Figure S1

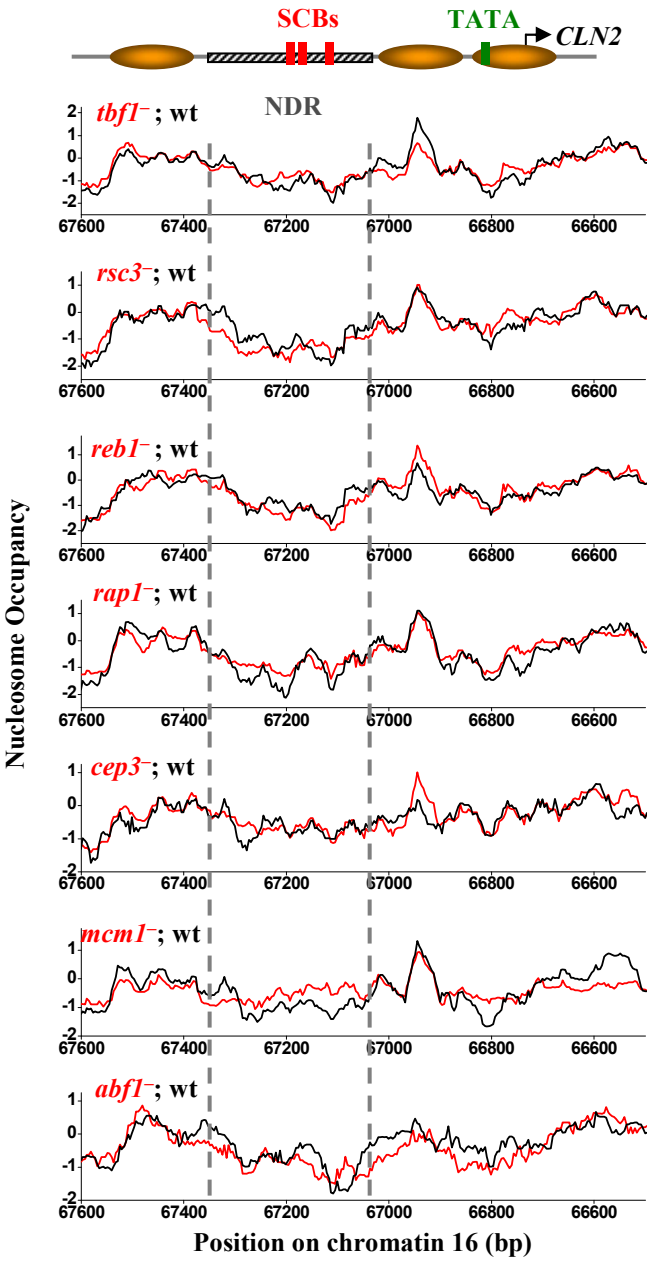

Figure S1. Nucleosome occupancy data on *CLN2pr* from Badis et al. (2008) in various temperature-sensitive strains. The two curves in each plot represents the measurements conducted before (black) or after (reb) the switch to non-permissive temperature. The NDR is in between the two dashed lines. Mcm1 deletion moderately increase the nucleosome density in the NDR; none of the other deletions has significant effect on the NDR.

