Cell Reports, Volume *32*

Supplemental Information

INO80C Remodeler Maintains Genomic Stability by Preventing Promiscuous Transcription at Replication Origins

Salih Topal, Christopher Van, Yong Xue, Michael F. Carey, and Craig L. Peterson

Figure S1

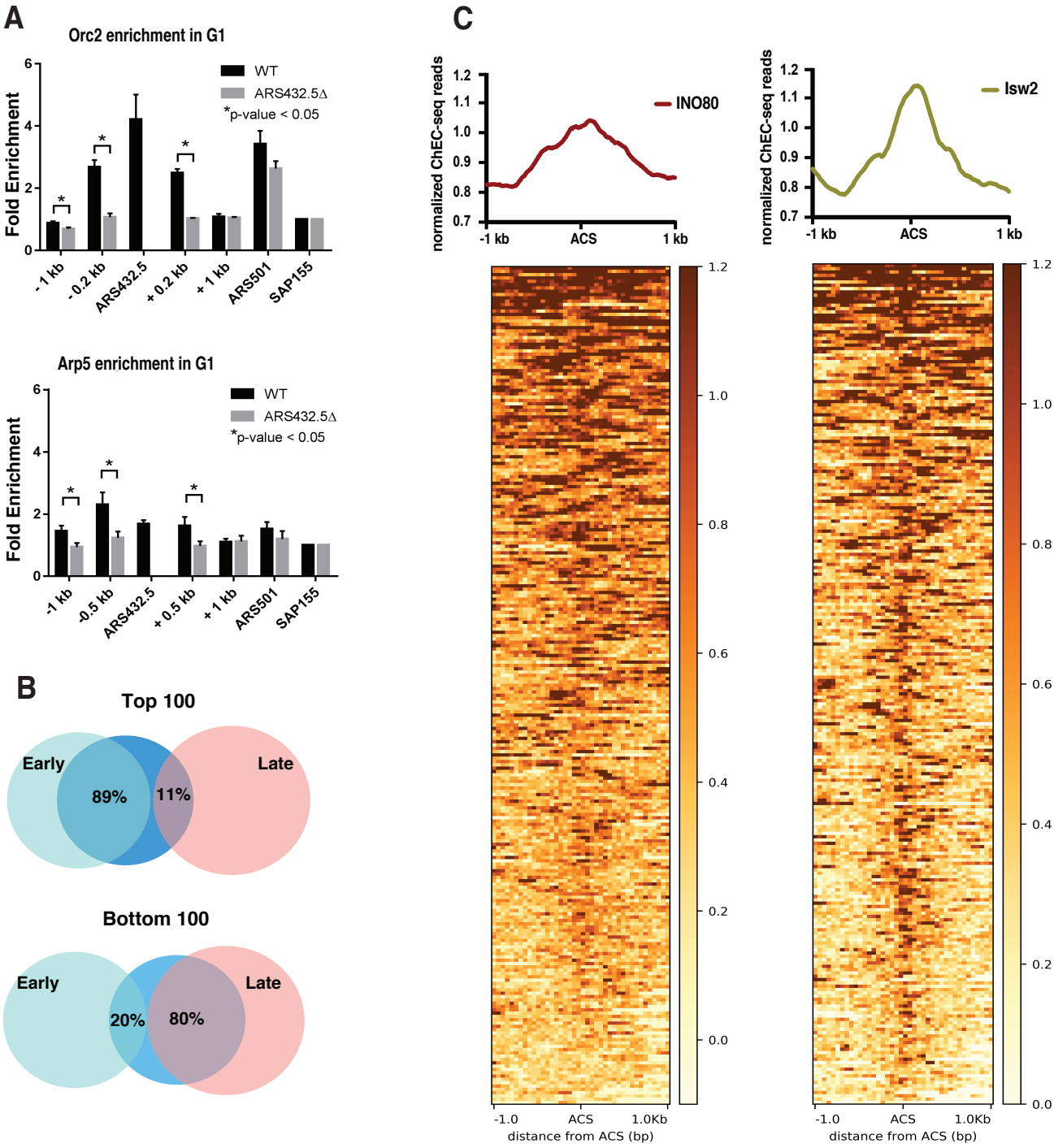
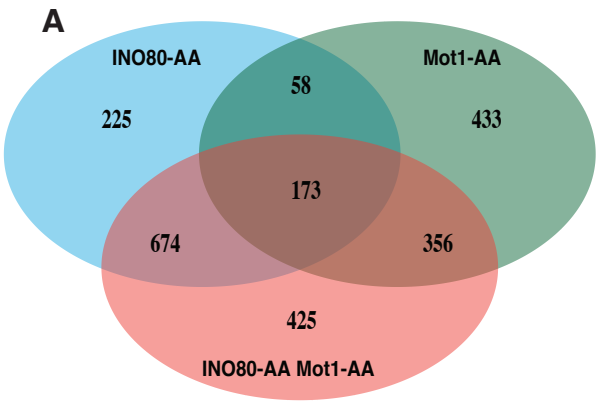
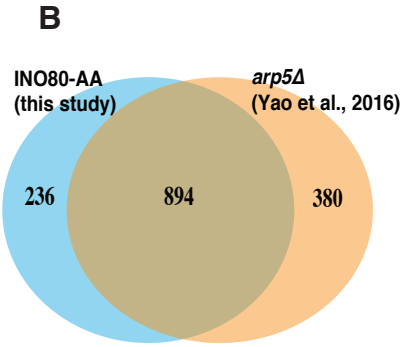
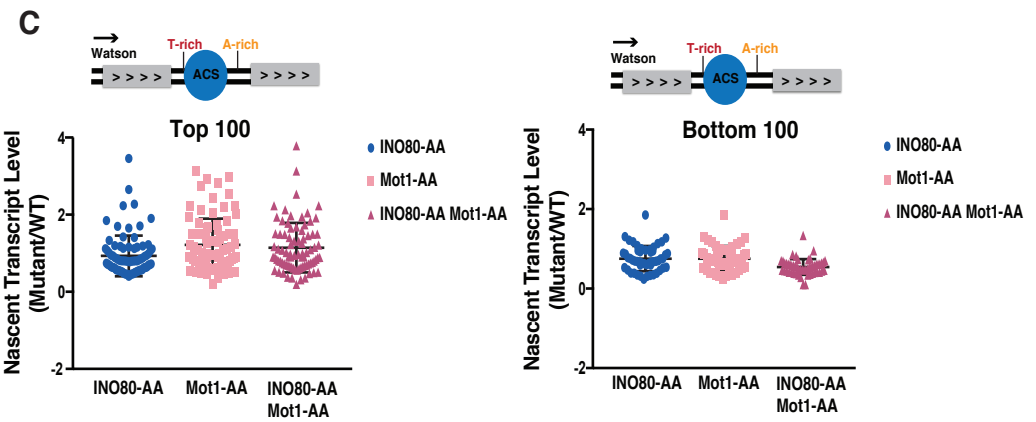

