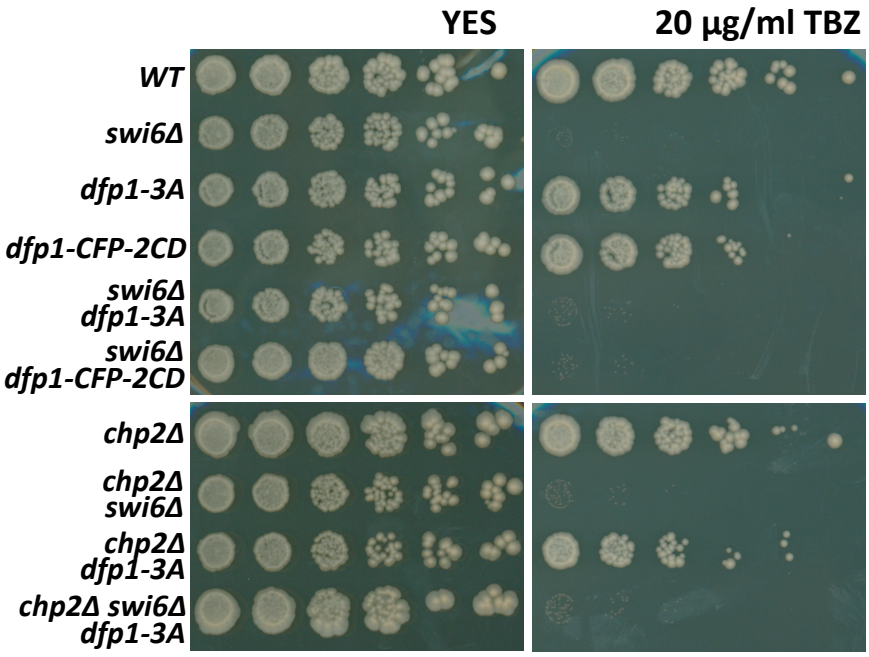

Figure S1. *swi6Δ dfp1-CFP-2CD* mutants are sensitive to TBZ
Strains from Figure 3A were tested for TBZ sensitivity. *dfp1-3A*, d*fp1-CFP-2CD* and *chp2Δ* single mutants are more sensitive to TBZ comparing to wild type. Moreover, TBZ sensitivity slightly increased in *chp2Δ dfp1-3A* double mutant compared to *chp2Δ* and *dfp1-3A* single mutants. Cells were grown at 32˚C for 5 days.

