Expanded View Figures

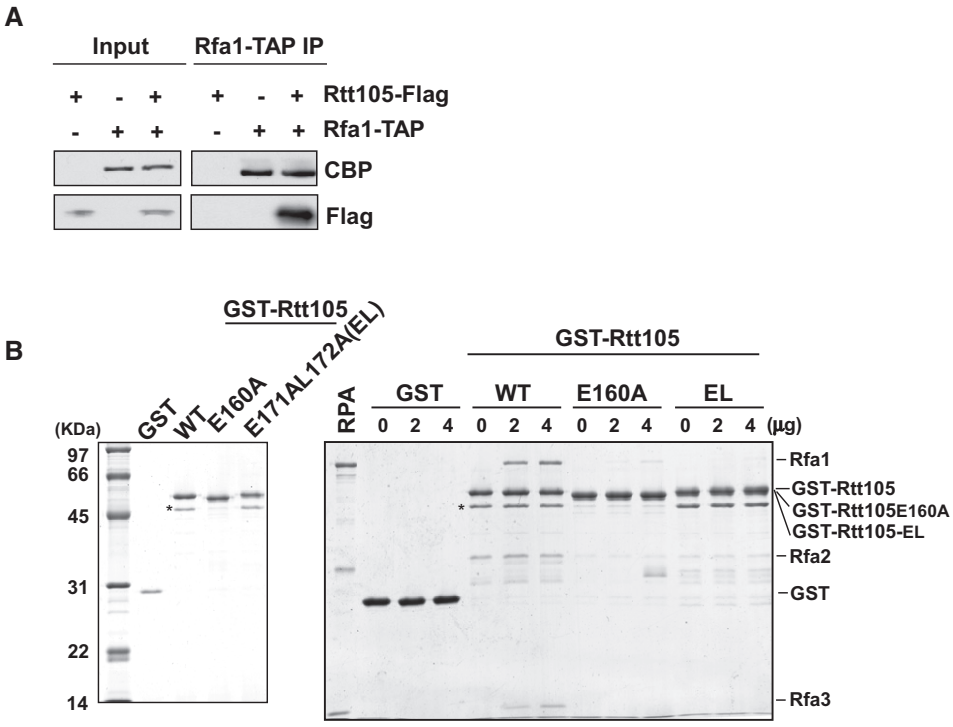

Figure EV1. Mapping key amino acids for the Rtt105–RPA interaction.

A Identification of RPA–Rtt105 interaction by tandem affinity purification (TAP). TAP-tagged Rfa1 was purified from yeast cells with or without Flag-tagged Rtt105 (Rtt105-Flag), and the co-purified proteins were resolved on SDS–PAGE and analyzed by Western blotting using antibodies against calmodulin binding peptide (CBP) and Flag. A no-TAP tag strain was used as a negative control.

B The Rtt105 E160A and E171AL172A (EL) mutations attenuate the RPA–Rtt105 interaction. Left panel: purified recombinant GST-tagged Rtt105 WT and mutant proteins analyzed by SDS–PAGE with CBB staining. Asterisk (*) indicates non-specific band; right panel: GST pull-down assay was performed to determine the interaction of RPA with Rtt105 mutants. The bead-bound proteins were resolved by SDS–PAGE gels and subjected to silver staining.

Figure EV2. Rtt105 is important for the association of RPA with replication forks.

A An outline for ChIP assays for Rfa1 and Rfa2 subunits. G1 yeast cells were released into fresh YPD medium containing 0.2 M HU to arrest cells at early S phase. Equal amounts of cells were collected prior to G1 (0 min) or at different time points following release. Rfa1 and Rfa2 ChIP was performed using IgG beads for TAP-tagged strains, or anti-Rfa2 antibody.

B, C RPA binding was dramatically decreased at HU-stalled replication forks upon deletion of *RTT105*. ChIP DNA and input DNA were analyzed by quantitative real-time PCR using primers against the replication origins *ARS607* and *ARS305* and their corresponding distal regions (*ARS607* + 14 kb and *ARS305* + 12 kb), and the percentage of ChIP DNA over the input DNA was calculated. The mean and standard error (SE) of at least two biological replicates are shown, with *P*-values derived from two-way analysis of variance (ANOVA; $^{**}0.001 \leq P\text{-value} < 0.01$, $^{***}P\text{-value} \leq 0.001$).

