Supplemental Information

Orc4 spatiotemporally stabilizes centromeric chromatin

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Supplemental references

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Movie S1

Dataset S1

Supplemental Materials and Methods

Construction of conditional orc4 mutants

In order to create a conditional null mutant of *orc4* in *C. albicans*, a deletion cassette was constructed as follows: a 368 bp fragment (Ca21Chr5 480170-479721) upstream to *ORF19.4221* was amplified using the primers ORC4_13 and ORC4_14 from the genomic DNA of SC5314 and cloned as a KpnI-XhoI fragment into pSFS2a (Reuss et al. 2004) to create pLSK1. A 490 bp fragment (Ca21Chr5 478025-477535) downstream to *ORF19.4221* was amplified using ORC4_15 and ORC4_16 and cloned as a SacII-SacI fragment into pLSK1 to generate pLSK2. pLSK2 was digested using KpnI and SacI, and was used to transform YJB8675 (Joglekar et al. 2008) to obtain the strain CaLS328 (*ORC4/orc4::SAT1*). The marker was recycled to obtain the strain CaLS329 (*ORC4/orc4::FRT*). To inactivate the remaining allele, a construct was generated by amplifying a fragment (Ca21Chr5 479720-479221) of *ORF19.4221* using the primers ORC4_11 and ORC4_12 and cloned as a BamHI-PstI fragment in pCaDIS (Care et al. 1999). The resulting plasmid pLSK3 was digested using BglII and was used to transform CaLS329 to obtain independent transformants of the conditional mutant CaLS330, CaLS331 (*MET3prORC4/orc4::FRT)*. Similar deletions were performed in SN148 (Noble and Johnson 2005) to obtain the *orc4* conditional mutants CaLS322, CaLS323 and CaLS324. Each of these strains was transformed with the *CSE4-TAP-HIS* cassette (Mitra et al. 2014) to obtain CaLS325, CaLS326 and CaLS327, respectively. The desired transformants were confirmed by PCR and western blot analysis.

Construction of conditional mcm2 mutants

In order to create a conditional null mutant of *mcm2* in *C. albicans*, a deletion cassette was constructed as follows: a 474 bp fragment (Ca21ChrR 857151-856675) upstream to *ORF19.4354* was amplified using the primers MCM2_13/MCM2_14 from the genomic DNA of SC5314 and cloned as a KpnI-XhoI fragment in pSFS2a to generate pLSK4. A 468 bp fragment (Ca21ChrR 853962-853494) downstream to *ORF19.4354* was amplified using MCM2_15 and MCM2_16 and cloned as a SacII-SacI fragment in pLSK4 to generate pLSK5. The plasmid was digested using KpnI and SacI, and was used to transform YJB8675 (Joglekar et al. 2008) to obtain the strain CaLS309 *(MCM2/mcm2::SAT1)*. The marker was recycled to obtain CaLS310 *(MCM2/mcm2::FRT)*. To inactivate the remaining allele, a construct was created by amplifying a fragment (Ca21ChrR 856674-856164) of *ORF19.4354*

using the primers MCM2_11 and MCM2_12 and cloned as a BamHI-PstI fragment in pCaDIS (47). The resulting plasmid pLSK7 was digested using BglII and was used to transform CaLS310 to obtain independent transformants of the conditional mutant CaLS311, CaLS312 and CaLS313 *(MET3prMCM2/mcm2::FRT)*. Similar deletions were performed in the SN148 (Noble and Johnson 2005) to obtain the *mcm2* conditional mutants CaLS303, CaLS304 and CaLS305. Each of these strains was transformed by the *CSE4-TAP-HIS* cassette (Mitra et al. 2014) to obtain CaLS306, CaLS307 and CaLS308. Desired transformants were confirmed by PCR and western blot analyses.

Construction of conditional scm3 mutants

In order to create a conditional null mutant of *scm3* in *C. albicans*, a deletion cassette was constructed as follows: a 598 bp fragment (Ca21Chr3 390264-390708) upstream to *ORF19.1668* was amplified using the primers ASB25 and ASB26 from the genomic DNA of SC5314 and cloned as a KpnI-XhoI fragment in pSFS2a to create pASB1. A 305 bp fragment (Ca21Chr3 387626-388030) downstream to *ORF19.1668* was amplified using the primers ASB27 and ASB28 and cloned as a SacII-SacI fragment in pASB1 to generate pASB2. The plasmid was digested using KpnI and SacI and was used to transform *C. albicans* SN148 (Noble and Johnson 2005) to obtain the strain CaASB1 *(SCM3/scm3::SAT1)*. The marker was recycled to obtain CaAB2 *(SCM3/scm3::FRT)*. To inactivate the remaining allele, a construct was generated by amplifying a fragment (Ca21Chr3 389106-390089) of *ORF19.1668* using the primers ASB34 and ASB35 and cloned as a BamHI-PstI fragment in pCaDIS (47). The resulting plasmid pAB3 was digested by KpnI and was used to transform CaAB2 to obtain independent transformants of the *scm3* conditional mutant CaAB3 *(MET3prSCM3/scm3::FRT)*. The conditional mutants were further confirmed by PCR. Similar deletions were performed in the strains YJB8675 (*CSE4-GFP-CSE4*) (Joglekar et al. 2008) and CaKS102 (*CSE4-TAP(URA3)/CSE4*) (Mitra et al. 2014). To construct the *MET3*pr-*SCM3* cassette with a *HIS1* marker, a *HIS1* fragment was cloned from pBS-HIS into the EcoRI site of the plasmid pASB3 to obtain pASB4. The plasmid pASB4 was digested with KpnI and was used to transform CaAB9 to obtain CaNV52. The conditional mutants were confirmed by their inability to grow in non-permissive media.

