**Materials and Methods**

**Antibodies.** Rabbit monoclonal antibody against ubiquityl-Histone H2B (Lys120)(Cell Signaling, 5546), and rabbit polyclonal to histone H2B (Abcam, ab1790) (Van Oss et al., 2017) were used to probe for the specific ubiquitin mark and the underlying nucleosome, respectively. For TAP tagged strains, rabbit IgG (Sigma) conjugated to Dynabeads was used. Protein A module of the TAP tag was the target.

**Chromatin preparation.** Indicated mutant strains were grown in selective CSM-his media, while the WT strains were grown in CSM+all media to an O.D600 of 0.8 at 25°C. Cells were crosslinked with formaldehyde for 15 min at room temperature at a final concentration of 1%, and quenched with glycine for 5 min at a final concentration of 125 mM. Cells were then centrifuged and washed with ST buffer (10 mM Tris-HCl, pH 7.5; 100 mM NaCl) at 4°C. Supernatant was removed and cell pellets were flash frozen and stored at -80°C until further use.

50 ml culture aliquots were resuspended and lysed in 750 μl FA Lysis Buffer (50 mM Hepes-KOH, pH 7.5; 150 mM NaCl; 2 mM EDTA; 1% Triton X-100; 0.1% sodium deoxycholate and complete protease inhibitor (Roche) along with 1 ml of 0.5 mm zirconia beads by bead beating for 4 cycles of 3 min on/7 min off in a Mini-Beadbeater-96 machine (Biospec). Whole cell lysates were transferred to microcentrifuge tubes and spun at 14,000 rpm for 3 min at 4°C to obtain chromatin pellets. Supernatant (representing cytoplasmic fraction) was discarded and the chromatin pellet was resuspended in 200 μl FA Lysis Buffer supplemented with 0.1% SDS and 75 μl 0.1 mm zirconia beads. Resuspended chromatin sample was sonicated in a Bioruptor (Diagenode) for 4 cycles of 30s on/30 s off. The tubes were centrifuged at 14,000 rpm for 10 minutes to collect the sonicated chromatin fraction.

**ChIP-exo 5.0.** Chromatin obtained from 50 ml culture was used for a single ChIP experiment. Reb1-TAP tagged strain (Open Biosystem) was used a positive control to determine the success of the ChIP experiments. Appropriate antibodies (5 μg per ChIP) were conjugated to protein A Magnetic Sepharose resin (GE Healthcare Life Sciences) at 4°C for 6-8 hours. Unconjugated antibody was removed and chromatin sample was added and incubated at 4°C overnight to allow for immunoprecipitation. All steps after immunoprecipitation were essentially performed as described previously (Rossi, M. J., Lai, W. K. M. and Pugh, B. F., Nature Communications **9,** 2842 (2018)). Prepared libraries were gel purified and were sequenced in paired-end mode with a NextSeq 500. Sequence reads were aligned to the Saccharomyces cerevisiae genome (sacCer3) using bwa-mem (v0.7.9a) (Li, H., arXiv:1303.3997v1[q-bio.GN], **1303** (2013)). Aligned reads obtained were filtered to remove PCR duplicates and any non-unique alignments. ChIP-exo Read-1 5’ ends had their coordinates shifted by 6 bp in the 3’ direction, to reflect to offset of exonuclease stops and the site of crosslinking. At least two biological replicates were performed for ChIP-exo experiments, unless otherwise indicated.

**Data availability.** Sequencing files generated from this study are available at NCBI Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo/>) under the accession number GSE131639. Custom codes, script parameters and coordinate files can be downloaded from: <https://github.com/CEGRcode/Gallego_2019>.