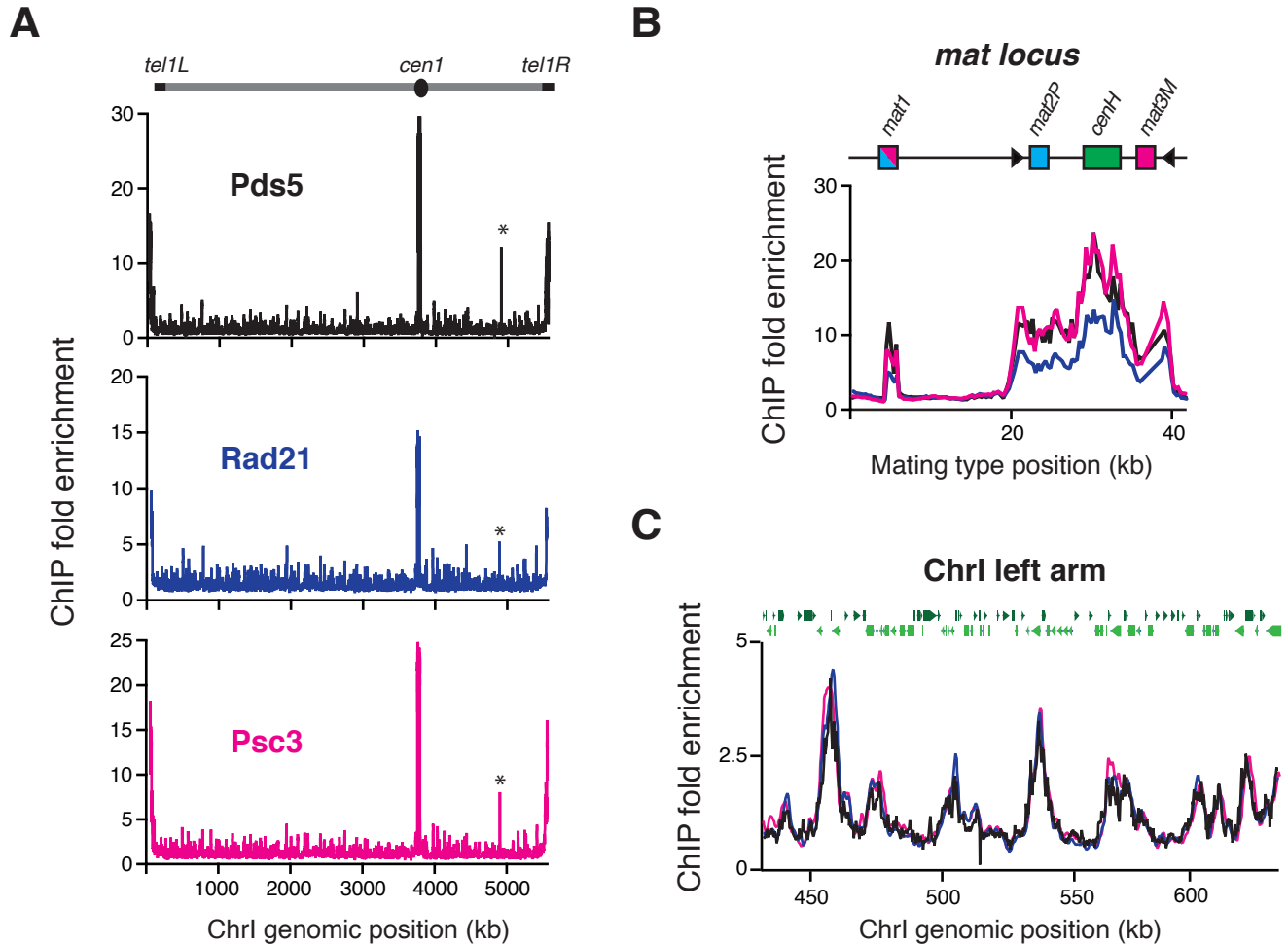


Figure S4. Pds5 colocalizes with cohesin. (A-C) ChIP-chip analysis of cohesin subunit distribution in WT strain backgrounds. Pds5-GFP, Rad21-GFP and Psc3-GFP localization along ChrI (A), *mat* locus (B) and a euchromatic chromosome arm region (C) are shown. Rad21 and Psc3 data were previously published (FOLCO *et al.* 2017). Enrichments at *mae1*, marked by asterisks, reflect cross-hybridization of this locus to subtelomeric sequences. Green bars represent open reading frames according to the 2007 *S. pombe* genome assembly.

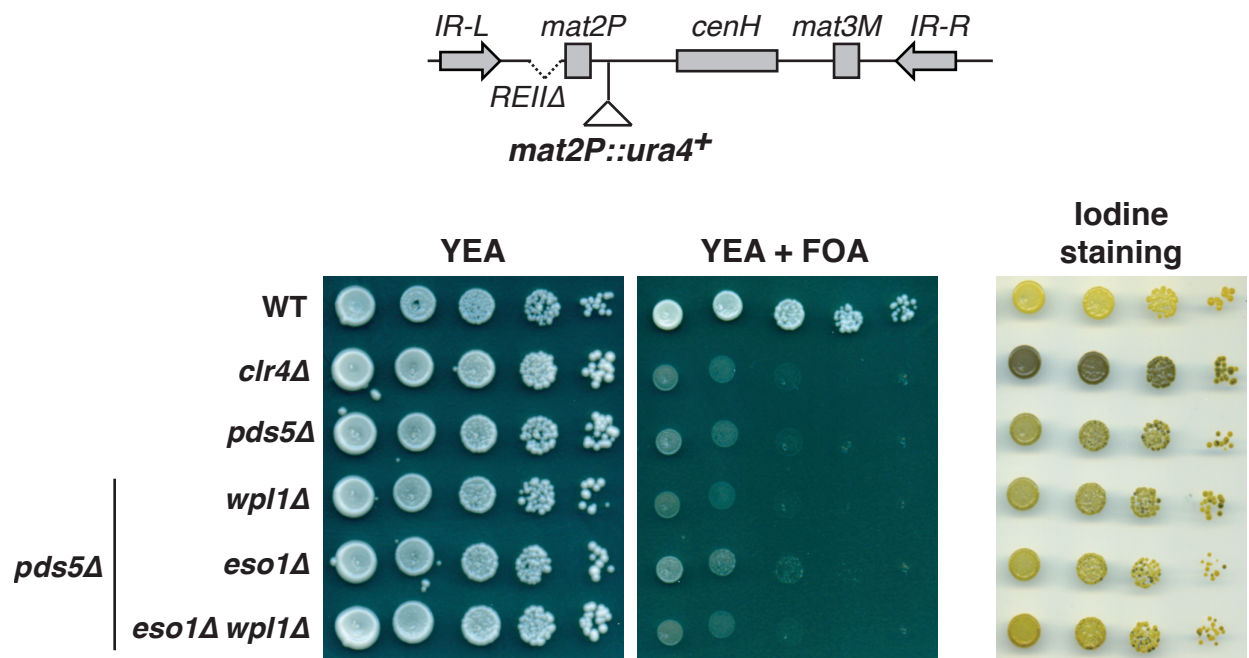


Figure S5. Effect of *eso1* and *wpl1* deletions in cells lacking Pds5. Ten-fold serial dilutions of the indicated strains were spotted on YEA rich media, with or without FOA, and PMG5S minimal media and grown at 32°C. The PMG5S plate was stained with iodine vapor.