Figure S2

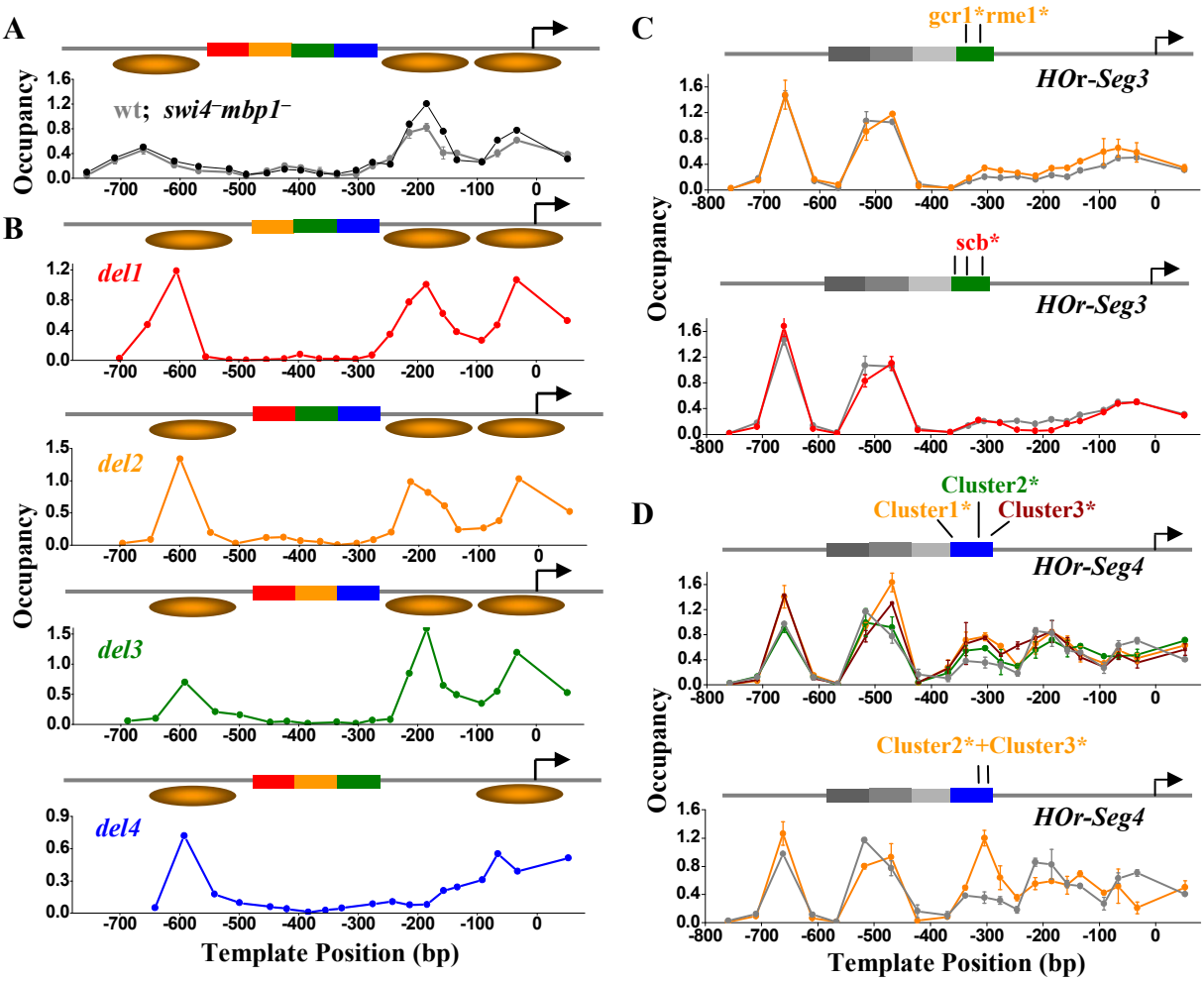

Figure S2, related to Figure 3; Multiple factors other than SBF/MBF contribute to *CLN2pr* NDR formation. **A)** Measured nucleosome occupancy and inferred nucleosome positioning on wt *CLN2pr* in the wt (gray) or *swi4⁻mbp1⁻* strain (black). **B)** Measured nucleosome occupancy and inferred nucleosome positioning on *CLN2pr*-del1-4 templates. *CLN2pr*-del1-4 are constructed by dividing the *CLN2pr* NDR into four segments (see the diagram in A), and delete one at a time. **C)** Nucleosome occupancy on *HOr-Seg3* with mutations in both Gcr1 and Rme1 binding sites (orange curve in the upper panel), or three SCBs (red curve in the lower panel), in comparison with the wt *HOr-Seg3* (gray curves). **D)** Nucleosome occupancy on *HOr-Seg4* with mutations in Cluster 1-3 (one cluster at a time; upper panel colored curves), or mutations in both Cluster2 and 3 (orange curve in the lower panel). Wt *HOr-Seg4* nucleosome occupancy is shown in gray curves.

