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Supplemental Information

Acute stress drives global repression through two independent RNA polymerase II stalling events in *Saccharomyces*

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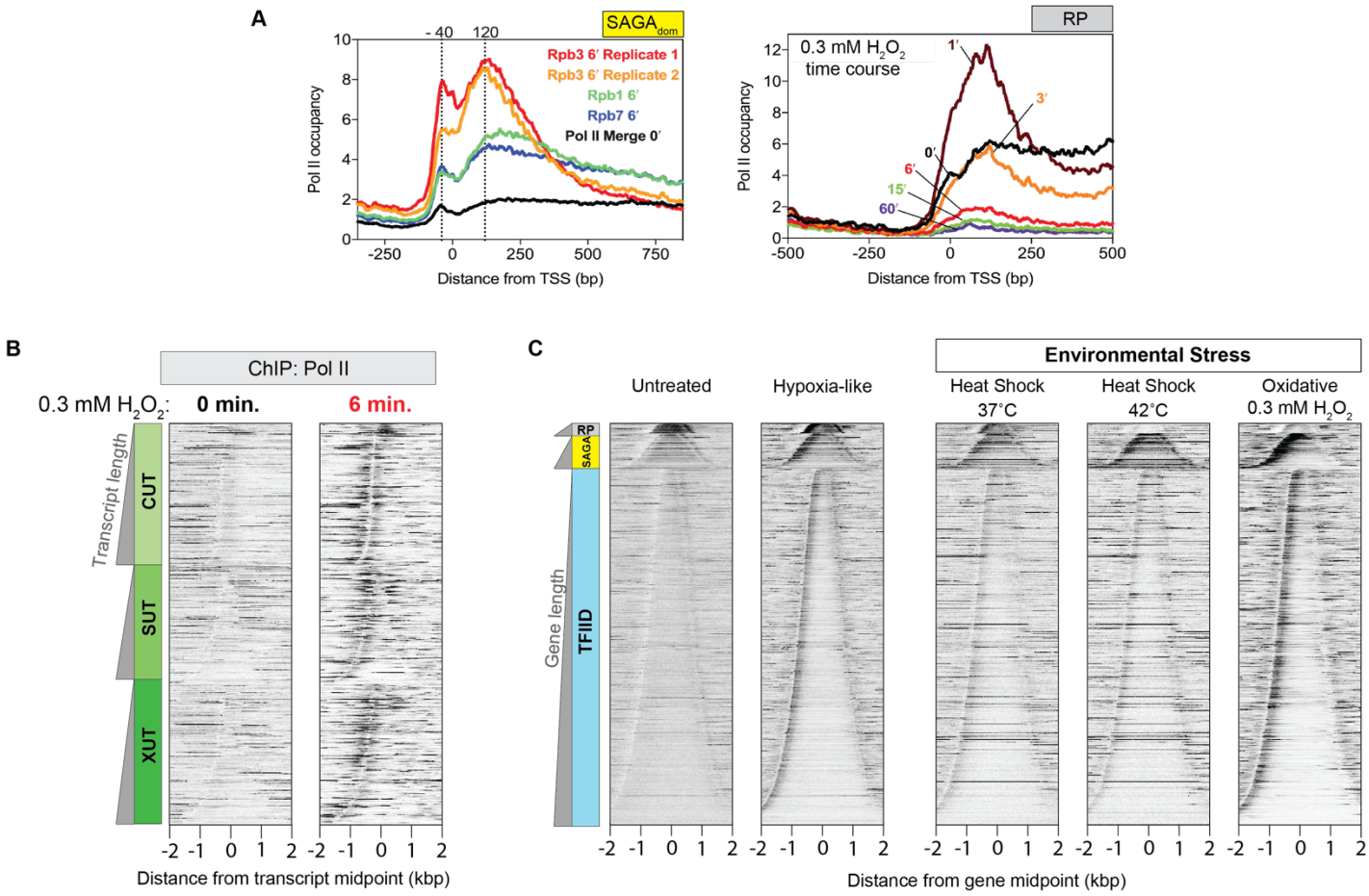