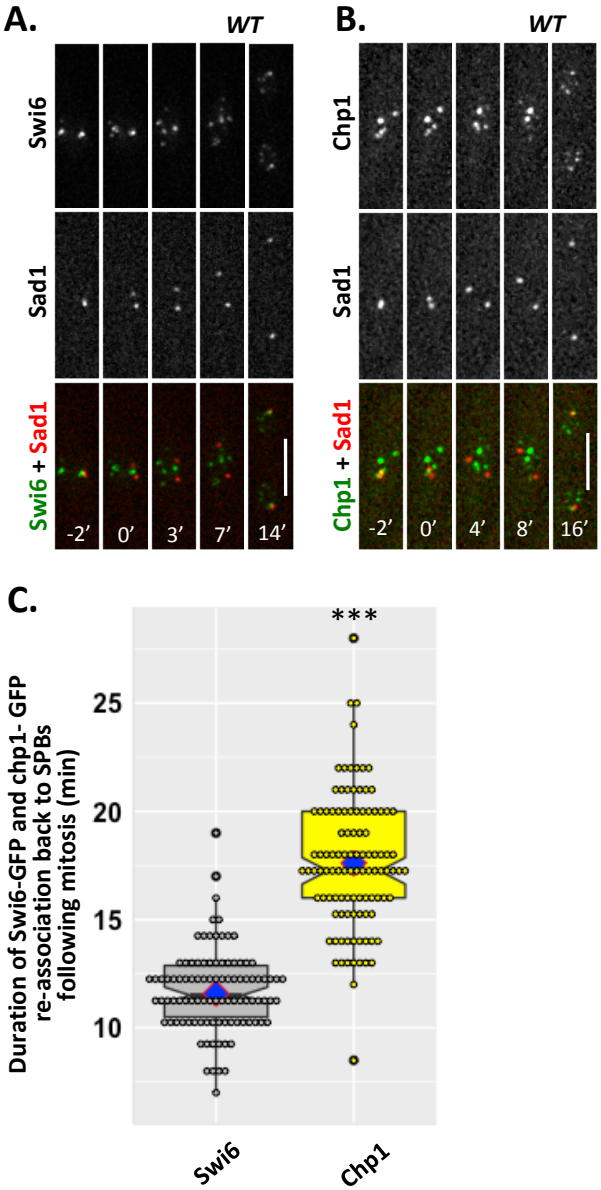

Figure S2. Swi6 moves to spindle poles earlier than Chp1 following mitosis

The position of (A) Swi6-GFP (FY7814) or (B) Chp1-GFP (FY5911) in relation to the centromere defined by the SPB marker Sad1-DsRed was observed in live cells. Time 0' corresponds to the timepoint that precedes the first observed separation of the SPB and defines initiation of mitosis. (C) Quantification of duration of re-association of Swi6-GFP and Chp1-GFP to the SPB after mitosis shows re-association of Swi6-GFP to the SPB following mitosis occurs earlier than Chp1-GFP. Two-tailed student's *t* test was used to determine significance. *p* values are reported as the follow: ***, $p<0.001$.