A
Orc2 enrichment in G1
WT
ARS432.5Δ
*p-value < 0.05
Fold Enrichment
- 1 kb
- 0.2 kb
ARS432.5
+ 0.2 kb
+ 1 kb
ARS501
SAP155
Arp5 enrichment in G1
WT
ARS432.5Δ
*p-value < 0.05
Fold Enrichment
-1 kb
-0.5 kb
ARS432.5
+ 0.5 kb
+ 1 kb
ARS501
SAP155
B
Top 100
Early
Late
89%
11%
Bottom 100
Early
Late
20%
80%
C
normalized ChEC-seq reads
INO80
-1 kb
ACS
1 kb
normalized ChEC-seq reads
Isw2
-1 kb
ACS
1 kb
-1.0
ACS
1.0Kb
distance from ACS (bp)
-1.0
ACS
1.0Kb
distance from ACS (bp)
D
INO80
ChEC-seq reads
(Kubik et al., 2019)
ARS1114
ARS1114.5
ARS1115
E
Top 100
ChIP-seq
(this study)
72%
ChEC-seq
(Kubik et al., 2019)
Bottom 100
ChIP-seq
(this study)
89%
ChEC-seq
(Kubik et al., 2019)

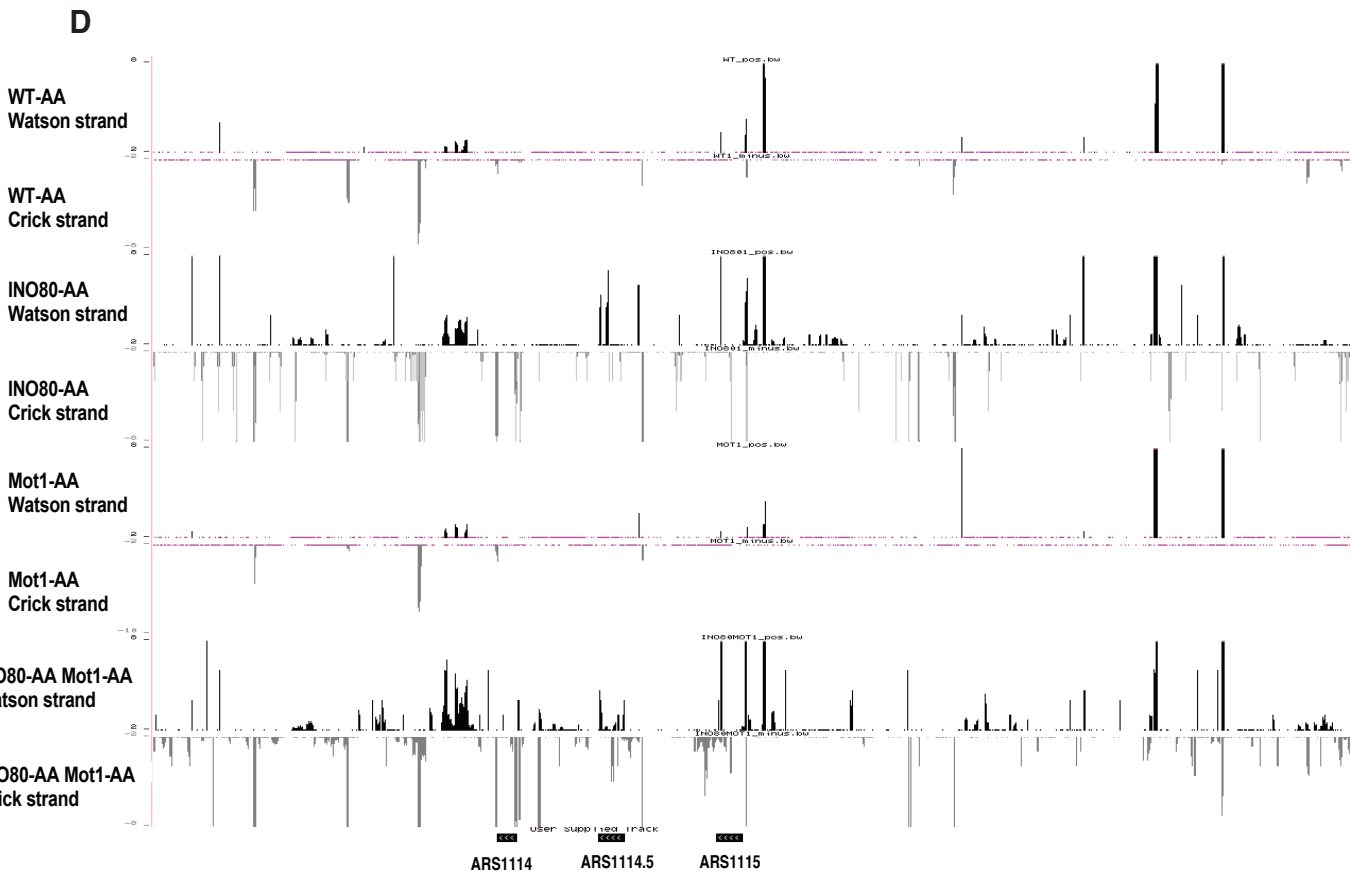
Figure S1, related to Figure 1. (A) Fold enrichment levels for Orc2 (upper panel) or Arp5 (lower panel) at SAP155 (a control gene), ARS501 (a late firing origin), ARS432.5 (an early firing origin), 0.2 kb and 1 kb upstream and downstream of ARS432.5 in wild type or ARS432.5Δ by ChIP-qPCR. **(B)** Venn diagrams showing correlations of early- or late-firing origins with Top 100 origins (upper panel) and with Bottom 100 origins (lower panel). **(C)** Heatmaps showing $\log_2$ mean intensity values for all yeast origins (n=253) for Ino80 (left) and Isw2 (right) by ChEC-seq (Kubik et al., 2019). The moving averages of $\log_2$ Ino80 and Isw2 enrichments were plotted by the distance from ACS (flanking a region of 1 kb upstream and downstream of ACS). Plots above the heatmaps show normalized ChEC-seq reads over ACS (flanking a region of 1 kb upstream and downstream of ACS) for Ino80 (left) and Isw2 (right) enrichments. **(D)** Representative genome browser view for normalized Ino80 enrichment signals by ChEC-seq for a highlighted region in yeast Chr XI for the replication origins ARS1114, ARS1114.5, and ARS1115. **(E)** Venn diagrams showing correlations between ChIP-seq (this study) and ChEC-seq (Kubik et al., 2019) for Top 100 (left panel) or Bottom 100 (right panel) for Ino80 enrichment.

