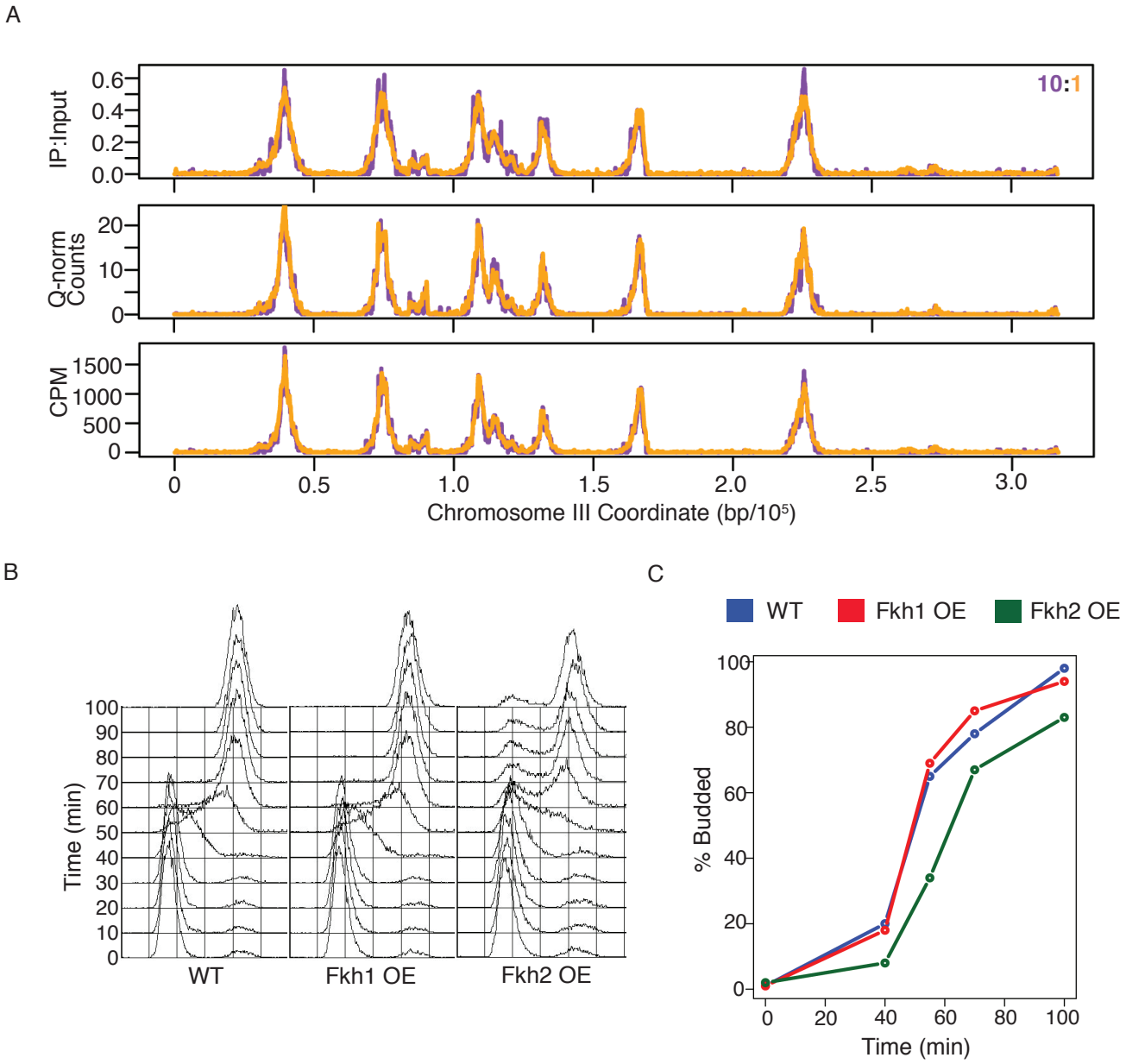


Figure S1. Comparison of data normalization methods and cell cycle progression. A. Input, Quantile, and CPM normalization of IP data from the experiment described in Fig. 1B. B. DNA content analysis of strains JPy88 (WT), JPy89 (Fkh1-OE), and JPy90 (Fkh2-OE) grown in YEP-raffinose (non-inducing), blocked in G1 phase by incubation with α-factor for 3 hrs, followed by incubation in YEP-galactose plus α-factor for 2 hrs to induce expression while maintaining the G1 block. Cells were released from G1 block into S phase by incubation in YEP-galactose without α-factor and aliquots were incubated with BrdU for the indicated time intervals and harvested. C. Budding morphology of cells analyzed in (B).