D The C-terminus of Rtt105 is important for RPA binding at replication forks. pRS313-based plasmids expressing Flag-tagged WT Rtt105 and mutants (Δ4 and EL) were transformed into the *rtt105*Δ strain. ChIP was performed using Rfa2 antibodies. Vc: pRS313 vector; WT: pRS313-Rtt105-Flag; Δ4: pRS313-Rtt105 (Δ155–208)-Flag; EL: pRS313-Rtt105E171AL172A-Flag. The mean and standard error (SE) of two biological replicates are shown. Statistical significance was evaluated based on Student's *t*-tests ($^{**}0.001 \leq P\text{-value} < 0.01$, $^{***}0.0001 \leq P\text{-value} < 0.001$; $^{****}P\text{-value} < 0.0001$).

E Snapshot of Rfa1 ChIP-seq at chromosome III under HU synchronized cells is shown. Yeast cells were synchronized at G1 phase and then released into fresh medium containing 0.2 M HU and 400 μg/ml BrdU for 45 min at 25°C. Equal amounts of cells were collected just prior to G1 (0 min) or at 45 min following release into early S phase in the presence of HU. Rfa1 ChIP DNA was processed for sequencing. The sequencing reads were mapped to the yeast reference genome.

F Snapshot of Rfa2 ChIP-seq at chromosome III at S phase without HU treatment is shown. G1-arrested yeast cells were released into S phase at low temperature (16°C) without HU. Yeast cells were collected for Rfa2 ChIP and were further processed for sequencing. The sequencing reads were mapped to the yeast reference genome.