Figure S2


A
INO80-AA
Mot1-AA
INO80-AA Mot1-AA
225
58
433
173
674
356
425


B
INO80-AA
(this study)
arp5Δ
(Yao et al., 2016)
236
894
380


C
Watson
T-rich
A-rich
ACS
Top 100
Bottom 100
Nascent Transcript Level
(Mutant/WT)
INO80-AA
Mot1-AA
INO80-AA Mot1-AA


D
WT-AA
Watson strand
WT-AA
Crick strand
INO80-AA
Watson strand
INO80-AA
Crick strand
Mot1-AA
Watson strand
Mot1-AA
Crick strand
INO80-AA Mot1-AA
Watson strand
INO80-AA Mot1-AA
Crick strand
ARS1114
ARS1114.5
ARS1115

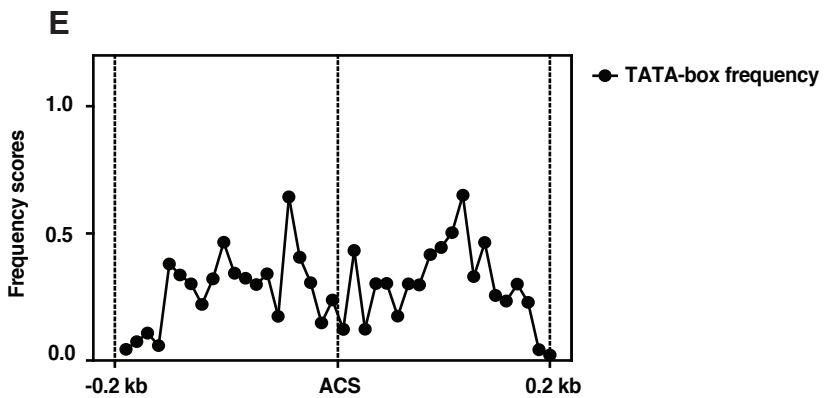
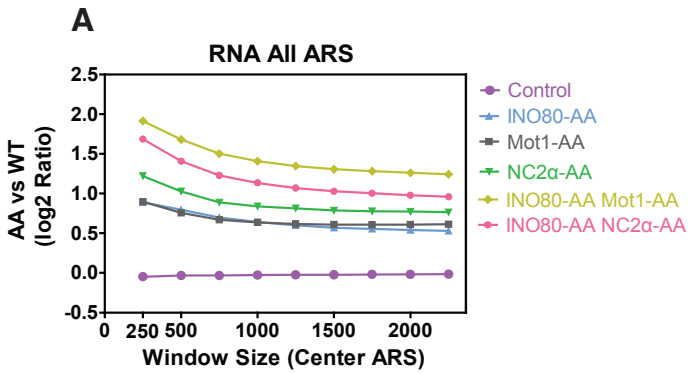
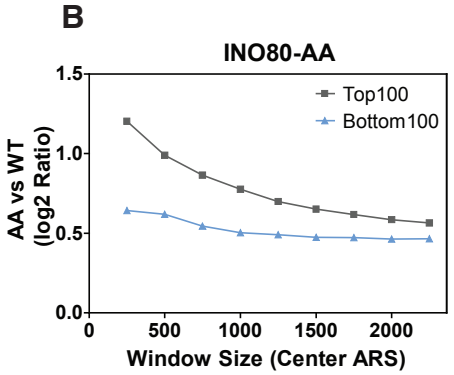
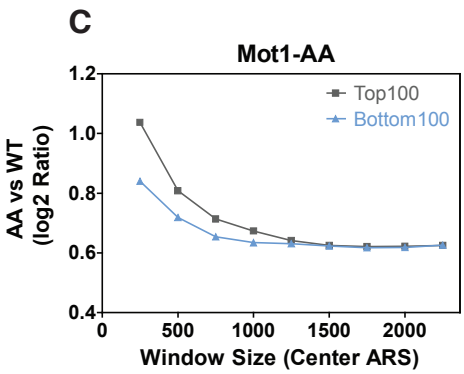