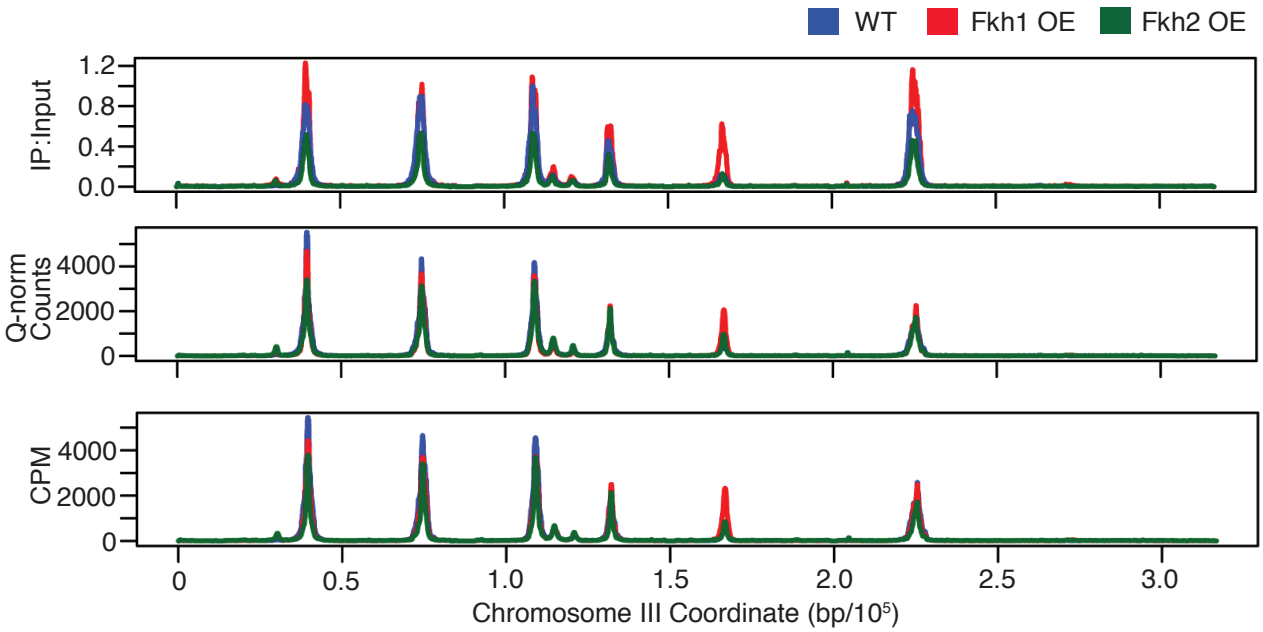


Figure S3. Comparison of data normalization methods. Input, Quantile, and CPM normalization of IP data from the experiment described in Fig 2A.

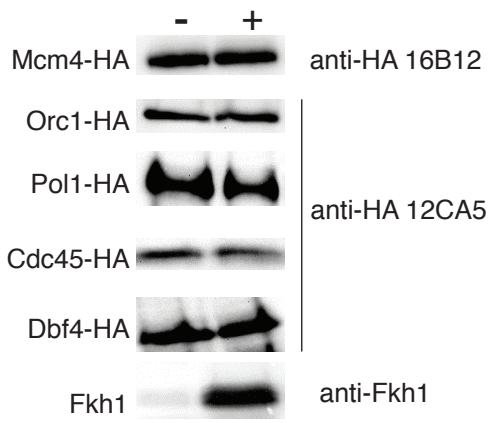


Figure S4. Fkh1-OE does not alter levels of several initiation proteins. Immunoblot analysis of replication initiation proteins in the strains OAy535 (Mcm4-HA3), OAy503 (Orc1-HA3), OAy644 (Pol1-HA3), OAy617 (Cdc45-HA3), and OAy606 (Dbf4-HA3) harboring pGAL (–) and pGAL-FKH1 (+), G1-blocked and GAL-induced as described in Figure 2A.