Construction of a C-terminal Protein-A tagged strain of Mcm2

The strain CaKS107 (*MCM2/MCM2-TAP*) was constructed by integrating a C-terminal Protein A tagging cassette with the *NAT* marker created by overlap extension

PCR using the primers M1 to M6 in BWP17 (Wilson et al. 1999). To delete the remaining allele of *MCM2*, the deletion cassette pLSK5 was modified as follows: The *SAT1* marker from pLSK5 was released by digesting with BamHI-PstI and a *URA3* fragment digested with the same enzymes was cloned into this backbone to generate pLSK6. This plasmid was digested using KpnI and SacI and was used to transform CaKS107 to generate CaLS334 (*MCM2-TAP(NAT))/mcm2::URA3*). *URA3* was recycled from this strain to obtain CaLS335, CaLS336 and CaLS337 (*MCM2-TAP(NAT)/mcm2::FRT*). The deletion was confirmed by PCR with primers MCM2_16 and PJ3 FRTrev, and the expression of the tagged protein was confirmed by western blot using anti-Protein A antibodies. To replace the endogenous promoter of *MCM2* with that of *MET3*, pLSK7 (described previously) was digested with BglII and was used to transform CaLS335, CaLS336 and CaLS337 to generate CaLS338, CaLS339 and CaLS340 (*MET3prMCM2-TAP(NAT)/mcm2::FRT*). The desired transformants were confirmed by their inability to grow in non-permissive media.

Construction of a C-terminal GFP tagged strain of Mcm2

To tag the endogenous copy of Mcm2 with GFP at the C-terminus, a 487 bp fragment (Ca21ChrR 853966-854452) from *MCM2* ORF was amplified using the primers Mcm2-GFP FP and Mcm2-GFP RP, and cloned as a SacII-SpeI fragment in pBS-GFP-HIS (Chatterjee et al. 2016) to generate pLSK8. The plasmid was digested with PacI and was used to transform SN148 to generate CaLS341 (*MCM2-GFP(HIS1)/MCM2*). The transformants were screened using fluorescence microscopy.

Construction of a C-terminal 2× GFP tagged strain of Scm3

To tag the endogenous copy of Scm3 with GFP at the C-terminus, a 770 bp fragment (Ca21Chr3 388221- 388991) from *SCM3* ORF was amplified using the primers NV158 and NV159, and cloned as a SacII-SpeI fragment in pBS-GFP-URA3 to obtain pNV31. Another fragment of the *GFP* ORF was amplified using primers NV250 and SR67 and inserted into the SpeI site of pNV31 to obtain pNV32. After confirming the orientation of GFP by HpaI, the plasmid was linearized with SwaI and was used to transform SN148 (Noble and Johnson 2005) to obtain CaNV50 (*SCM3-2×GFP(URA3)/SCM3*). To simultaneously localize Scm3 and Ndc80, we transformed CaNV50 with pNdc80-RFP-ARG4 (Varshney and Sanyal 2019) after digesting it with XhoI to obtain CaNV51 (*SCM3-2×GFP(URA3)/SCM3 NDC80-RFP(ARG4)/NDC80*).

The transformants were screened by microscopy. The remaining copy of *SCM3* was deleted by transforming CaNV51 with pASB2 to generate CaLS342 and CaLS343 (*SCM3-2×GFP(URA3)/scm3::SAT1 NDC80-RFP(ARG4)/NDC80*). Desired transformants were confirmed using PCR with primers ASB41 and PJ3 FRT rev. Localization of Scm3 was confirmed by microscopy. Similarly, YJB8675 was transformed with pNdc80-RFP-ARG4 to obtain CaLS344 (*CSE4-GFP-CSE4/CSE4 NDC80-RFP(ARG4)/NDC80*).

Western blotting

Approximately 3 O.D. equivalent cells of the relevant *C. albicans* strain were harvested and precipitated by 12.5% TCA overnight at -20°C. The pellet was spun down at 13000 rpm and washed with 80% acetone. The pellet obtained was then dried and resuspended in lysis buffer (1% SDS, 1N NaOH) and SDS loading dye. Samples were boiled for 5 min and electrophoresed on a 10% polyacrylamide gel. Protein transfer was performed by semi-dry method for 30 min at 25V. Following protein transfer, the blot was blocked with 5% skimmed milk for an hour. The blot was incubated with primary antibodies in the following dilutions: rabbit anti-Protein A (1:5000), rabbit anti-Orc4 (1:1000), and mouse anti-PSTAIRE (1: 5000). The blot was washed thrice in PBST (1x PBS + 0.05% Tween) and incubated with goat anti-rabbit IgG-HRP (1:10,000) or goat anti-mouse IgG-HRP (1:10,000). Following three PBST washes, the blot was developed using chemi-luminescence method. For quantifying protein level with respect to PSTAIRE, band intensity of the desired protein was divided with that of PSTAIRE in the corresponding lane and ratio was calculated using densitometric analysis.

Flow cytometry

Cells from the strain CaLS330 were harvested at different time points (as described previously) and processed as described before (Sanyal and Carbon 2002). Prior to injection of the sample into the flow cytometer, the cell suspension was sonicated briefly (30% amplitude, 7s pulse). The sonicated samples were diluted to a desired cell density in 1X PBS and injected into the flow cytometer (BD FACS Aria) for analysis. The output was analyzed using the FLOWJO software.