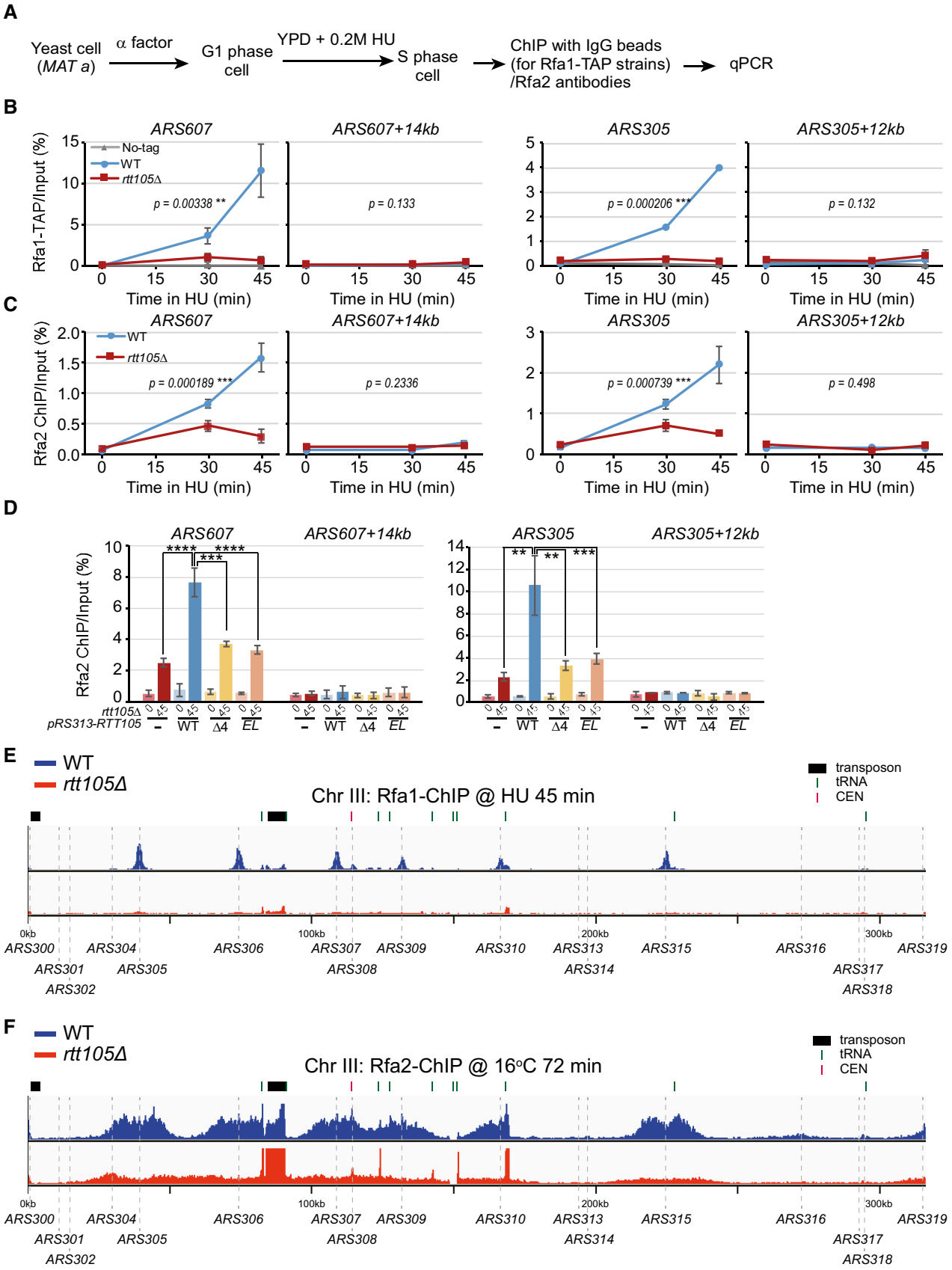

Figure EV2.