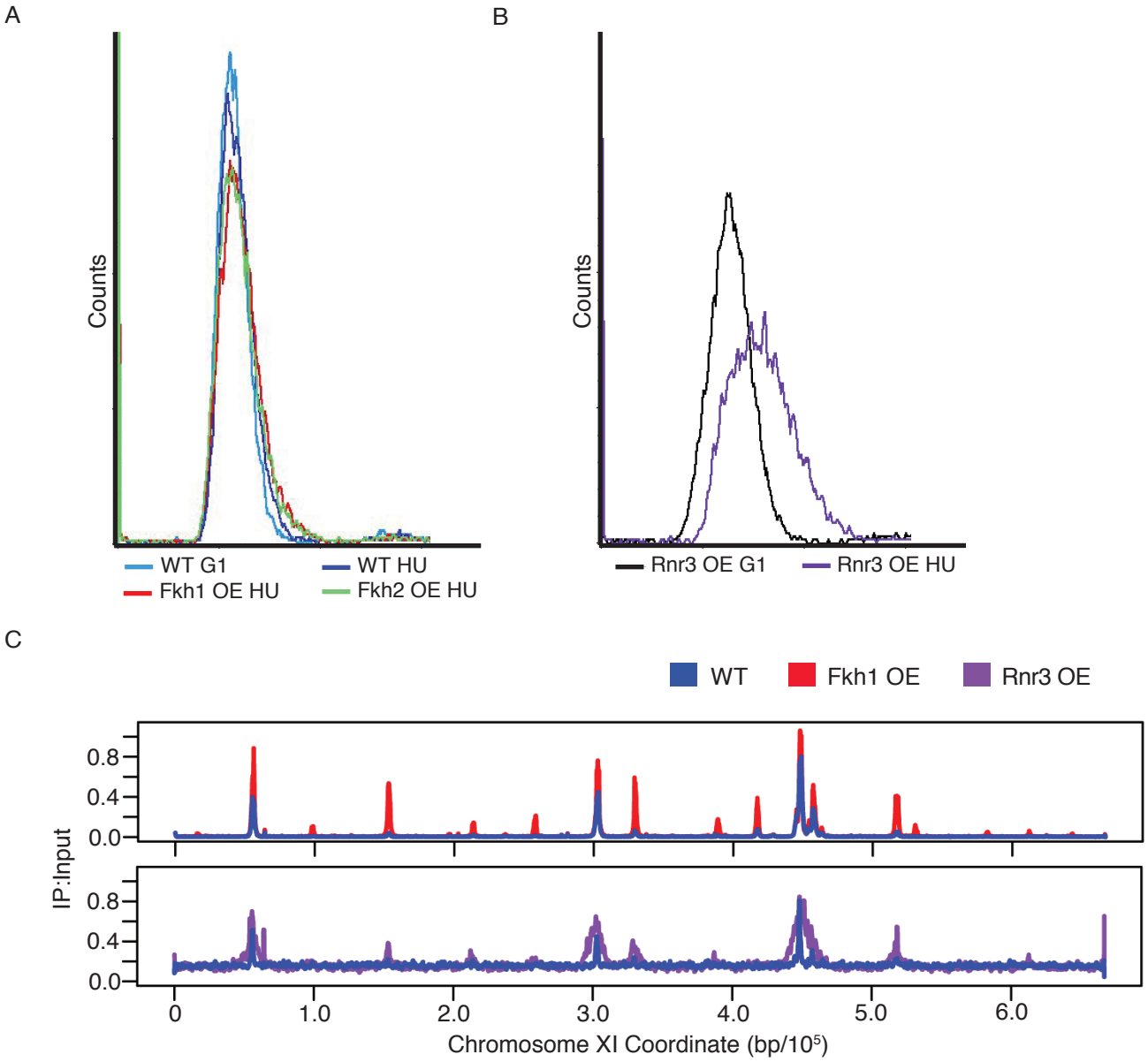
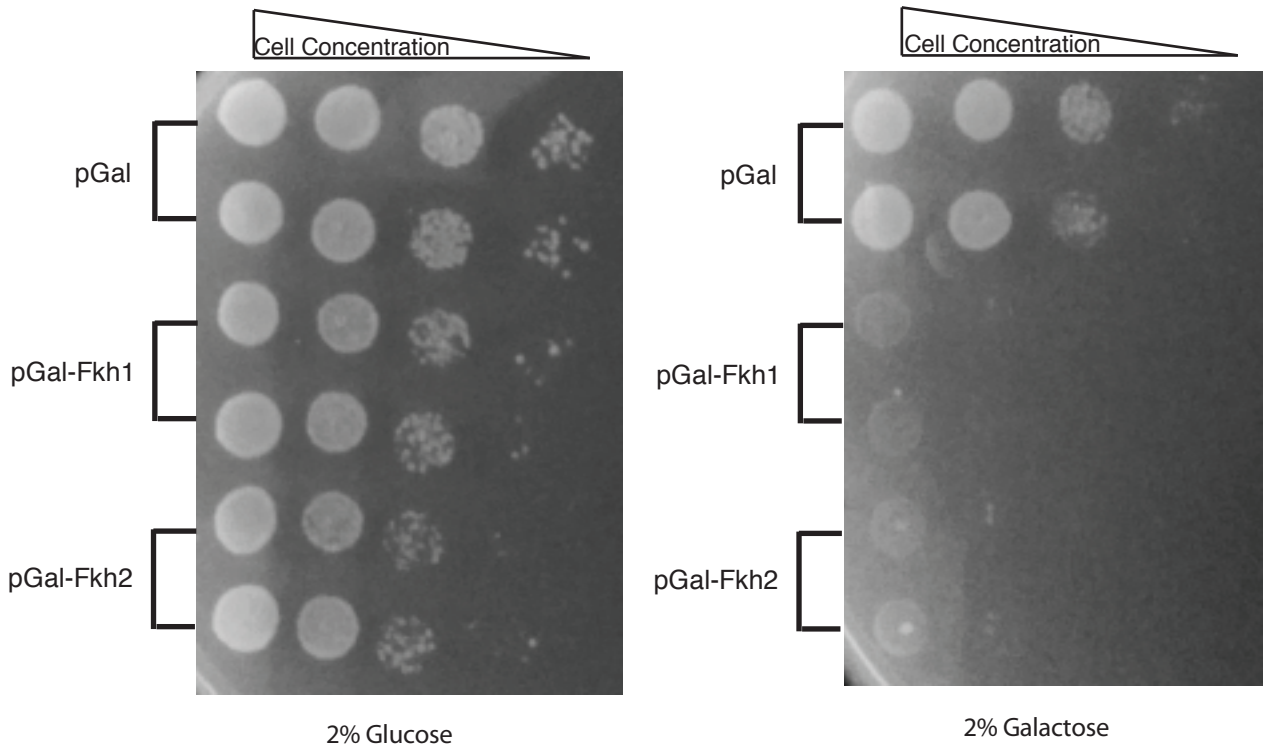