Figure S1. Pol II enrichment at SAGA$_{dom}$, RP genes and non-coding genes, Related to Figure 1. (A) Left, ChIP-exo pattern of multiple Pol II subunits are shown. Right, A time course of Pol II (Rbp3) ChIP-exo response to 0.3 mM hydrogen peroxide. (B) 5' ends of Rpb3 ChIP-exo tags are plotted relative to each transcription unit midpoint. Rows are sorted by transcript unit length and grouped by class: Cryptic Unstable transcripts (CUTs), Stable Unannotated Transcripts (SUTs), Xrn1-sensitive Unstable Transcripts (XUTs). All rows across datasets are linked. (C) Distribution of Pol II under a variety of stress conditions. "Untreated" reflects normal log-phase growth. For creating hypoxia-like conditions, non-crosslinked cells were harvested at 4 ºC for 5 minutes and crosslinked with 1% v/v final concentration of formaldehyde for 15 minutes in 1 ml of ST buffer (100 mM NaCl and 10 mM Tris, pH 7.5) before quenching with 125 mM of glycine. Heat shock at 37˚C was from (Vinayachandran et al., 2018) and was followed when applying a 42˚C heat shock here. Data are plotted as in Figure 1B. A single replicate was performed for heat sock at 42 ºC since the observed ChIP-exo pattern was similar to 37 ºC heat shock datasets. All datasets are normalized using total tag count.

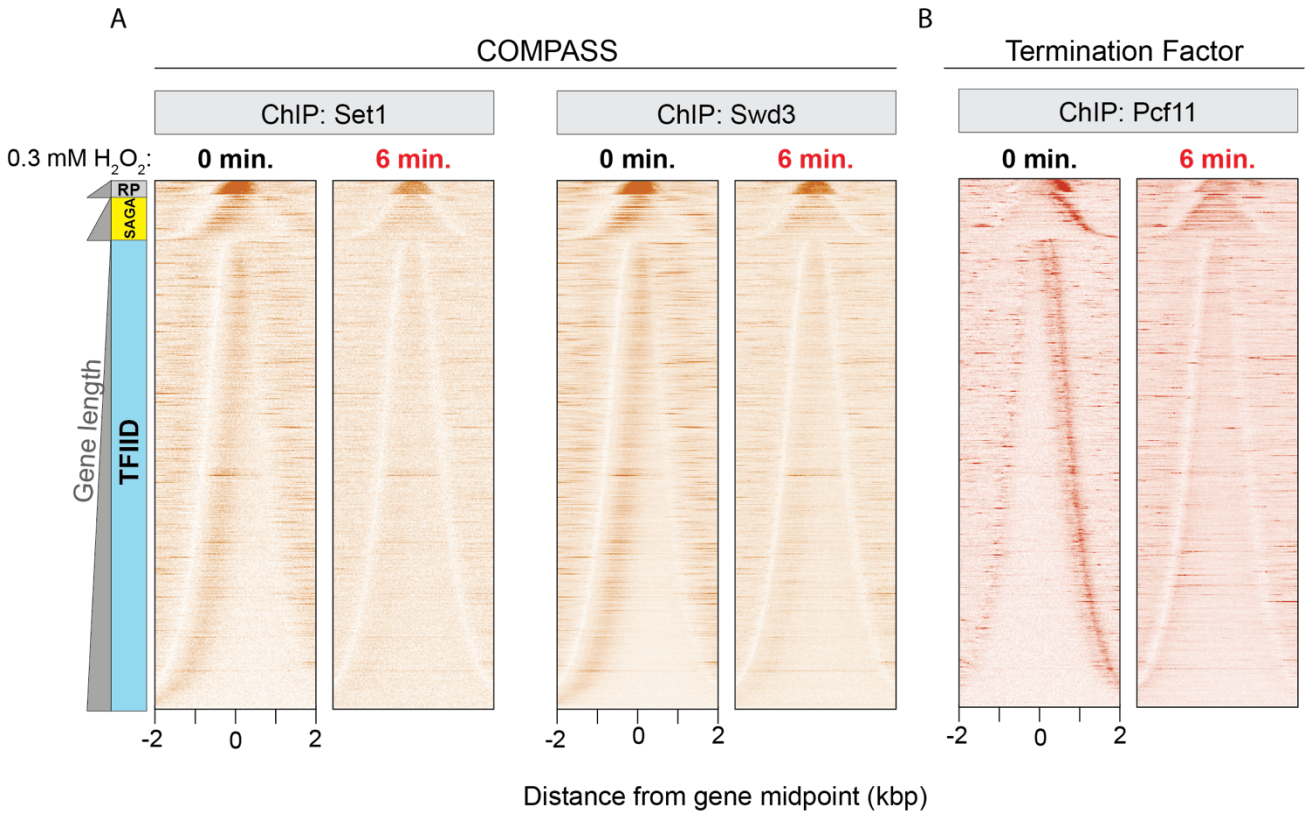

Figure S2. Distribution of COMPASS (Set1, Swd3) and termination factor Pcf11 at genes, Related to Figure 6. Data are plotted as in Figure 1B. Datasets are normalized using total tag count.