Indirect immuno-fluorescence

Exponentially grown cultures of SC5314 (*ORC4/ORC4*) and CaLS335 (*MCM2-TAP/mcm2::FRT*) were fixed with 37% formaldehyde. Spheroplasts were prepared using

lysing enzyme and cells were fixed on polyL-lysine coated slides using methanol and acetone and then incubated with 2% skimmed milk to block non-specific binding. Following 10 times washes with PBS, cells were incubated with anti-Orc4 antibodies (1:500) or anti-Protein A antibodies (1:1,000) for 1 h in a humid chamber. Post PBS washes, cells were incubated with Alexa Fluor goat anti-rabbit IgG 568 (1:500) or Alexa Fluor goat anti-rabbit IgG 488 (1:500) for 1 h. The coverslip was mounted on a slide using DAPI (10 ng/µl). Microscopic images were captured by a laser confocal microscope (Zeiss) using LSM 880 META software with He/Ne laser (bandpass 565-615 nm) for Alexa Fluor 568 and a 2-photon laser near IR (bandpass~780 nm) for DAPI. Z-stacks were collected at 0.5 µm intervals and stacked projection images were processed in ImageJ and Adobe Photoshop.

Fluorescence microscopy

For conditional expression of genes under the *MET3* promoter, cells were grown in permissive media overnight and shifted to non-permissive media for the indicated number of hours. In each case, the cells were washed twice with PBS and resuspended in PBS and imaged on a glass slide. DeltaVision System (Applied Precision) was used for CENPA (GFP) localisation upon Scm3 depletion. Zeiss Axio Observer Calibri was used for the localization of Scm3 (GFP) and Mcm2 (GFP), and the localization of CENPA (GFP) upon Mcm2 and Orc4 depletion. To visualize the nuclear mass in CaLS341 (*MCM2-GFP/MCM2*), cells were harvested, washed in PBS and 50 ng/ml of Hoechst was added to the cell suspension before imaging. Images were processed using ImageJ and Adobe Photoshop.

GFP quantification: For CENPA (GFP) intensity quantification in *C. albicans*, the *C. albicans* strain CaLS344 *(CSE4-GFP-CSE4/CSE4 NDC80-RFP/NDC80)* was mixed with the *S. cerevisiae* strain JBY254 (*ura3-1::pRS306-112XtetO)* (Wisniewski et al. 2014) and imaged using GFP (488 nm) and RFP (568 nm) channels. Images were taken from multiple fields (n>40) under identical conditions (exposure time = 200 ms and 300 ms for 488 nm and 568 nm, respectively). For quantification of relative GFP intensity, pixel values (arbitrary units) from the background fluorescence were subtracted from the pixel values obtained from the CENPA (GFP) cluster from individual cells. From each field, an unbudded cell of JBY254 was used for the normalization of *C. albicans* cells from the corresponding field. Likewise, 100 cells from each category: G1 (unbudded), S (small budded, with a budding index ≤ 0.3), metaphase (budding index ≥ 0.3, but unsegregated kinetochore) and anaphase (segregated kinetochore) were considered for analysis. Plots were generated using GraphPad Prism. For

CENPA (GFP) quantification upon HU treatment, similar analysis was performed using treated cells of YJB8675 *(CSE4-GFP-CSE4/CSE4)* and compared to small-budded cells of the control population. The values obtained were plotted in a scatter plot with standard error of mean (SEM). Students' unpaired *t*-test was used to determine statistical significance.

Hi-C analysis

Wild-type *C. albicans* Hi-C data files were downloaded from PRJNA308106 (Burrack et al. 2016). To examine interactions between the Orc4 binding regions, Hi-C interactions were analyzed according to the chromosome coordinates, different modes identified by DIVERSITY and based on replication timing (orc^{E}, orc^{M} and orc^{L}). The heatmap for the full genome was plotted using log-scaled values with a pseudocount of 0.000001 (10^{-6}). The heatmap for the "ORC-only" was plotted using values for the 2 kb windows overlapping with the midpoints of the Orc4 binding regions, using the same scaling and color scale as the full-genome heatmap. The violin plots were calculated for 1,000 randomizations of each dataset, where for each randomization, the chromosomal distribution and lengths of the regions were preserved.

Motif analysis by DIVERSITY

For motif analysis, the *de novo* motif discovery tool DIVERSITY (Mitra et al. 2018) was used with default web-server options on the 417 Orc4 ChIP-seq peaks. DIVERSITY is specially developed for ChIP-seq experiments profiling proteins that may bind DNA in more than one way.

Replication timing analysis

To analyze the replication timing of the Orc4 binding regions, fully processed timing data available in GSE17963_final_data.txt (Koren et al. 2010) was used. A larger replication time value implies earlier replication timing. All the 414 genomic origins were aligned according to their timing scores, and categorized as early, mid and late replicating regions based on the tertile distribution.

Supplemental figures:

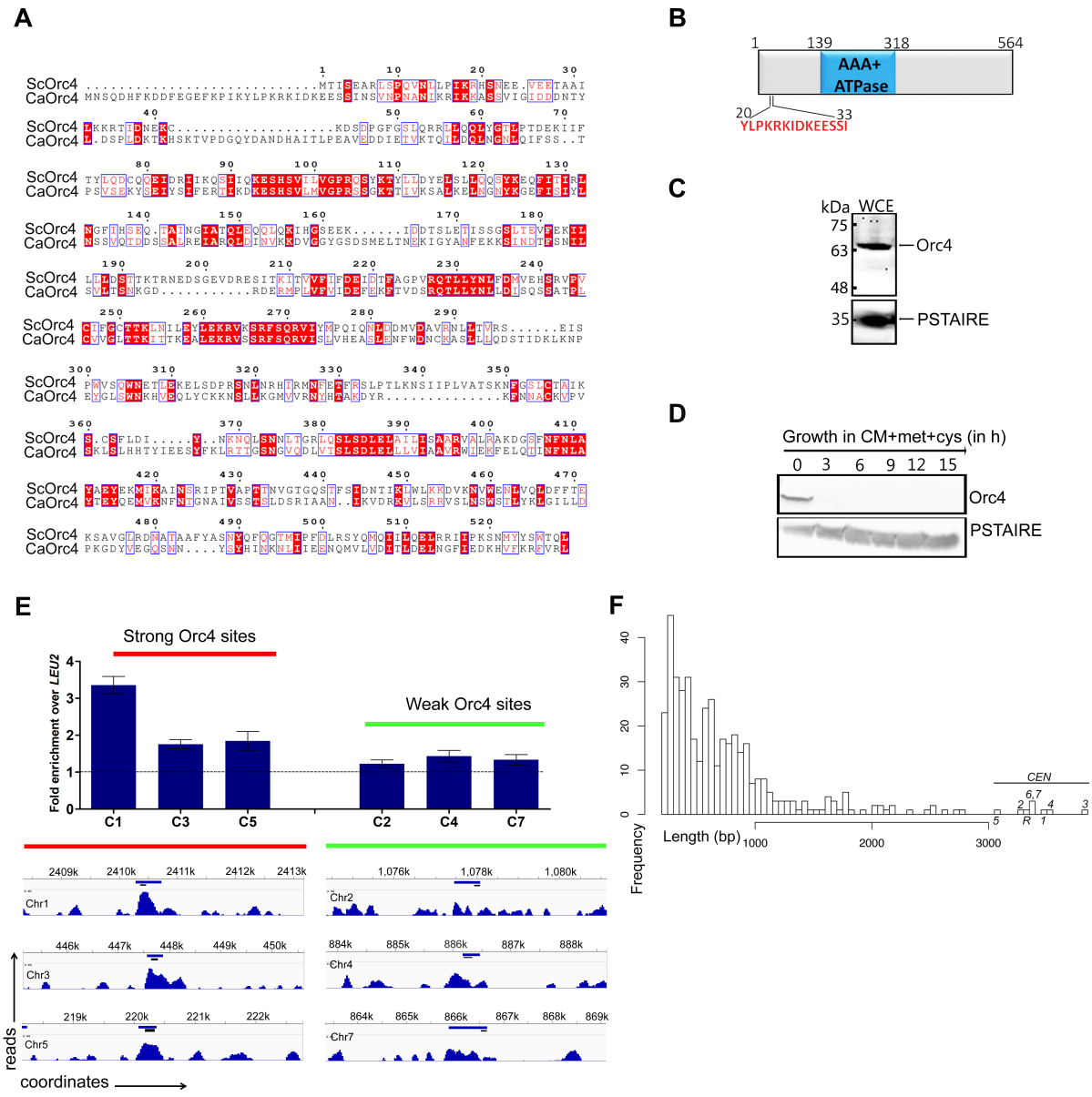

Fig. S1. Expression and *in vivo* localization of Orc4 in *C. albicans*. (**A**) A pair-wise alignment of Orc4 proteins from *S. cerevisiae* and *C. albicans* revealed conserved stretches of amino acids constituting the AAA+ ATPase domain (aa 139-318). (**B**) The domain architecture of Orc4 showed a 564 aa-long polypeptide consisting of a central AAA+ ATPase domain in *C. albicans*. The peptide sequence chosen to raise the antibodies has been highlighted in red letters (aa 20-33). (**C**) Expression of Orc4 was verified by western blot with anti-Orc4 antibodies using the whole cell extract (WCE) from the *C. albicans* strain SC5314 (*ORC4/ORC4*). Anti-Orc4 antibodies yielded a band at the expected molecular

weight of ~64 kDa in a denaturing SDS PAGE. PSTAIRE was used as the loading control. (**D**) Western blot analysis using anti-Orc4 antibodies indicates time course depletion of Orc4 in the conditional mutant CaLS330 (*MET3prORC4/orc4::FRT*) when the strain was grown for the indicated time (0, 3, 6, 9, 12, 15 h) in the presence of 5 mM met + 5 mM cys. PSTAIRE was used as the loading control. (**E**) Orc4 ChIP-qPCR assays were used to validate the binding of Orc4 in strong (red) and weak (green) enrichment loci with fold enrichment of >1.35 and <1.35, respectively. Fold enrichment values for each primer pair was plotted with respect to *LEU2* (black dashed line). (n=3)The genomic locations of these primer binding site have been depicted as snapshots below, with the blue lines indicating the Orc4 peak and the black lines indicating the PCR amplicon. (**F**) Frequency distribution of the length of Orc4 binding sites in the *C. albicans* genome.