E
TATA-box frequency
Frequency scores
1.0
0.5
0.0
-0.2 kb
ACS
0.2 kb

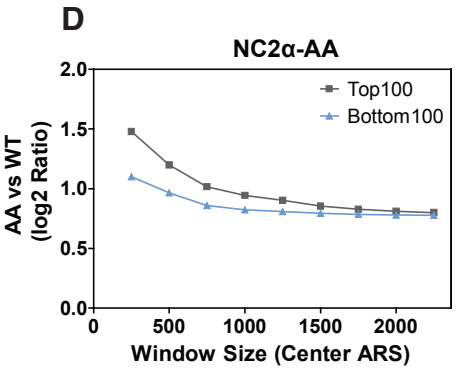
Figure S2, related to Figures 2 and 3. (A) Venn diagram showing correlation in numbers of down-regulated genes (≥1.5 FC, FDR≤0.05) between INO80-AA, Mot1-AA, and INO80-AA Mot1-AA. **(B)** Venn diagram showing correlation in numbers of genes (≥1.5 FC, FDR≤0.05) for INO80-AA between this study and a previously published study (Yao et al., 2016). **(C)** A scatter dot plot showing nascent transcription levels (mutant/wildtype) of genes flanking replication origins for rapamycin-treated INO80-AA (blue), Mot1-AA (pink), and INO80-AA Mot1-AA (fuchsia) for Top 100 (left graph) or Bottom 100 (right graph). The schematics above each graph indicate the orientation of ACS. Significance and p-values were calculated by using Mann-Whitney U-test. **(D)** Representative genome browser views for NET-seq for both strands (Watson strand – upper tracks, Crick strand – lower tracks) for WT-AA, INO80-AA, Mot1-AA, and INO80-AA Mot1-AA for a highlighted region in yeast Chr XI for the replication origins ARS1114, ARS1114.5, and ARS1115. **(E).** Frequency scores for TATA-box (the canonical TATAWAWR motif with a threshold of $p<0.001$) over all yeast origins (flanking a region of 200 bp upstream and downstream of ACS).

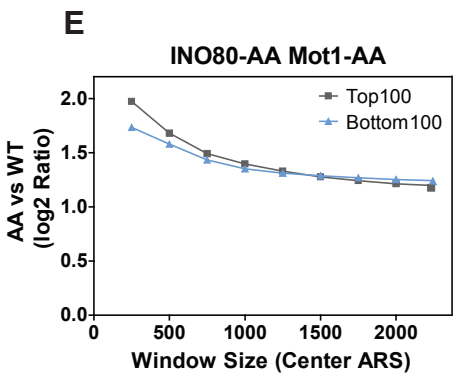
Figure S3


A
RNA All ARS
AA vs WT (log2 Ratio)
Window Size (Center ARS)
Control
INO80-AA
Mot1-AA
NC2α-AA
INO80-AA Mot1-AA
INO80-AA NC2α-AA


B
INO80-AA
AA vs WT (log2 Ratio)
Window Size (Center ARS)
Top100
Bottom100


C
Mot1-AA
AA vs WT (log2 Ratio)
Window Size (Center ARS)
Top100
Bottom100


D
NC2α-AA
AA vs WT (log2 Ratio)
Window Size (Center ARS)
Top100
Bottom100


E
INO80-AA Mot1-AA
AA vs WT (log2 Ratio)
Window Size (Center ARS)
Top100
Bottom100