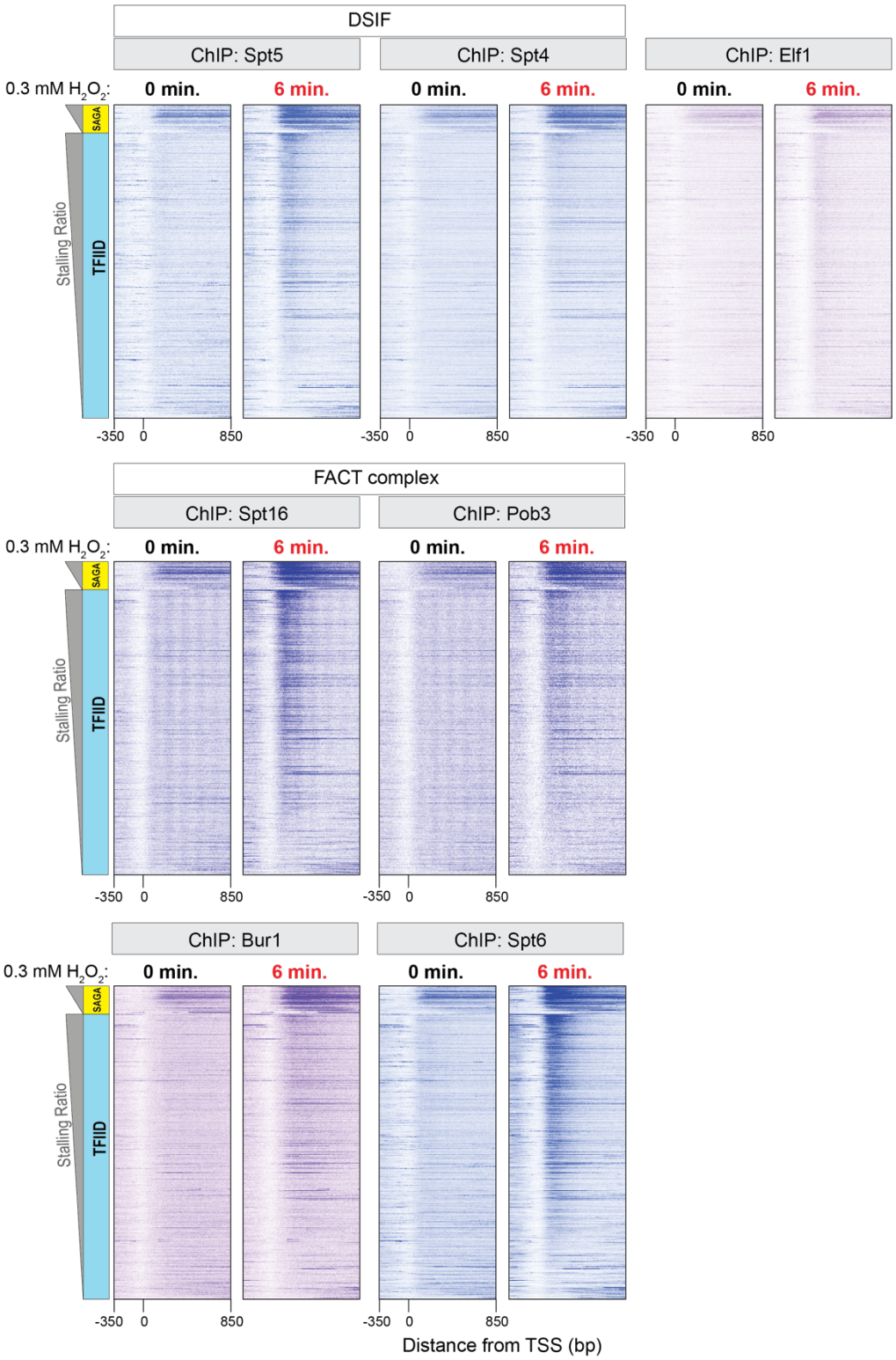

Figure S3. Distribution of elongation factors around stalled Pol II, Related to Figure 6. 5' ends of ChIP-exo tags are plotted. Rows are sorted by high to low stalling ratio (SR) and grouped by class: $SAGA_{dom}$ (SAGA) and $TFIID_{dom}$ (TFIID) genes. All rows across datasets are linked. Datasets are normalized using total tag count.