Figure S3

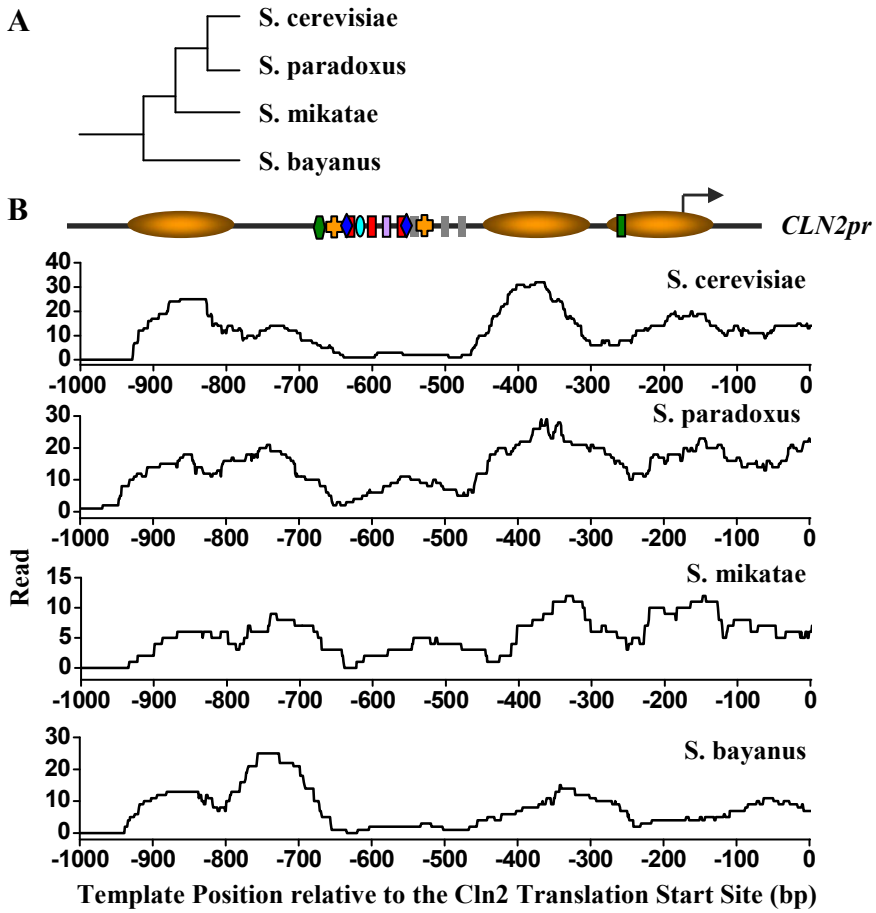

Figure S3. Related to Figure 4; The nucleosome occupancy on *CLN2pr* in close yeast species. **A)**.The four yeast species we compared, and the corresponding phylogenetic tree. **B)** The nucleosome occupancy on *CLN2pr* in the four yeast species (data from Tsankov et al.).