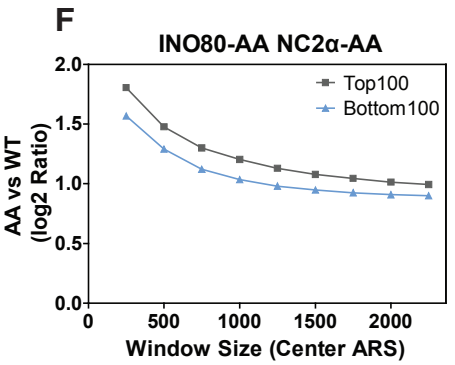

F
INO80-AA NC2α-AA
AA vs WT (log2 Ratio)
Window Size (Center ARS)
Top100
Bottom100

Figure S3, related to Figures 2 and 3. RNA-seq analyses. (**A-F)** Plots showing $\log_2$ ratio values for an average of two biological replicates (n=2) for mutant versus WT for INO80-AA, Mot1-AA, NC2α-AA, INO80-AA Mot1-AA and INO80-AA NC2α-AA over all yeast origins in **(A)**, for INO80-AA over Top 100 and Bottom 100 origins in **(B)**, for Mot1-AA over Top 100 and Bottom 100 origins in **(C)**, for NC2α-AA over Top 100 and Bottom 100 origins in **(D)**, for INO80-AA Mot1-AA over Top 100 and Bottom 100 origins in **(E)**, and for INO80-AA NC2α-AA over Top 100 and Bottom 100 origins in **(F).** Window size indicates the covered region centered at ARS elements. Control is MJE7 (Rapamycin versus DMSO).