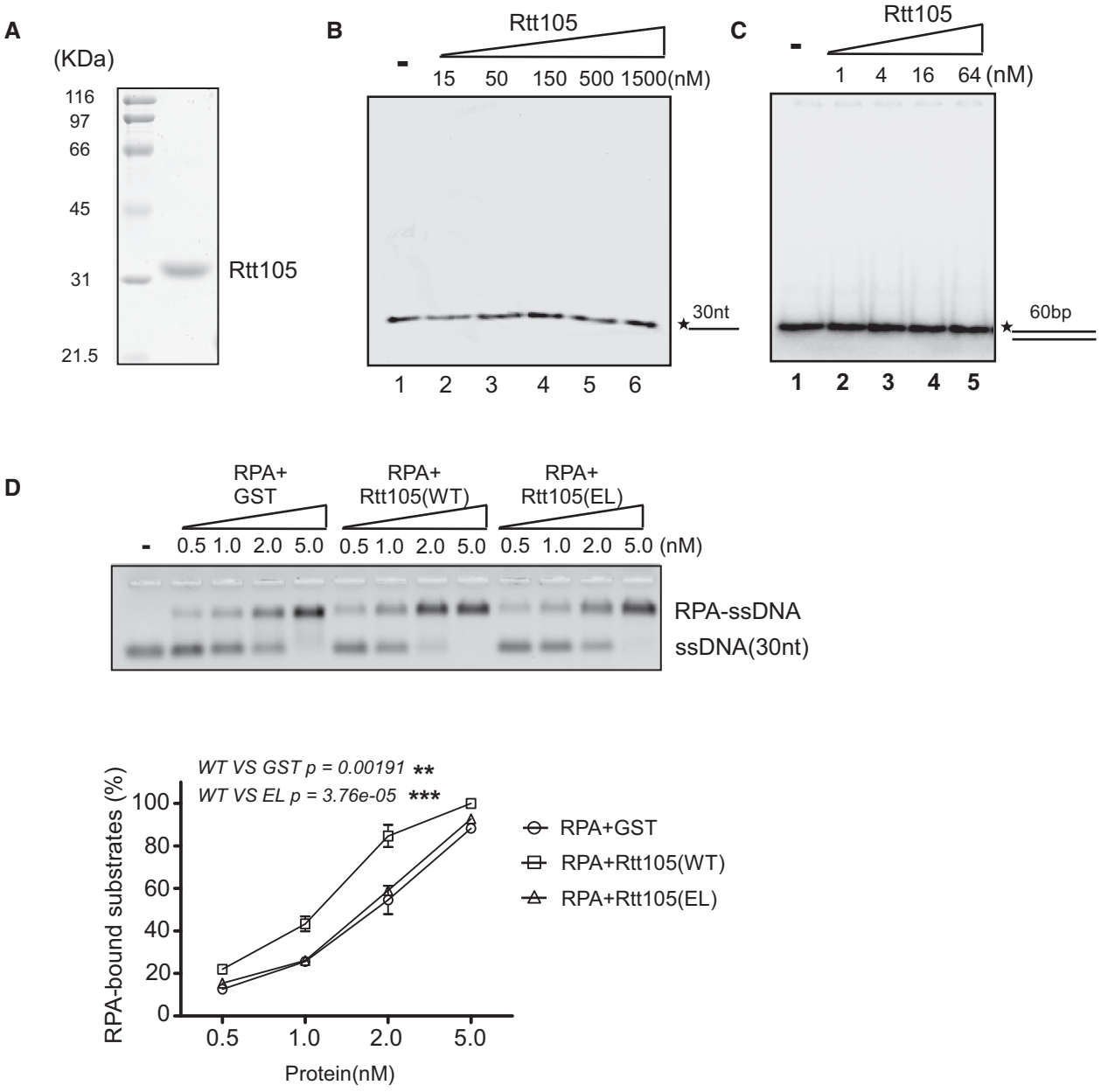

Figure EV3. Rtt105 does not possess DNA binding ability, but promotes RPA binding to ssDNA.

A Recombinant His-tagged Rtt105 used in EMSAs. Purified protein was resolved on 10% SDS–PAGE and stained with CBB.

B Rtt105 does not bind ssDNA. 30-nt ssDNA was 5′-labeled with [γ-^{32}P] ATP. Rtt105 and DNA were incubated at room temperature (RT) for 20 min. The reaction products were resolved on 4% native PAGE on ice. The asterisk denotes the γ-^{32}P label.

C Rtt105 does not bind dsDNA. A 60-bp dsDNA substrate was 5′-labeled with [γ-^{32}P] ATP. Rtt105 and DNA were incubated at room temperature (RT), and the reaction products were resolved on 4% native PAGE on ice. The asterisk denotes the γ-^{32}P label.

D The Rtt105-EL mutant protein is deficient in promoting RPA–ssDNA binding. Recombinant GST-tagged WT and mutant were used in EMSAs. After incubation with RPA on ice for more than 2 h in titrations, Cy3-labeled 30-nt ssDNA was added as the substrate. The reaction mixtures were resolved on 2% native agarose gels, and the Cy3 signals were detected. At least three independent experiments were performed. The mean values ± SD from three independent experiments are shown. *P*-values were derived from two-way analysis of variance (ANOVA; **$0.001 \leq P$-value < 0.01, ***P-value ≤ 0.001).