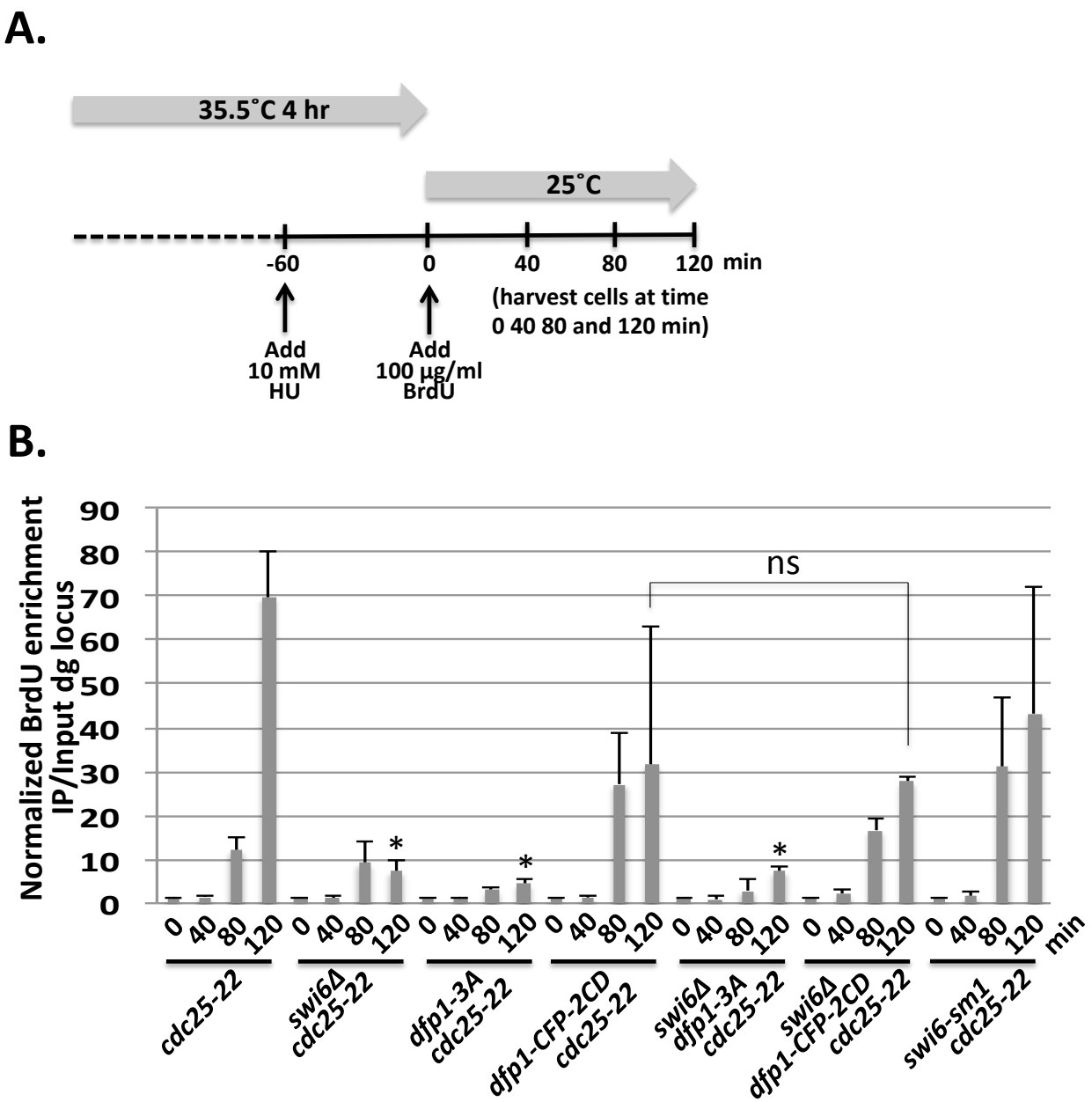

Figure S3. Replication timing in *dfp1-3A* and *dfp1-CFP-2CD swi6Δ*

(A) Schematic diagram of experimental protocol. Indicated strains FY4255, FY4574, FY7583, FY7585, FY7631, FY7687 and FY8444 contained a temperature sensitivity *cdc25-22* mutation to arrest the cell cycle at G2/M. Cultures were grown at 25˚C in the air shaker until cell density reached 0.3 OD_{595} and then shifted to 35.5˚C for 4 hours. An hour before downshifting to the permissive temperature (25˚C), 10 mM of HU was added into the culture. Upon release, freshly prepared 100 µg/ml BrdU was added into the culture for labeling newly synthesized DNA. Cells were harvested at time-points 0, 40, 80, and 120 minutes. BrdU-incorporated DNA fragments were isolated by ChIP using an anti-BrdU antibody and enrichment of BrdU was then measured by a real-time quantitative PCR. Two independent experiments were done. (B) The normalized ratio of BrdU-enriched IP versus input at the *dg* locus was plotted. Similar to *swi6Δ*, the pericentromere replicates late in *dfp1-3A* mutants. As expected, *dfp1-CFP-2CD* partially restores early pericentromeric replication in the *swi6*-deleted cells. Two-tailed student *t* test was used to determine statistical significance at 120 min, compared to wild type. *p* values were represented as the follow: *: $p < 0.05$. ns: not statistically significant.

