

Supplementary Information

Replication licensing regulated by a short linear motif within an intrinsically disordered region of origin recognition complex

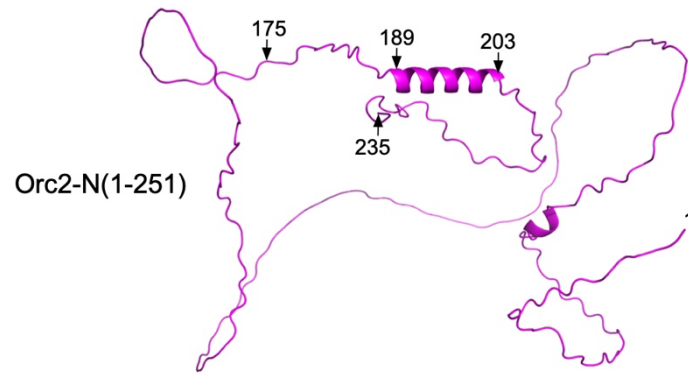
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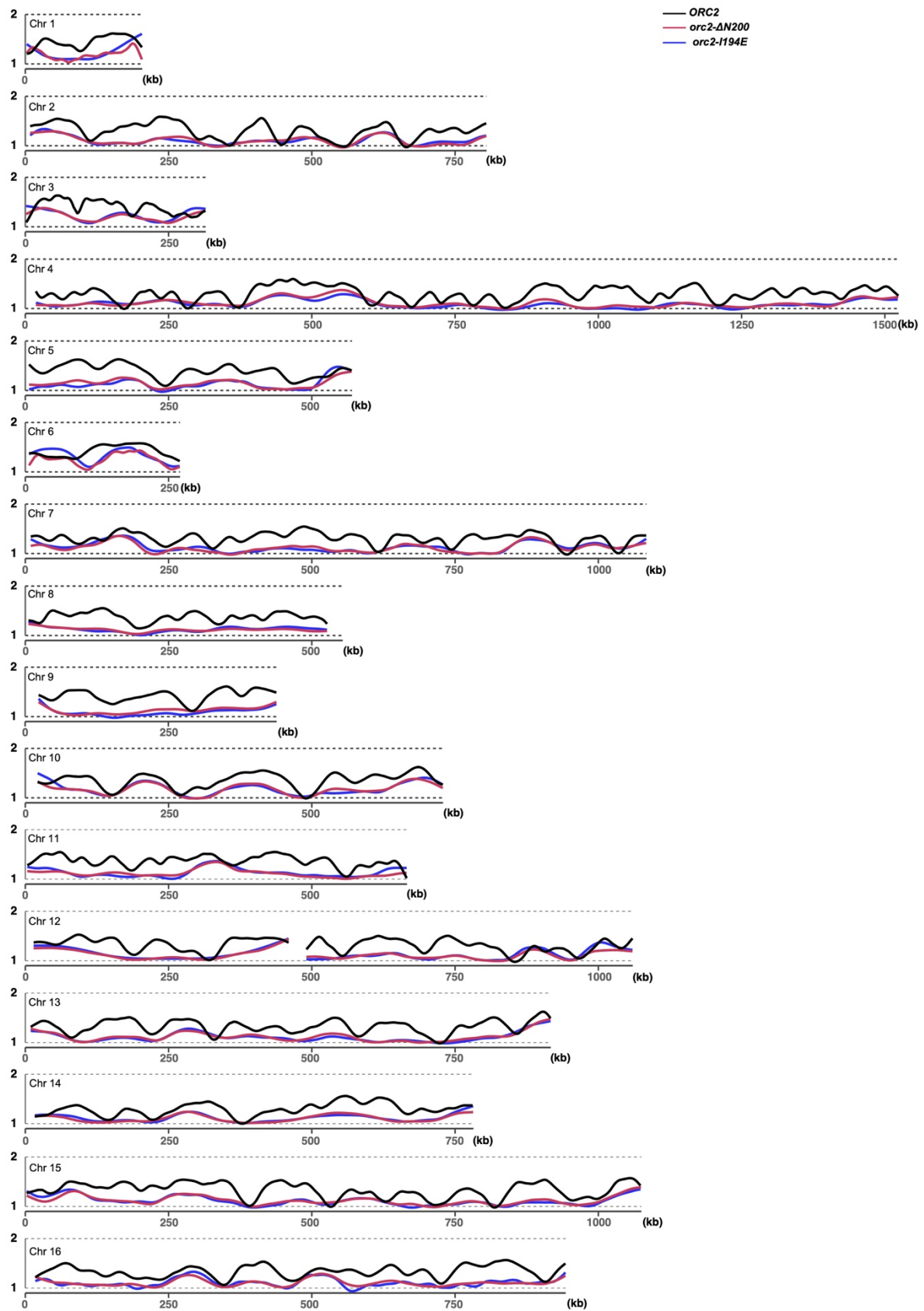
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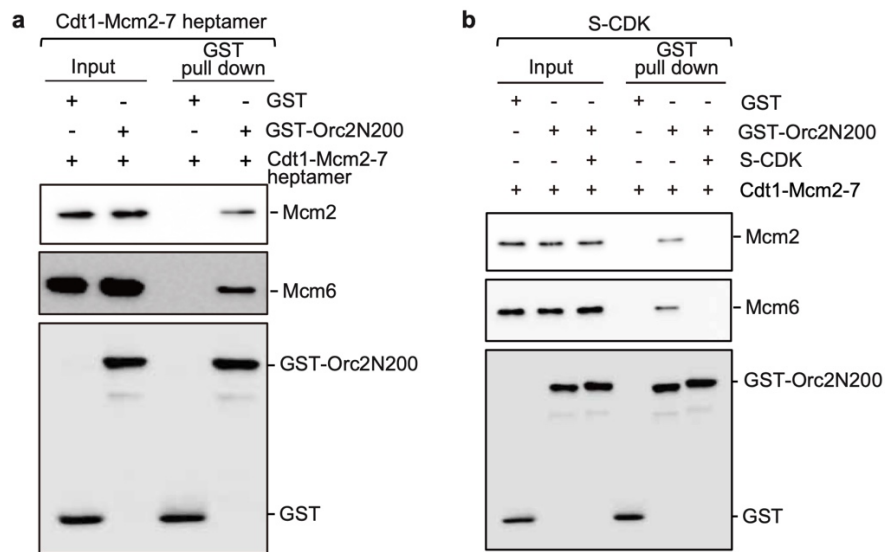
This document contains 6 Figures and 1 Table