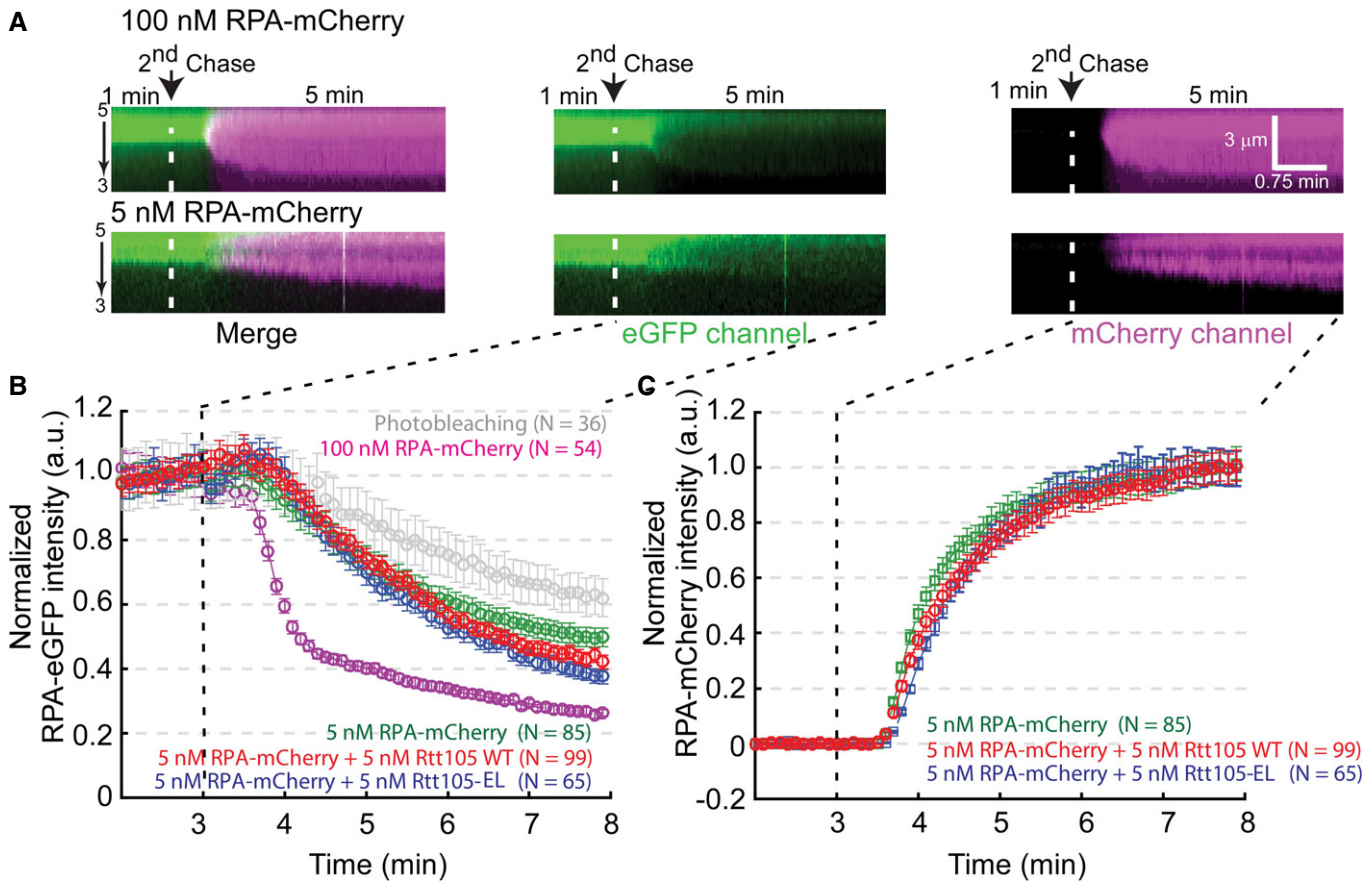

Figure EV4. RPA-eGFP can be rapidly replaced on ssDNA by RPA-mCherry.

A The exchange of RPA-eGFP with free RPA-mCherry is dependent on the concentration of RPA-mCherry. Each kymogram shows a single RPA–ssDNA complex at an indicated experimental condition. Each kymogram with two channels was also split into two single-color kymograms, in which the green color represents eGFP signal and the red color represents mCherry signal.

B Rtt105 does not significantly affect the disassociation rate of RPA-eGFP from ssDNA, as indicated by a 5 nM RPA-mCherry chase. Quantitation of the green color signal intensity versus time for each experimental condition of the second chase. Each curve represents the normalized average calculated from the indicated numbers of ssDNA molecules. Error bars represent the standard deviation for each data set.

C Rtt105 increased the RPA-mCherry binding to ssDNA. Quantitation of the mCherry signal intensity versus time for each indicated the second chase condition. Each curve represents the normalized average calculated from the indicated numbers of ssDNA molecules. Error bars represent the standard deviation for each data set.