Figure S5. Rnr3-OE affects replication differently than Fkh1/2-OE. Strains JPy88 (WT), JPy89 (Fkh1-OE), JPy90 (Fkh2-OE) and JPy103 (Rnr3-OE) were treated as described in Fig. 2A and subjected to DNA content analysis (A and B) and qBrdU-seq analysis (C).

A



B

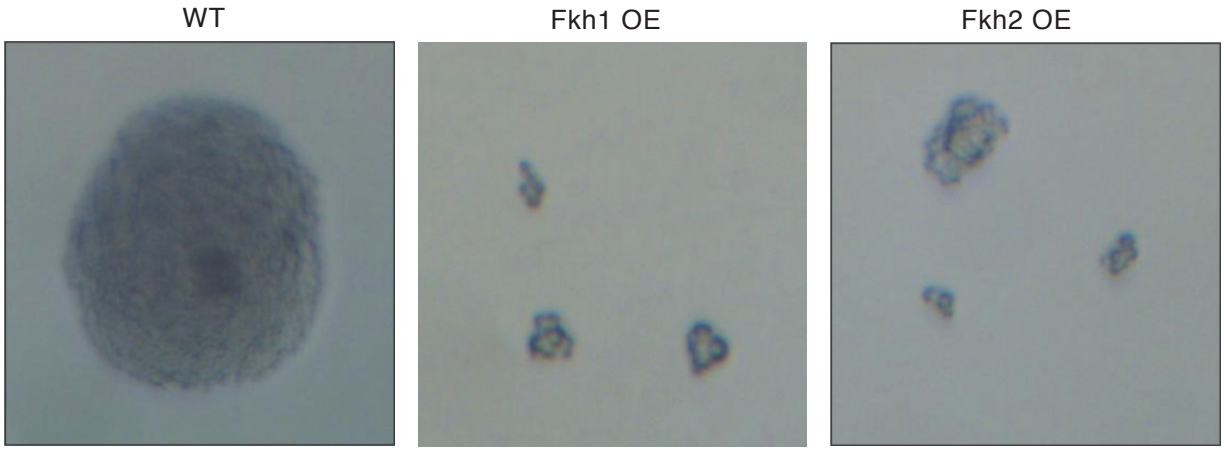


Figure S7. Cells recover from acute Fkh1-OE. A. Viability analysis of strains JPy88 (WT), JPy89 (Fkh1-OE), and JPy90 (Fkh2-OE) G1-blocked and GAL-induced as described in Figure 2A legend and plated on the indicated media in ten-fold serial dilutions. B. Images of colony formation of the indicated cells plated on galactose (inducing) medium and imaged after 72 hrs.