Supplementary Figure 1. The atomic model of Orc2-NTD (1-251) predicted by AlphaFold.



Supplementary Figure 2. Replication profiles in Orc2-IDR mutant cells. Plots showing relative DNA copy numbers in the sections of chromosome (chr) 1-16. Open circles indicate confirmed replication origins according to OriBD.

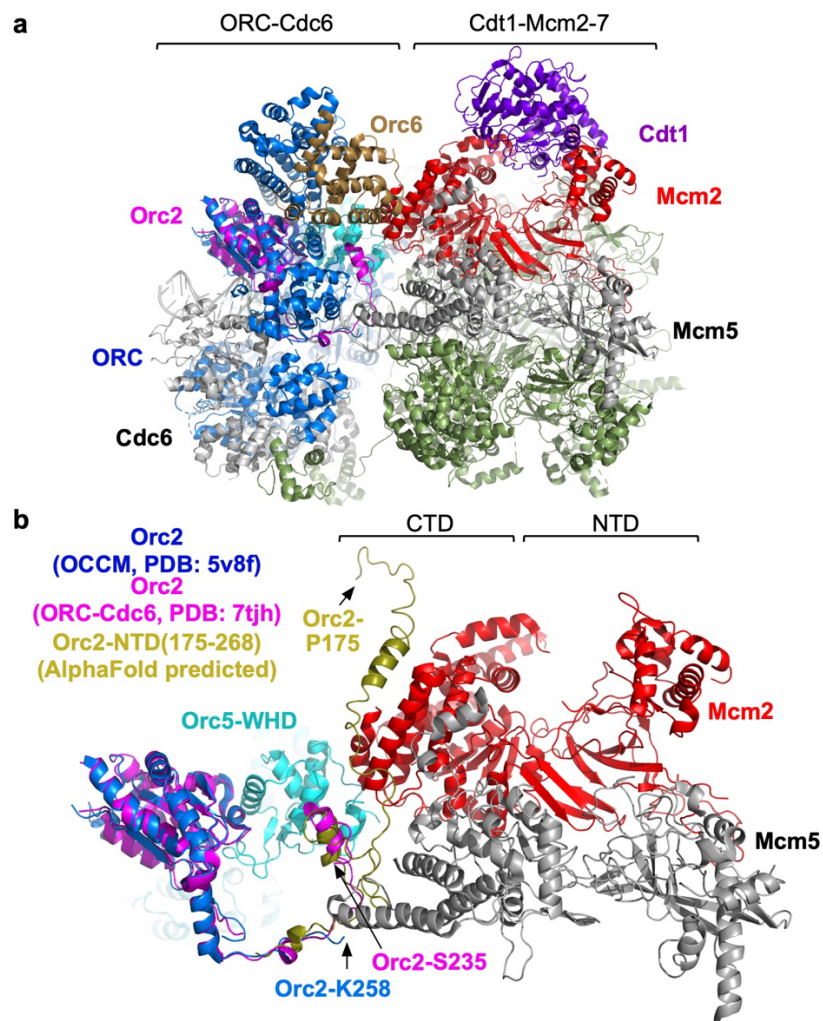


Supplementary Figure 3. S-CDK phosphorylation blocks the binding of Orc2-IDR to Mcm2-7.