Figure S4

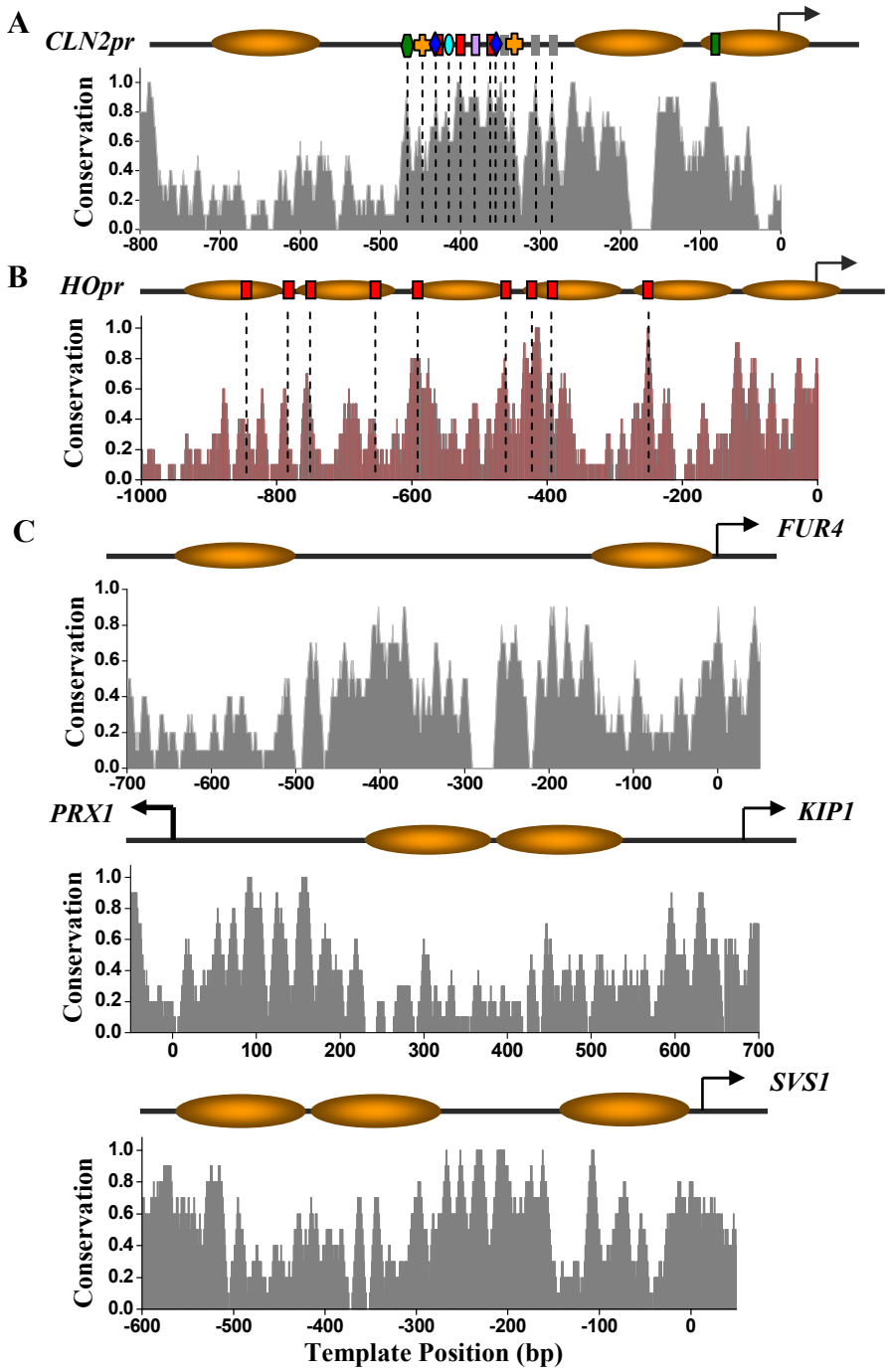

Figure S4. Related to Figure 6; The conservation of several promoter sequences. **A)** *CLN2pr* **B)** *HOpr*. C) *FUR4pr*, *PRX1pr* / *KIP1pr* and *SVS1pr*. In A and B, the locations of the functional sites and the corresponding conservation peak are highlighted.