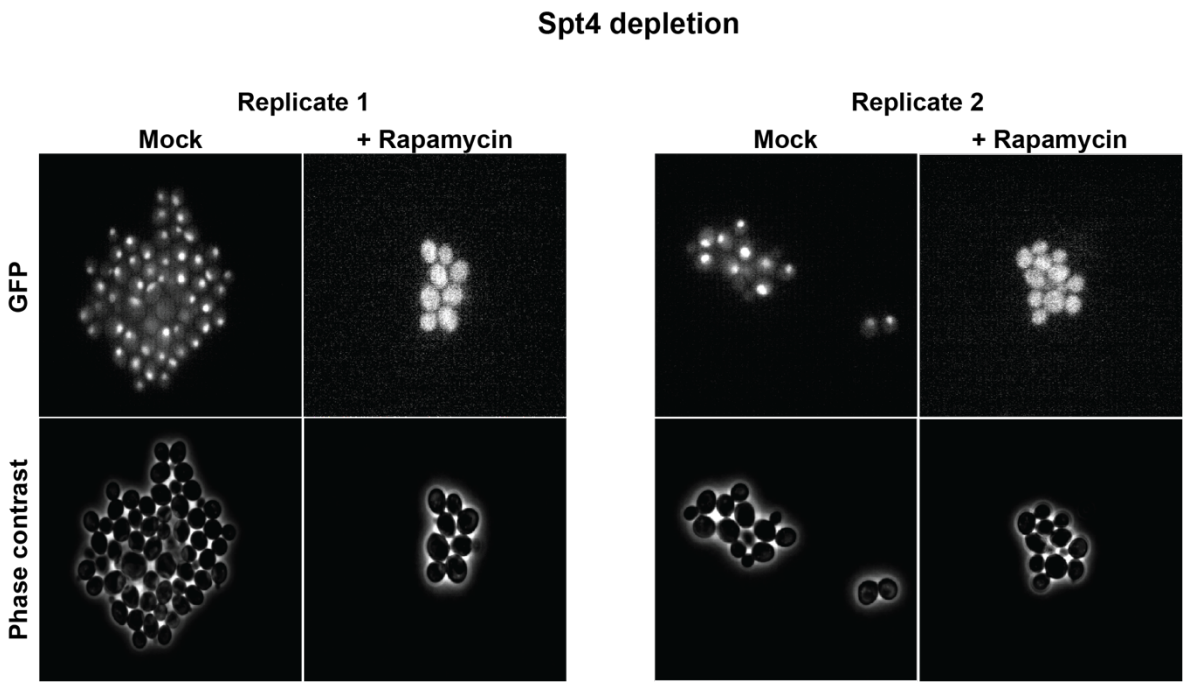

Figure S4. Nuclear depletion of Spt4 in an Spt4 depletion strain, Related to Figure 7. A culture of Spt4 depletion strain expressing Spt4-FRB-GFP was grown to log phase then treated with rapamycin for 30 minutes Fluorescence images show the nuclear depletion of Spt4 (change from bright focal spots to diffuse spots) by addition of rapamycin for 30 minutes. The bottom row shows the phase contrast signal.

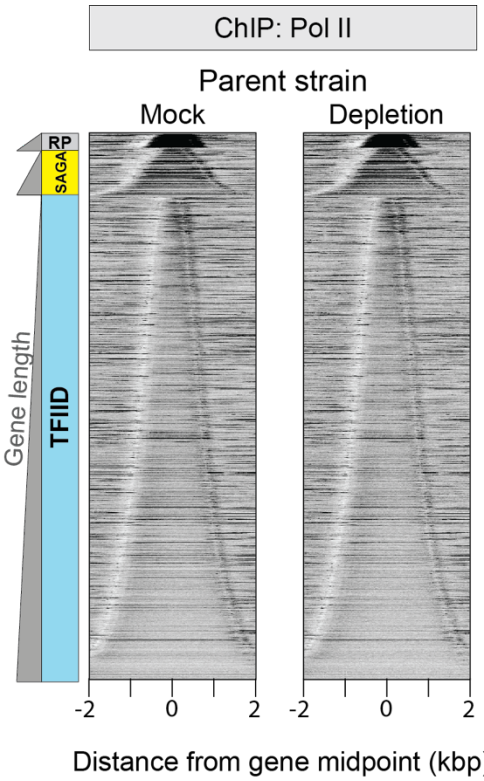

Figure S5. Distribution of Pol II in the parent strain of the dual tag depletion system before and after addition of rapamycin and indole-3-acetic acid, Related to Figure 7. Data were plotted as in Figure 1B. A 30 minute depletion was carried out with rapamycin and indoleacetic acid. Datasets are normalized using total tag count.