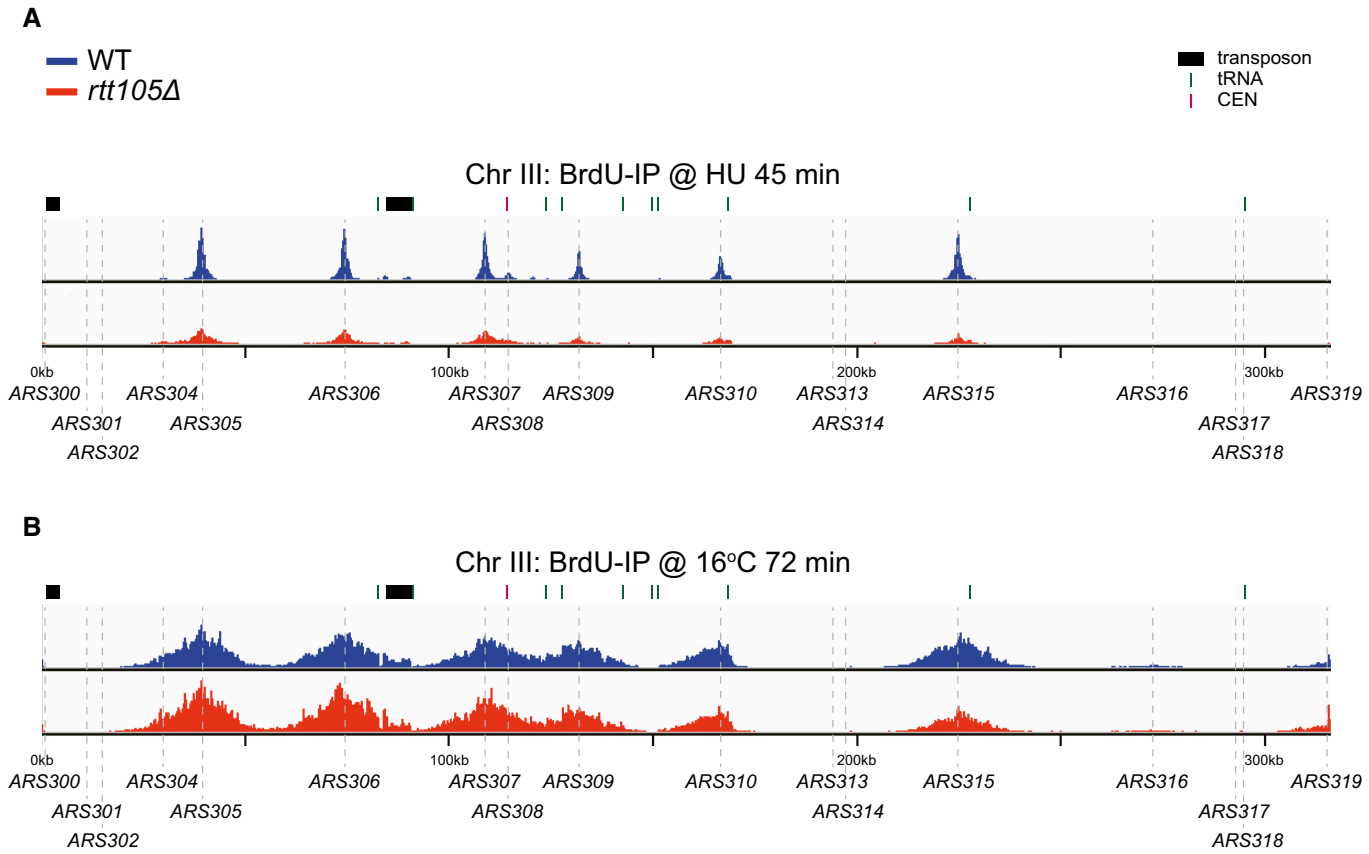

Figure EV5. Snapshots of BrdU IP-seq of chromosome III.

A Snapshot of BrdU IP-seq at chromosome III for cells under HU treatment is shown.

B Snapshot of BrdU IP-seq at chromosome III for cells at S phase without HU treatment (16°C, 72 min) is shown. The sequencing reads were mapped to the yeast reference genome.