Figure S5

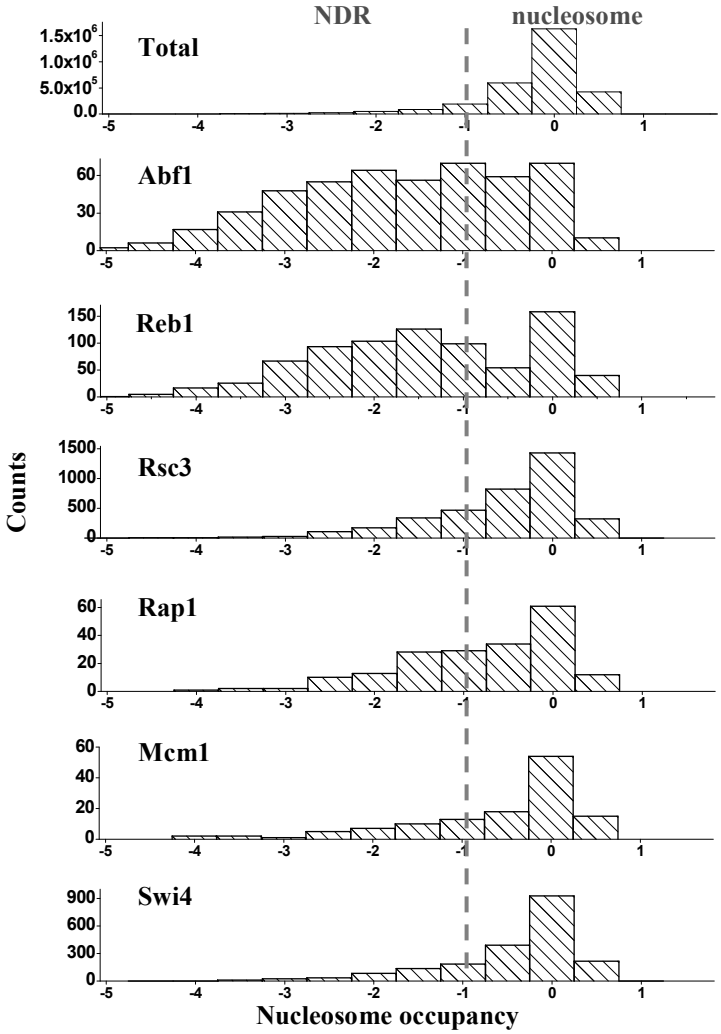

Figure S5. Related to Figure 7; The top panel is the histogram of genome-wide nucleosome occupancy in yeast (Lee et al., 2007), and the rest is the histogram of nucleosome occupancy at the Reb1, Abf1, Rsc3, Mcm1 and Swi4 consensus binding sites in the yeast genome. See "Methods" for detailed data analysis. Roughly, the regions with nucleosome occupancy <–1 can be grouped into NDR. Most of the Reb1 and Abf1 binding sites reside in NDR, whereas those of Rsc3, Rap1 and Mcm1 are only slightly biased towards NDR comparing with random distribution. Swi4 (DNA-binding subunit of SBF), which does not have significant effect on nucleosome depletion, also has a slightly biased distribution. This likely reflects co-localization of SBF binding sites with the NDF binding sites in the genome.

Table S1, related to Figure 3; Comparison of *CLN2pr* NDR sequence in *S. cerevisiae* with homologs from other close yeast species. As explained in the text, the sequence is divided into four segments, and the conserved bases in each segment are highlighted. The identified potential factor binding sites, their consensus, and the mutations we generated to disrupt the factor-binding are also shown.