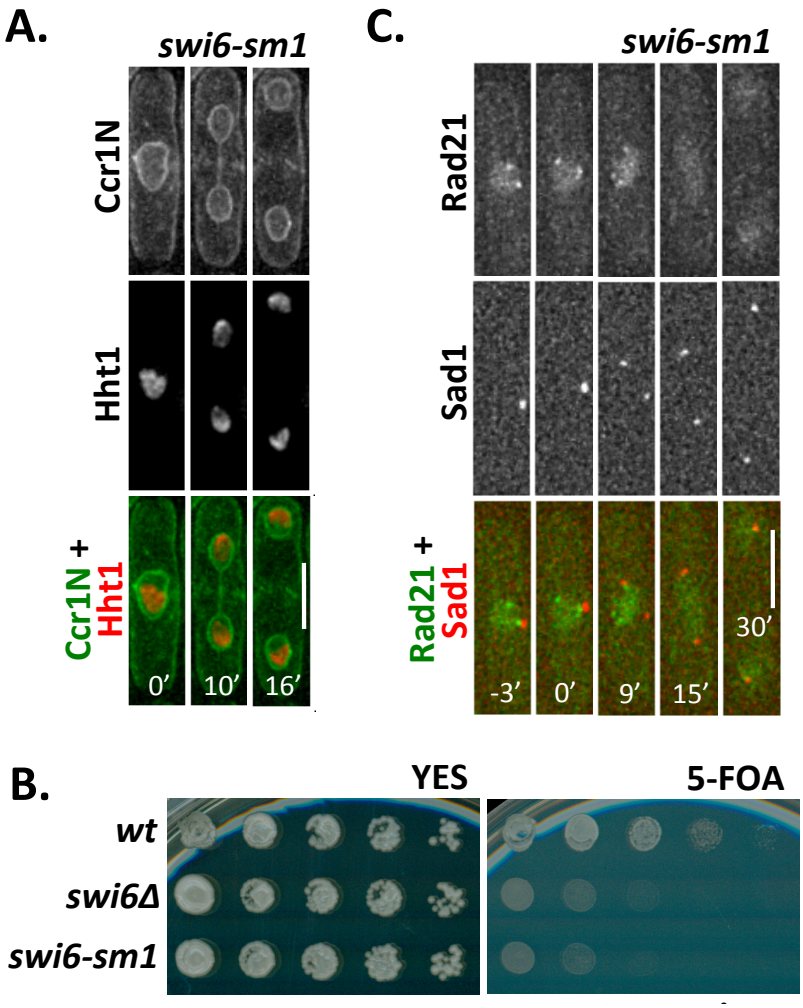

Figure S4. Centromeric Rad21 localization is not affected by *swi6-sm1*
(A) Time lapse imaging of Ccr1N-GFP in relation to Hht1-mRFP was captured in strain FY8513 carrying a *swi6-sm1* mutant allele that uncouples heterochromatin silencing from chromosome segregation. Mitosis was normal in *swi6-sm1* mutants. (B) Expression of the *ura4*$^{+}$ marker at *otr1L* in *wt* (FY4293), *swi6Δ* (FY4295) and *swi6-sm1 (*FY8688:) was determined by sensitivity to FOA. (C) Rad21-GFP localization in relation to Sad1-DsRed was followed in a *swi6-sm1* strain FY8958. Rad21-GFP colocalizes with Sad1-DsRed before mitosis in *swi6-sm1* mutants similar to wild type (compare to Figure 5)

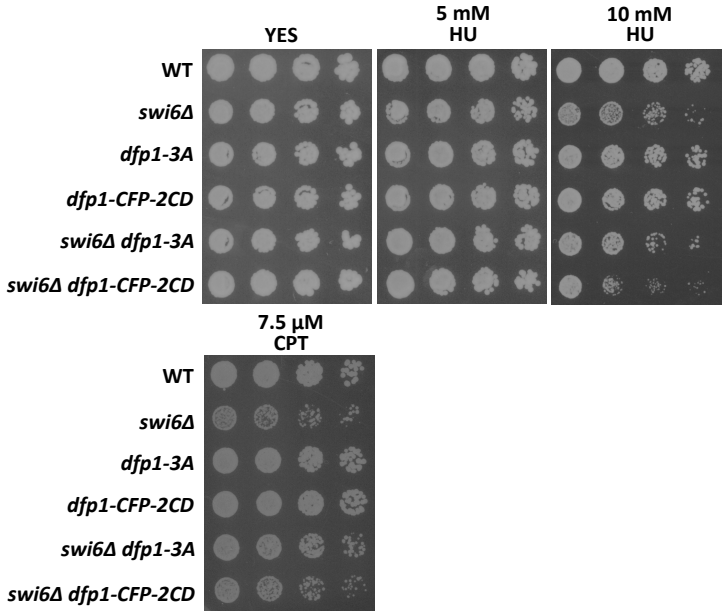

Figure S5. *dfp1-3A* and *dfp1-CFP-2CD* mutant cells are not sensitive to HU and CPT.
Strains FY4293, FY4295, FY8144, FY8221, FY8146, and FY8191 were tested for sensitivity to HU or CPT at the indicated concentrations. Cells were plated in a 1:5 serial dilution series and grown at 32 ˚C for 3 days.