Figure S4

A

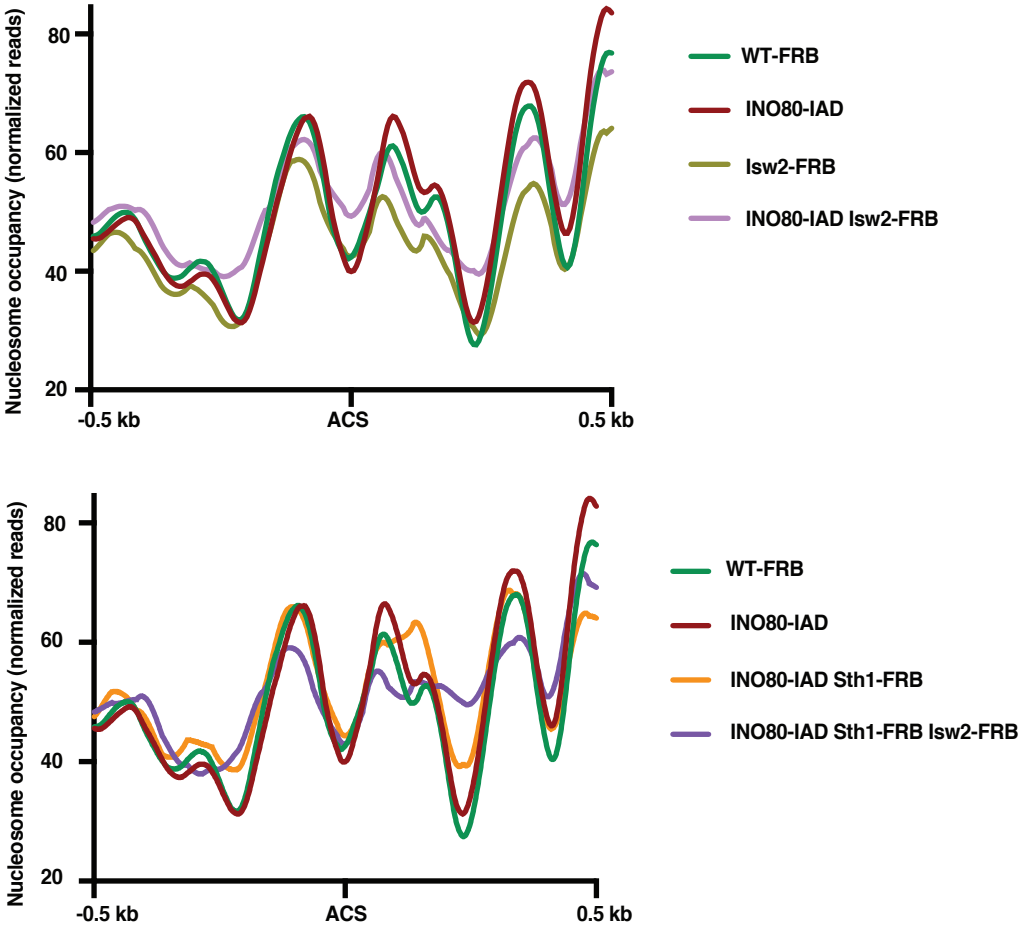

Nucleosome occupancy (normalized reads)
80
60
40
20
-0.5 kb
ACS
0.5 kb
WT-FRB
INO80-IAD
Isw2-FRB
INO80-IAD Isw2-FRB
Nucleosome occupancy (normalized reads)
80
60
40
20
-0.5 kb
ACS
0.5 kb
WT-FRB
INO80-IAD
INO80-IAD Sth1-FRB
INO80-IAD Sth1-FRB Isw2-FRB

Figure S4: Nucleosome occupancy is disrupted around yeast origins after depletion of Ino80, related to Figure 3. (A) Plots of nucleosome occupancies shown for WT-FRB, INO80-IAD, Isw2-FRB, INO80-IAD Isw2-FRB (upper panel) and for **(B)** WT-FRB, INO80-IAD, INO80-IAD Sth1-FRB, INO80-IAD Sth1-FRB Isw2-FRB (lower panel) for all yeast origins (flanking a region of 0.5 kb upstream and downstream of ACS) by MNase-seq (Kubik et al., 2019).