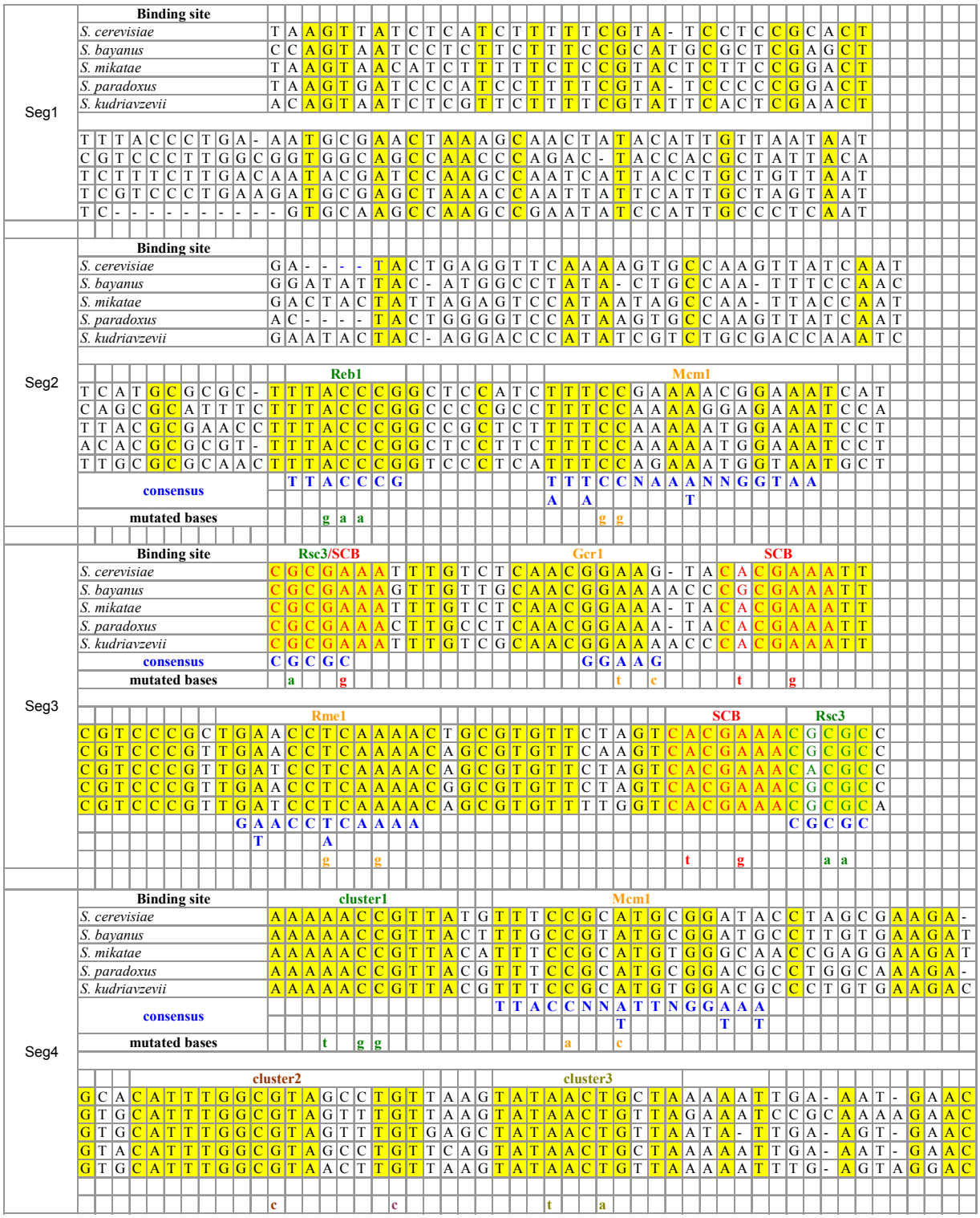

Table S2 Strain list

Name	**Genotype**
GC46-03	MATa, *MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB5	MATa, *CLN2:: wtCln2pr-GFP-Cln2pest-CaURA3, MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB6	MATa, *CLN2:: CLN2-del-GFP-Cln2pest-CaURA3,MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB21-3merNuc	MATa, *CLN2:: 3merNucpr-GFP-Cln2pest-CaURA3,MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
JB22-1A	MATα, *swi4::Leu2, mbp1::URA3, Gal-CLN2::TRP1, cdc20::Leu2, Gal-CDC20::ADE2*
yLB46-del1-4	MATa, *CLN2:: Cln2pr-del1-4: Cln2, my01:my01-mcherry-SpHis3, ADE2*
yLB56	MATa, *HO:: HO*, CLN2:: CLN2pr-ndr: Cln2*, *MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB57	MATa, *CLB2:: CLB2*, CLN2:: CLN2pr-ndr: Cln2*, *MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB58	MATa, *MYO1::MYO1-mCherry-SpHIS5*, *ADE2* + pCC67
yLB60	MATa, *MYO1::MYO1-mCherry-SpHIS5*, *ADE2, HOpr:KanMx*
yLB60-CLN2-HOr	MATa, *CLN2:: CLN2-HOr: Cln2, my01:my01-mcherry-SpHis3, ADE2*
yLB60-HOr-Seg1-4	MATa, *CLN2:: HOr-Seg1-4: Cln2, my01:my01-mcherry-SpHis3, ADE2*
yLB60-HOr-Seg2-reb1*	MATa, *CLN2:: HOr-Seg2-reb1*: Cln2, my01:my01-mcherry-SpHis3, ADE2*
yLB60-HOr-Seg2-mcm1*	MATa, *CLN2:: HOr-Seg2-mcm1*: Cln2, my01:my01-mcherry-SpHis3, ADE2*
yLB60-HOr-Seg3ds-rsc3*	MATa, *CLN2:: HOr-Seg3ds-rsc3*: Cln2, my01:my01-mcherry-SpHis3, ADE2*
yLB60-HOr-Seg3ds-gr*	MATa, *CLN2:: HOr-Seg3ds-gcr1rme1*: Cln2, my01:my01-mcherry-SpHis3, ADE2*