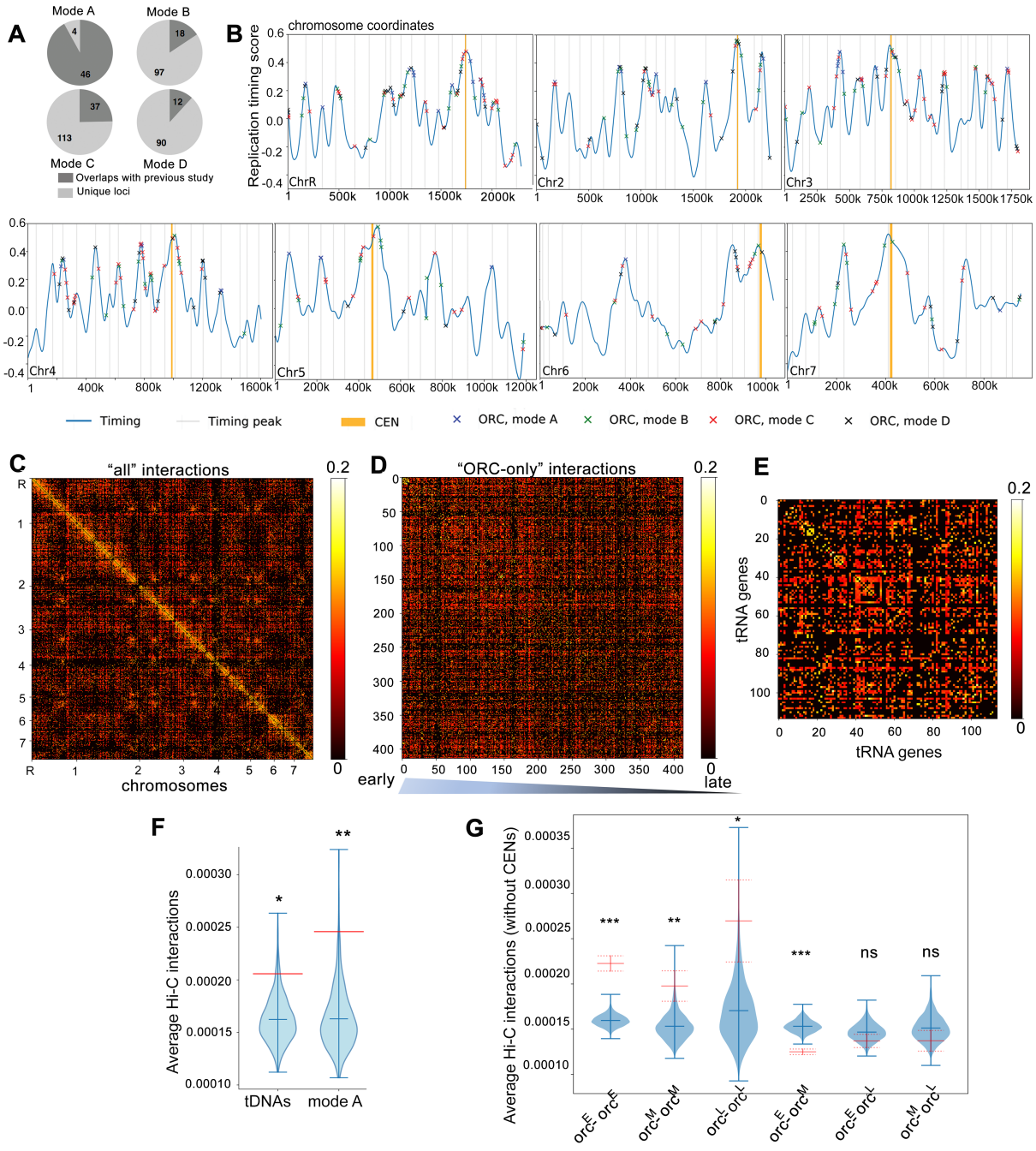

Fig. S2. Early replicating regions interact among themselves to form an early replication nuclear domain. (**A**) Pie charts depicting the number of Orc4 binding sites in the current study overlapping with binding sites reported earlier (Tsai et al. 2014). (**B**) Orc4 ChIP-seq peaks aligned to the replication timing profile of *C. albicans* chromosomes (Chr2-ChrR) from the previous study (Koren et al. 2010). (**C**) The Hi-C heatmap shows the whole-genome "all" heatmap representation of the Hi-C data (Burrack et al. 2016) as a 7145 x 7145 matrix with a 2 kb resolution. The maximum value in the data was 0.2015 and the minimum was zero. For plotting, the values were log-transformed with a pseudocount of 0.0001. (**D**)

The Hi-C "ORC-only" heatmap depicted interactions between the chromosomal ORC binding regions, ordered by timing (early to late), to the same color scale as in (C). The analysis was performed at a resolution of 2 kb. It indicates marginally higher interactions within orc^{E} (top-left quadrant) and within orc^{L} (square on bottom right). (**E**) Hi-C interactions for 62 tRNA-associated Orc4 peaks (1-62) and 64 tRNA genes not overlapping with Orc4 peaks (63-126). (**F**) Average Hi-C interactions compared to 1,000 randomizations for the 64 non-Orc4 tRNA genes and the Orc4 peaks, suggesting tRNA genes in general interact more than average, but less than the tRNA genes associated with Orc4 peaks. (**G**) Mean Hi-C interactions (solid red) with standard error (dotted red) within and across each of the three timing classes (orc^{E}, orc^{M} and orc^{L}) without the centromere peaks. These indicated higher interaction values within orc^{E} and within orc^{L} domains, similar to the pattern observed in Fig. 2E. Blue violins indicate mean interactions across 1,000 randomizations. (***p-value<0.0001, **p-value<0.001, *p-value<0.01, ns p-value>0.5)