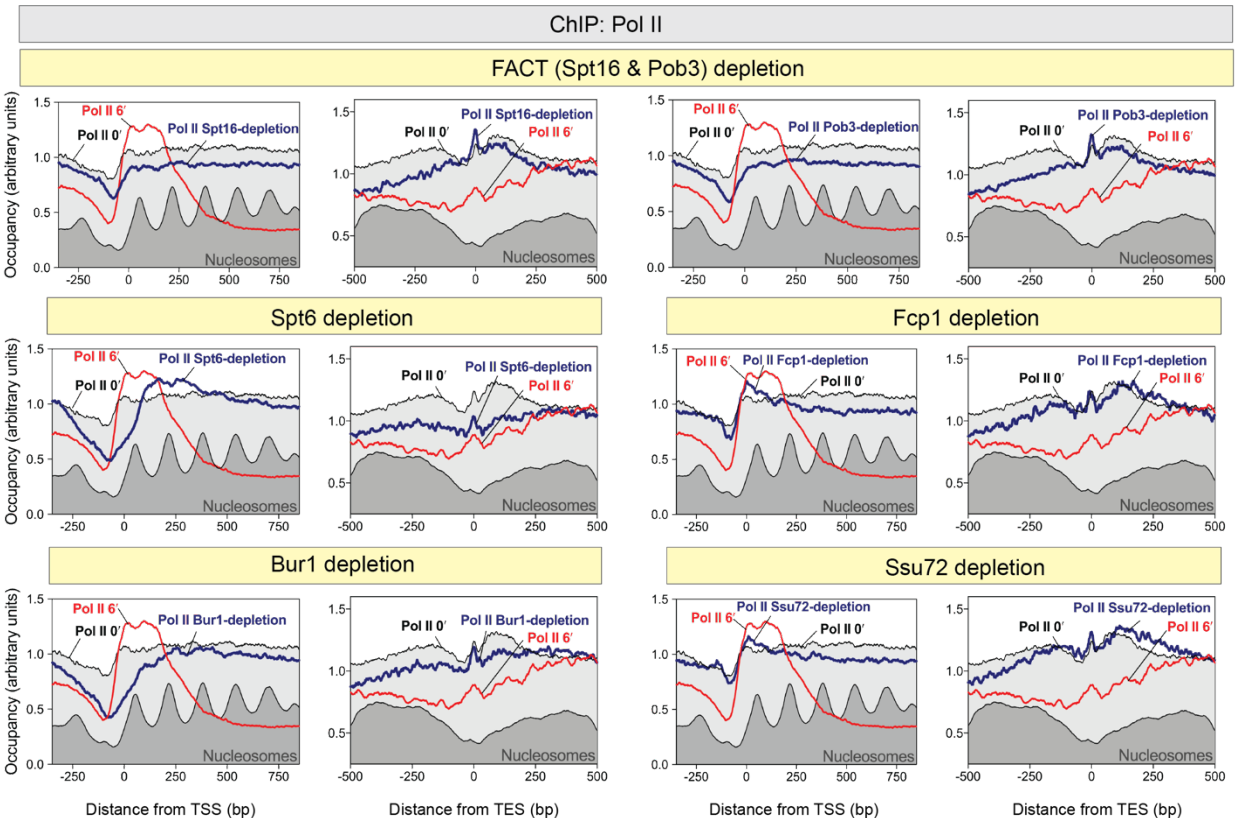

Figure S6. Anchor Away of elongation factors and CTD phosphatases, Related to Figure 7. Composite plots for Pol II ChIP-exo are shown for $TFIID_{dom}$ genes before and after depletion of the specified factor. Each pair of panels has a TSS (left) and TES (right) alignment. Nucleosome midpoints are shown in filled gray plots. A single replicate of each anchor-away depletion was performed. Light gray-filled traces reflect Pol II in wild type (undepleted) cells in unstressed conditions (absence of stalling), where red traces reflect Pol II in wild type cells that are subject to 6 minutes of 0.3 mM H_2O_2 stress (stalled state).