a GST pull-down assays showed that Cdt1-Mcm2-7 heptamer binds to GST-Orc2N200 but not GST.

b Analysis of Orc2-IDR binding to Cdt1-Mcm2-7 in the presence of S-CDK phosphorylation using GST pull-down assays.

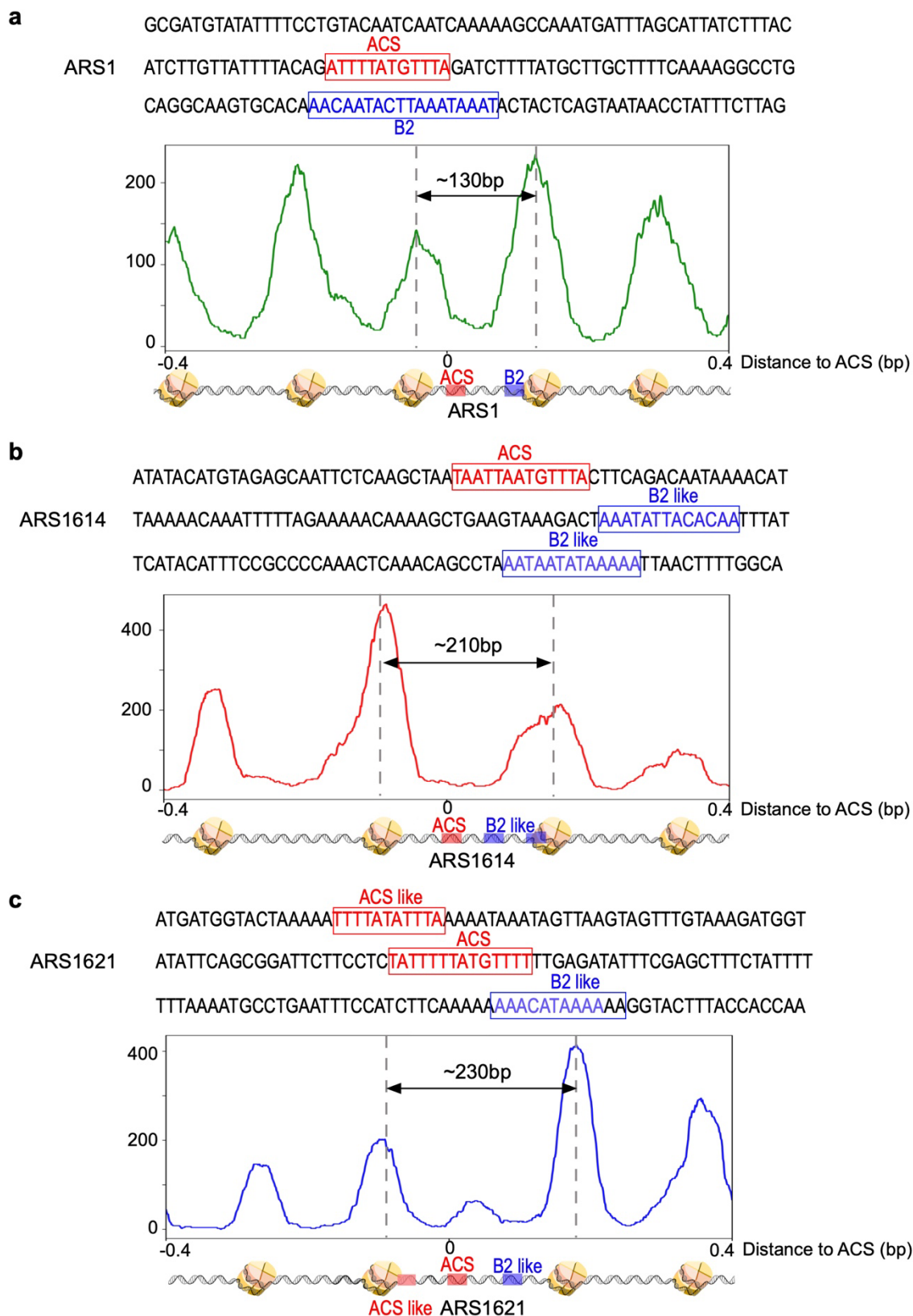
The relevant samples were analyzed by SDS-PAGE and immunoblotting of the indicated proteins. Similar results were obtained in at least two independent experiments. Source data are provided as a Source Data file.