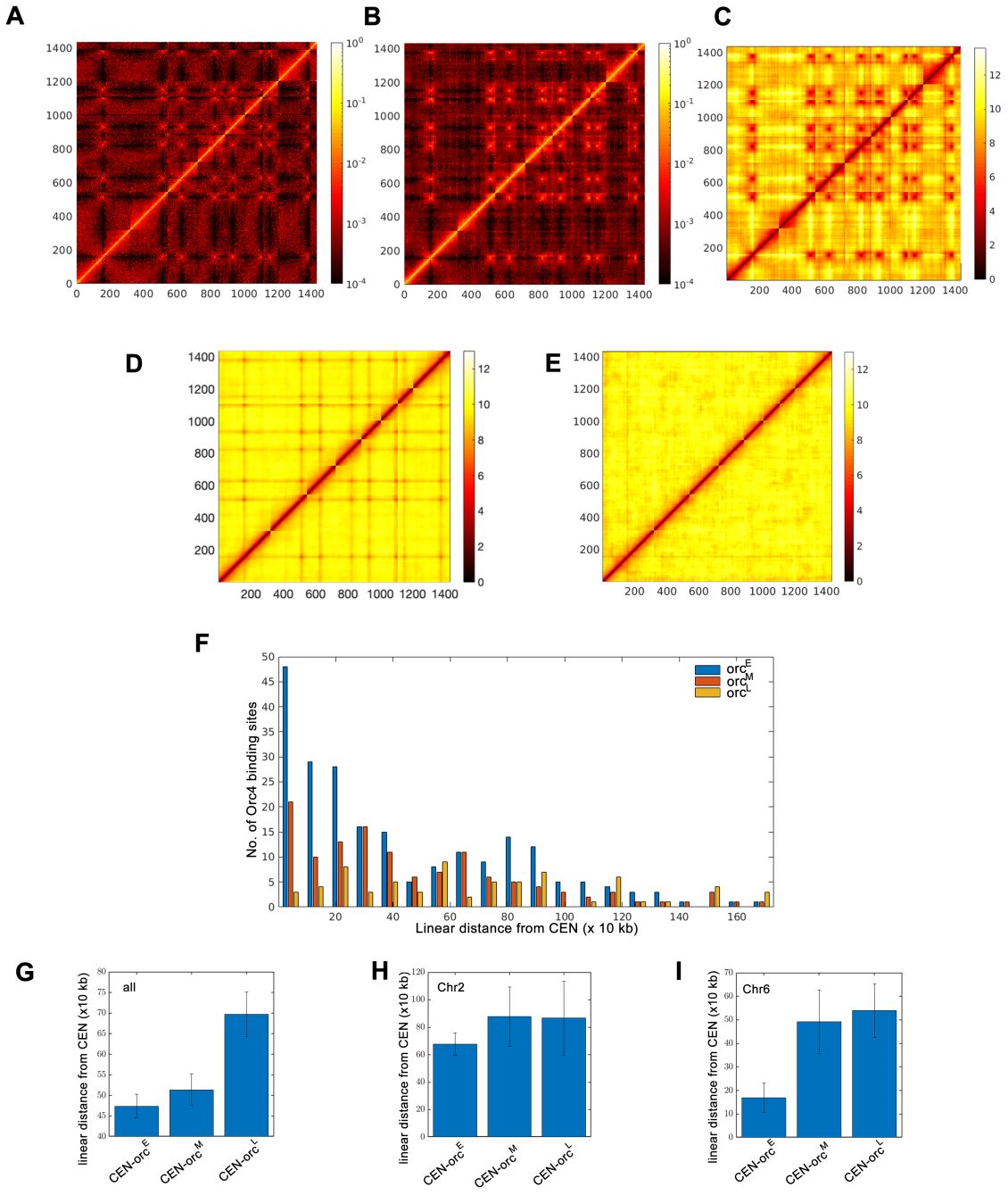

Fig. S3. Comparison between experimental and simulated Hi-C contact probabilities in *C. albicans*. (**A**) The Hi-C heatmap representing the whole genome of *C. albicans* from experimental data reported in the previous study (Burrack et al. 2016) analyzed at a resolution of 10 kb. (**B**) Heatmap depicting the contact probability calculated from the polymer model of the *C. albicans* genome, at a resolution of 10 kb. (**C**) Heatmap depicting the average spatial distances between individual beads. (**D**) Heatmap depicting the average spatial distances between only the Orc4 beads when the SPB tether to the centromere is removed. (**E**) Heatmap depicting the average spatial distances of a control simulation where

no SPB tethering and no Hi-C contacts were considered. For (A-E), the *x* and *y* axes represent the bead number along the polymer chain where the spatial distances or contact frequency was computed from the simulation based on the 1,000 independent configurations (**F**) The linear distance of Orc4 binding sites from centromeres (*x*-axis) plotted against the number of Orc4 -bound loci reveals that majority of orc^{E} are located closest to centromeres. (**G-I**) The average linear distances between CEN- orc^{E}, CEN- orc^{M}, and CEN- orc^{L} depicts the minimum distance between centromeres and orc^{E}. Plots depicting linear distances for all chromosomes (F), Chr2 (G), and Chr6 (H) are shown here. The *y*-axis indicates the linear distance between the given set of loci in the simulation. CEN, centromere.