A

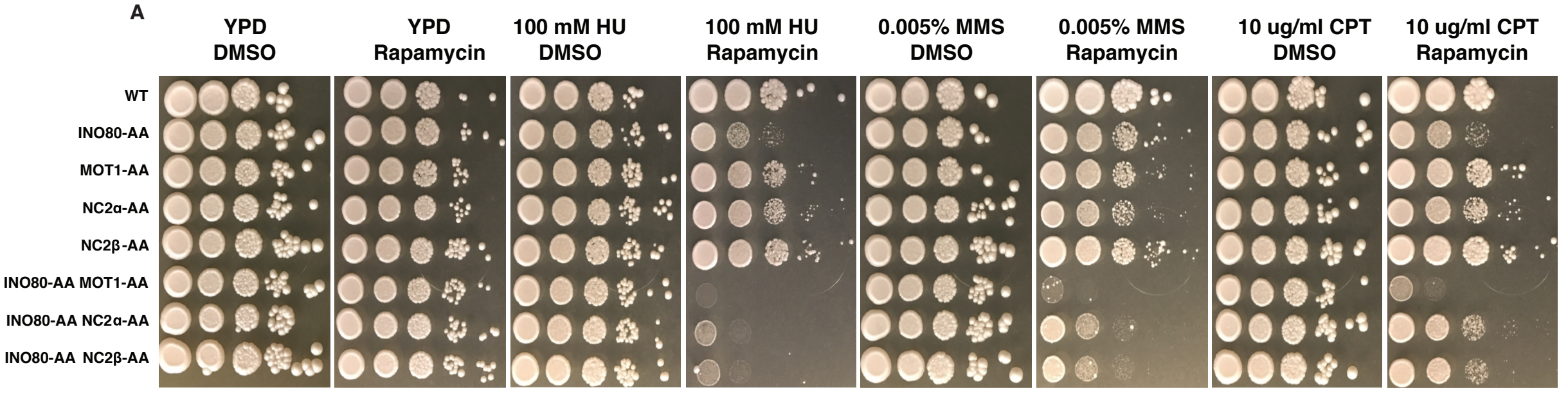

YPD DMSO
YPD Rapamycin
100 mM HU DMSO
100 mM HU Rapamycin
0.005% MMS DMSO
0.005% MMS Rapamycin
10 ug/ml CPT DMSO
10 ug/ml CPT Rapamycin
WT
INO80-AA
MOT1-AA
NC2α-AA
NC2β-AA
INO80-AA MOT1-AA
INO80-AA NC2α-AA
INO80-AA NC2β-AA

Figure S5: INO80C, Mot1, and NC2 contribute to genome stability, related to Figure 4. (A) Wild type and mutant strains were spotted (1/10 dilutions) on YPD media containing either DMSO or 8 μg/ml rapamycin in the presence or absence of 0.1M HU, 0.005% MMS, or 10 μg/ml CPT, then grown for 3 days at 30^{o}C.