Supplementary Figure 4. The hypothetical location of Orc2-IDR in the OCCM structure.

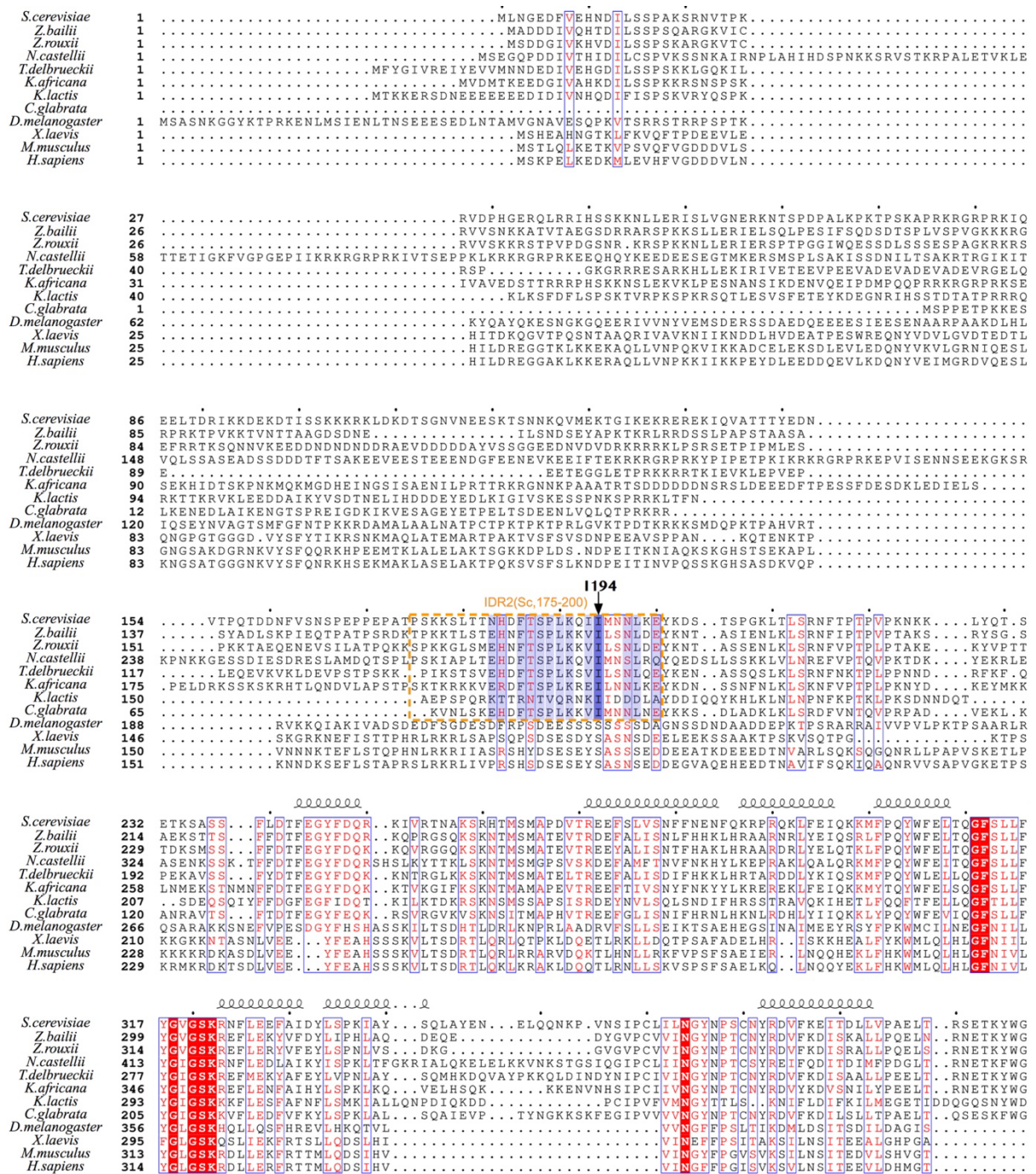
a, The atomic model of OCCM (PDB: 5v8f) superimposed with Orc2 from the structure of ORC-Cdc6 (PDB: 7tjh).

b, Magnified view of the ORC-MCM interface from **a** highlighting the location of Orc2-NTD relative to Mcm2 and Mcm5. Orc2-NTD (175-268) from the AlphaFold predicted Orc2 aligned with ORC-Cdc6 (PDB: 7tjh) using Orc2 as a reference.