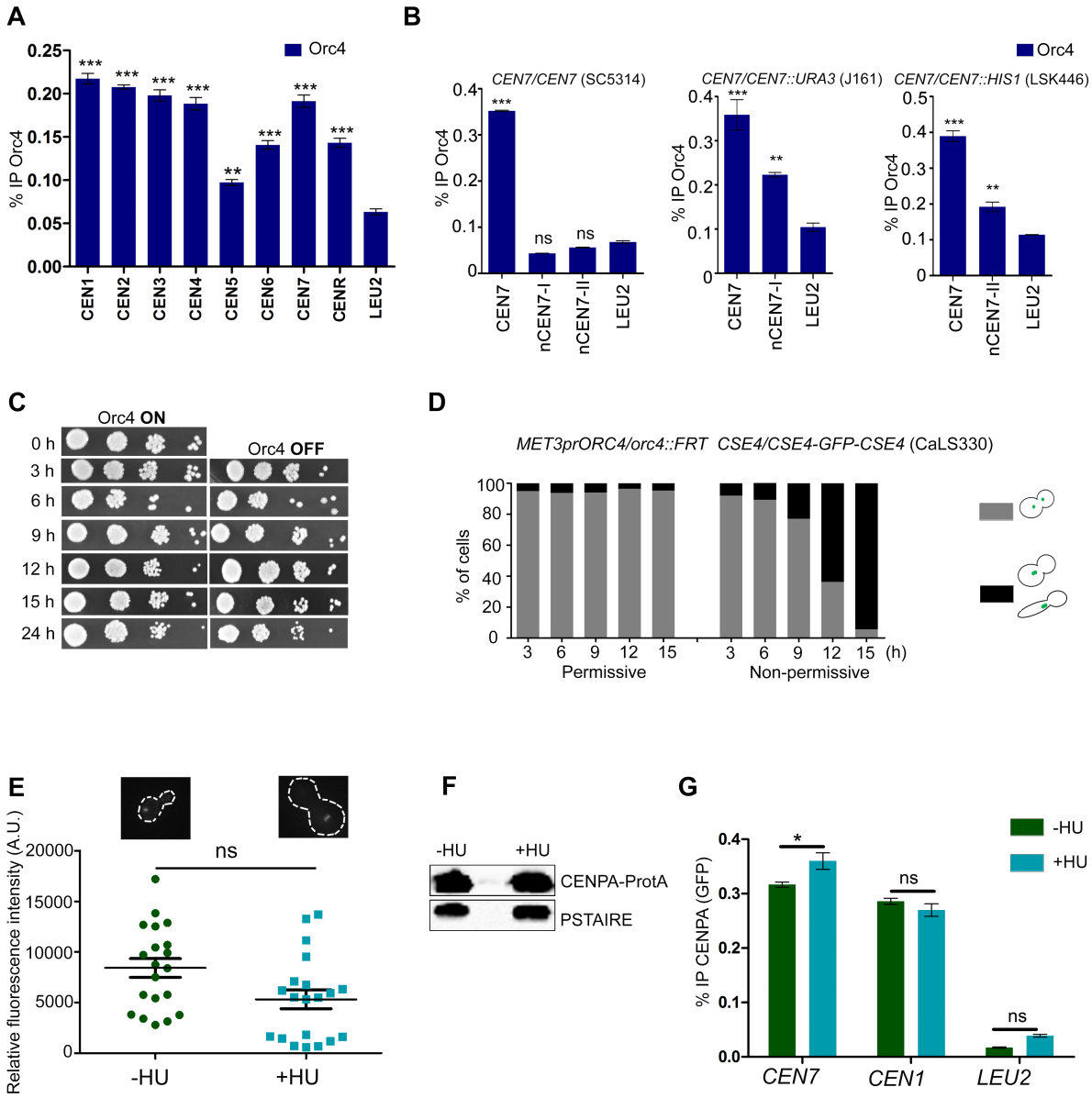

Fig. S4. Orc4 binds to active centromeres and facilitates kinetochore segregation in *C. albicans*. (**A**) Orc4 ChIP-qPCR assays in SC5314 was used to validate the enrichment of Orc4 at all *C. albicans* centromeres. *LEU2* was used as a control non-centromeric locus. (One-way ANOVA, *** *p*-value<0.001, ** *p*-value <0.01, ns: *p*-value >0.05). n=3 (**B**) Orc4 ChIP-qPCR in the wild type (*CEN7/CEN7*) (*left*), *CEN7* deletion strain J161 (Thakur and Sanyal 2013) (*CEN7/CEN7::URA3)* (*middle*) and LSK446 (Sreekumar et al. 2019) (*CEN7/CEN7::HIS1)* (*right*) indicates significant enrichment of Orc4 at *nCEN7-I* and *nCEN7-II,* the neocentromere hotspots, over the control region (*LEU2*). (One-way ANOVA, *** *p*-value<0.001, ** *p*-value<0.01, ns: *p*-value>0.05). n=2 (**C**) Spot dilution assays to indicate viability of the *orc4* conditional mutant CaLS330 (*MET3prORC4/orc4::FRT*) grown in permissive or non-permissive media for the indicated time and then spotted on permissive media. Plate photographs were captured after 48 h of incubation at 30ºC. (**D**) The segregation pattern of the kinetochores in large-budded cells was examined in CaLS330 when cells were grown in permissive or non-permissive media for the indicated time. n=100 (**E**) Normalized fluorescence intensity of cells in YJB8675 (*CSE4-GFP-CSE4/CSE4*) when treated with 200 mM HU for 2 h as compared to untreated cells (Unpaired *t*-test, ns: *p*-value>0.05; n=20) (**F**) Western blot analysis using anti-Protein A antibodies in the strain J200 (*CSE4/CSE4-TAP*) (Thakur and Sanyal 2013) revealed comparable levels of CENPA before (-HU) and after HU treatment (+HU). PSTAIRE was used as a loading control. (**G**) ChIP-qPCR analysis in YJB8675 using anti-GFP antibodies revealed no depletion of CENPA at centromeres (*CEN7* and *CEN1*) upon HU treatment. (Two-way ANOVA, * *p*-value<0.05, ns: *p*-value>0.05; n=3)