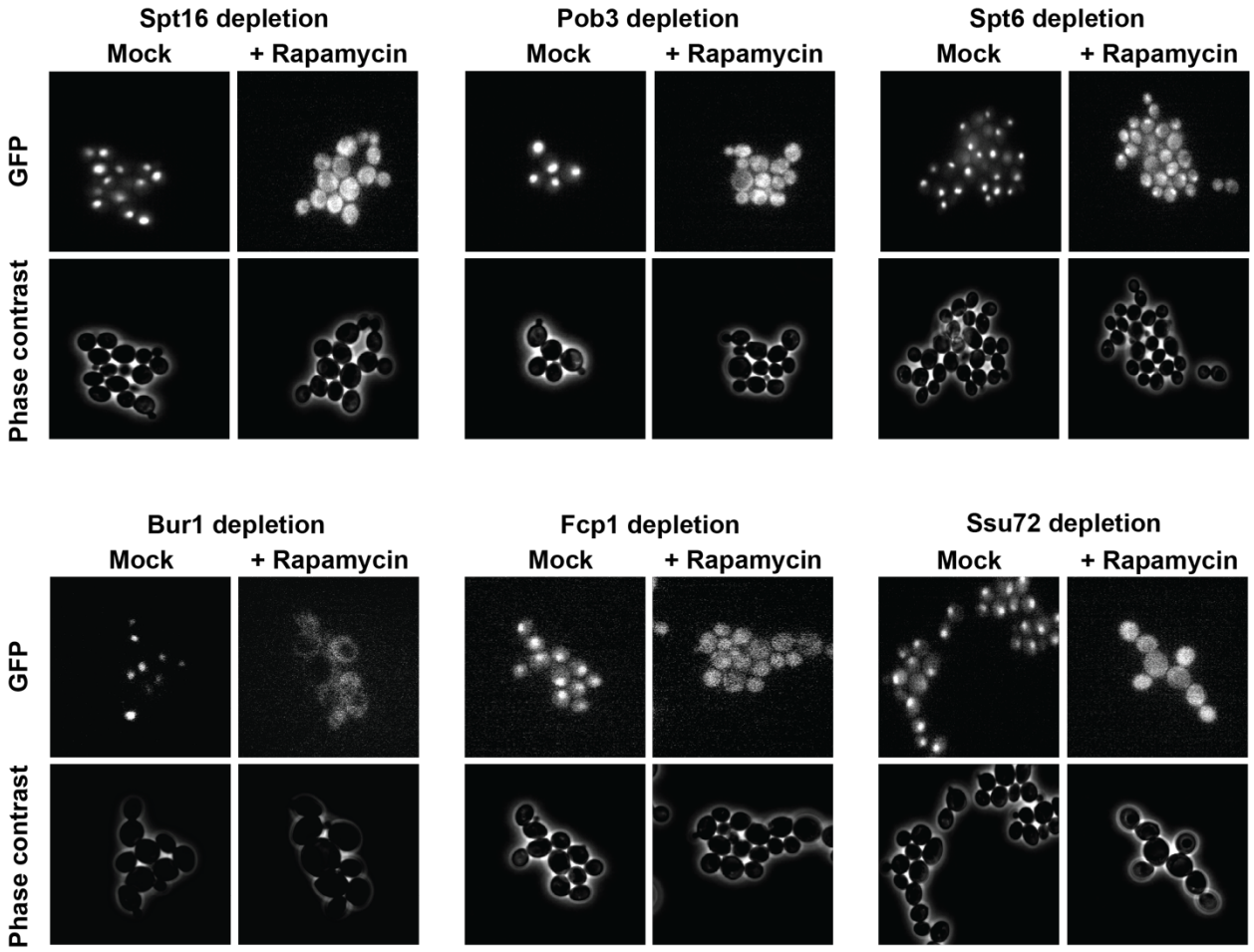

Figure S7. Anchor Away of elongation factors and CTD phosphatases, Related to Figure 7. A culture of the indicated depletion strains expressing Factor-FRB-GFP was grown to log phase then treated with rapamycin for 30 minutes before fluorescence imaging. The bottom row shows the phase contrast signal.