yLB60-HOr-Seg3ds-scb*	MATa, *CLN2:: HOr-Seg3ds-scb*: Cln2, my01:my01-mcherry-SpHis3, ADE2*
yLB60-HOr-Seg4-mcm1*	MATa, *CLN2:: HOr-Seg4-mcm1*: Cln2, my01:my01-mcherry-SpHis3, ADE2*
yLB60-HOr-Seg4-cluster1-3*	MATa, *CLN2:: HOr-Seg4-cluster1-3*: Cln2, my01:my01-mcherry-SpHis3, ADE2*
yLB76-all*	MATa, *CLN2:: Cln2pr-all*-GFP-Cln2pest-CaURA3, MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB76-all*-pt	MATa, *CLN2:: Cln2pr-all*-Cln2, MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB76-reb1*	MATa, *CLN2:: Cln2pr-reb1*-GFP-Cln2pest-CaURA3, MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB76-reb1*-pt	MATa, *CLN2:: Cln2pr-reb1*-Cln2, MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB76-mcm1*	MATa, *CLN2:: Cln2pr-mcm1*-GFP-Cln2pest-CaURA3, MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB76-mcm1*-pt	MATa, *CLN2:: Cln2pr-mcm1*-Cln2, MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB76-rsc3*	MATa, *CLN2:: Cln2pr-rsc3*-GFP-Cln2pest-CaURA3, MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB76-rsc3*-pt	MATa, *CLN2:: Cln2pr-rsc3*-Cln2, MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB76-part*	MATa, *CLN2:: Cln2pr-part*-GFP-Cln2pest-CaURA3, MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB76-part*-pt	MATa, *CLN2:: Cln2pr-part*-Cln2, MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB76-scb*	MATa, *CLN2:: Cln2pr-scb*-GFP-Cln2pest-CaURA3, MYO1::MYO1-mCherry-SpHIS5*, *ADE2*
yLB76-gr*	MATa, *CLN2:: Cln2pr-gr*-GFP-Cln2pest-CaURA3, MYO1::MYO1-mCherry-SpHIS5*, *ADE2*