Supplementary Figure 5. ARS1614 and ARS1621 exhibit extra ORC binding sites with wider nucleosome depleted regions.

a-c The distribution of ACS, ACS-like, B2, and B2-like elements within ARS1 (a), ARS1614 (b), and ARS1621 (c) and their relation with nucleosome positioning calculated using a previously published MNase-seq dataset (SRP041314)⁴⁰. Y-axis represents relative strength of nucleosome signal. Both ARS1614 and ARS1621 show a wider nucleosome depleted region between -1 and +1 nucleosomes compared to ARS1.



Supplementary Figure 6. Multiple sequence alignment of Orc2-NTD from various species.

Supplementary Table 1. Yeast strains used in this study

Yeast Strain	Genotype	Source
GA1680	<i>MATa ade2-1 trp1-1 his3-11,15 ura3-1 leu2-3,112 can1-100 orc2::His3MX6-UASGal-orc2-1</i>	Shimada and Gasser, 2006
ySDORC	<i>MATa ade2-1 trp1-1 his3-11,15 ura3-1 leu2-3,112 can1-100 bar1::Hyg pep4::KanMX his3::pRS303-GAL-ORC3/4 (HIS3) ura3::pRS306-GAL-CBP-ORC1/2 (URA3) trp1::pRS304-ORC5/6 (TRP1)</i>	Frigola et al., 2013
yJF38	<i>MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::Hyg pep4::KanMX his3::HIS3 pRS303-CDT1/GAL4 (HIS3) ura3::pRS306-MCM2/FLAG-MCM3 (URA3) trp1::pRS304-MCM4/MCM5 leu2::pRS305-MCM6/MCM7</i>	Frigola et al., 2013
yAE37	<i>MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::Hyg pep4::KanMX his3::HIS3 pRS303-CBP-TEV-CLB5/GAL4 (HIS3) ura3::pRS306-CKS1/CDC28(URA)</i>	Yeeles et al., 2015
yYW101	<i>GA1680 leu2::pRS405-ORC2</i>	This study
yYW102	<i>GA1680 leu2::pRS405- orc2ΔN100</i>	This study
yYW103	<i>GA1680 leu2::pRS405- orc2ΔN125</i>	This study
yYW104	<i>GA1680 leu2::pRS405- orc2ΔN150</i>	This study
yYW105	<i>GA1680 leu2::pRS405- orc2ΔN175</i>	This study
yYW106	<i>GA1680 leu2::pRS405- orc2ΔN200</i>	This study
yYW107	<i>GA1680 leu2::pRS405- orc2ΔN215</i>	This study
yYW108	<i>GA1680 leu2::pRS405- orc2ΔN175-200</i>	This study
yYW109	<i>GA1680 leu2::pRS405- orc2ΔN175-215</i>	This study
yYW1010	<i>GA1680 leu2::pRS405- orc2 all PC to A</i>	This study
yYW1011	<i>GA1680 leu2::pRS405- orc2 all HF to E</i>	This study
yYW1012	<i>GA1680 leu2::pRS405- orc2 I193E/I194E/M195E</i>	This study
yYW1013	<i>GA1680 leu2::pRS405- orc2 I193E/I194E</i>	This study
yYW1014	<i>GA1680 leu2::pRS405- orc2 I193E</i>	This study
yYW1015	<i>GA1680 leu2::pRS405- orc2 I194E</i>	This study
yYW1021	<i>GA1680 leu2::pRS405- orc2 I194V</i>	This study
yYW201	<i>ySDORC leu2::pRS405-GAL-FLAG-ORC2</i>	This study
yYW202	<i>ySDORC leu2::pRS405-GAL-FLAG- orc2ΔN200</i>	This study
yYW203	<i>ySDORC leu2::pRS405-GAL-FLAG- orc2ΔN175</i>	This study
yYW204	<i>ySDORC leu2::pRS405-GAL-FLAG- orc2ΔI194E</i>	This study
yYW205	<i>ySDORC leu2::pRS405-GAL-FLAG- orc2ΔN175-S188D</i>	This study