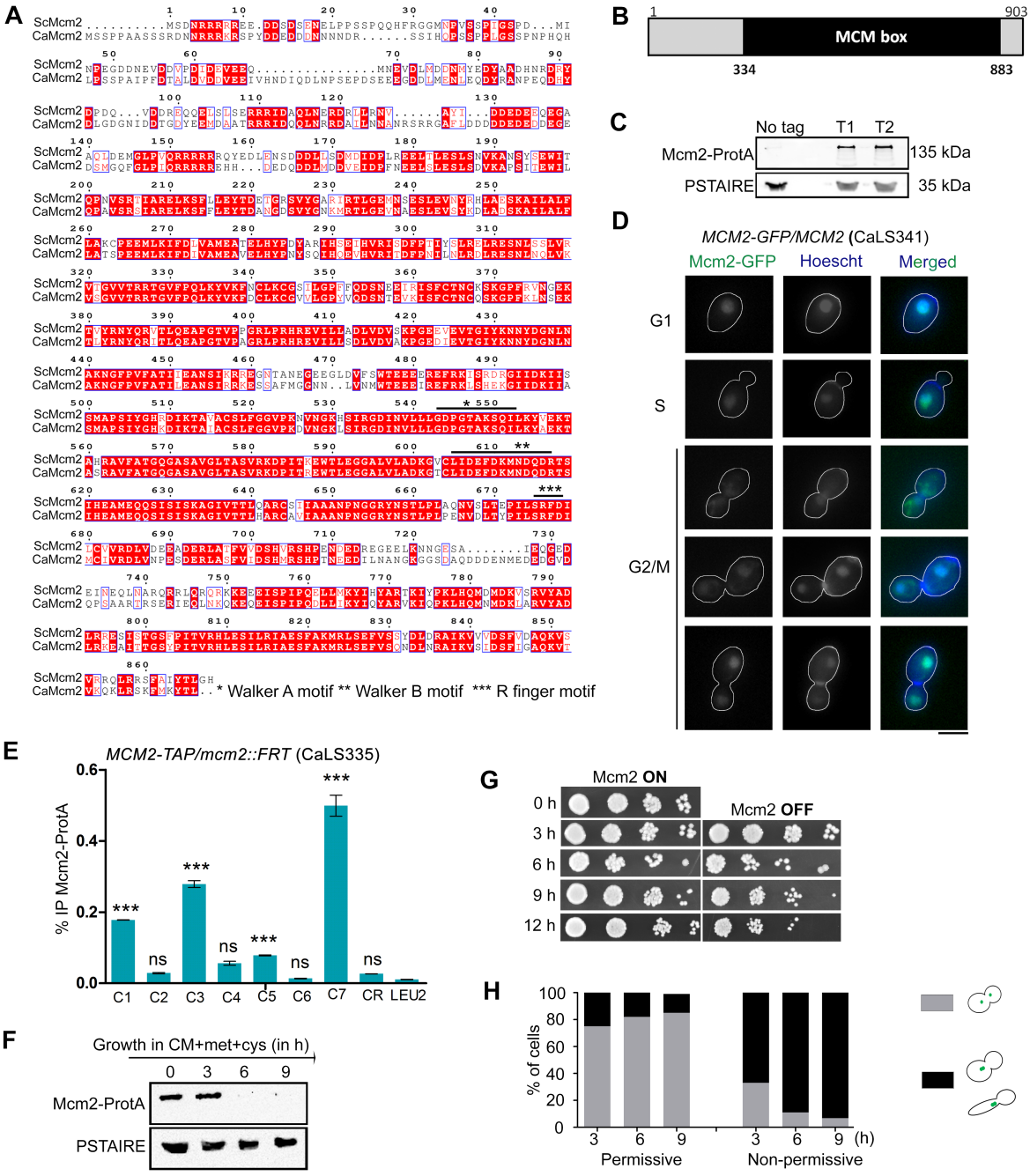

Fig. S5. Mcm2 is an evolutionarily conserved protein and is important for kinetochore segregation in *C. albicans* (**A**) A pair-wise comparison of the Mcm2 proteins from *S. cerevisiae* and *C. albicans* revealed the three conserved motifs, Walker A, Walker B and R finger. (**B**) Domain architecture of Mcm2 reveals a 903 aa-long polypeptide consisting of the catalytic helicase domain, MCM box (aa 334-883). (**C**) Western blot analysis of the whole cell extracts of CaLS335 *(MCM2-TAP(NAT)/mcm2::FRT)* using anti-Protein A antibodies displayed a specific band of Mcm2-TAP corresponding to 135 kDa, which was undetected in

the untagged strain. PSTAIRE was used as the loading control. (**D**) Intracellular localization of Mcm2-GFP in CaLS341 (*MCM2-GFP/MCM2*) cells stained with Hoechst dye to stain the nucleus. Scale bar, 5 µm. (**E**) ChIP-qPCR analysis to examine the binding of Mcm2 to Orc4-bound regions on all eight chromosomes in *C. albicans* compared to a control region (*LEU2*). (One-way ANOVA, *** p-value<0.001, ns: p-value>0.05; n=3) (**F**) Western blot analysis using anti-Protein A antibodies indicated time course depletion of CENPA in CaLS306 (*MET3prMCM2-TAP/mcm2::FRT*) when the strain was grown for the indicated time in presence of 5 mM met +5 mM cys. PSTAIRE was used as the loading control. (**G**) Spot dilution assays to indicate viability in the *mcm2* conditional mutant CaLS311 (*MET3prMCM2/mcm2::FRT*) grown in permissive or non-permissive media for the indicated time and then spotted on permissive media. Plate photographs were captured after 48 h of incubation at 30ºC. (**H**) The segregation pattern of the kinetochores was examined in large-budded cells of CaLS311 when the cells were grown in permissive or non-permissive media for the indicated time. (n=100)

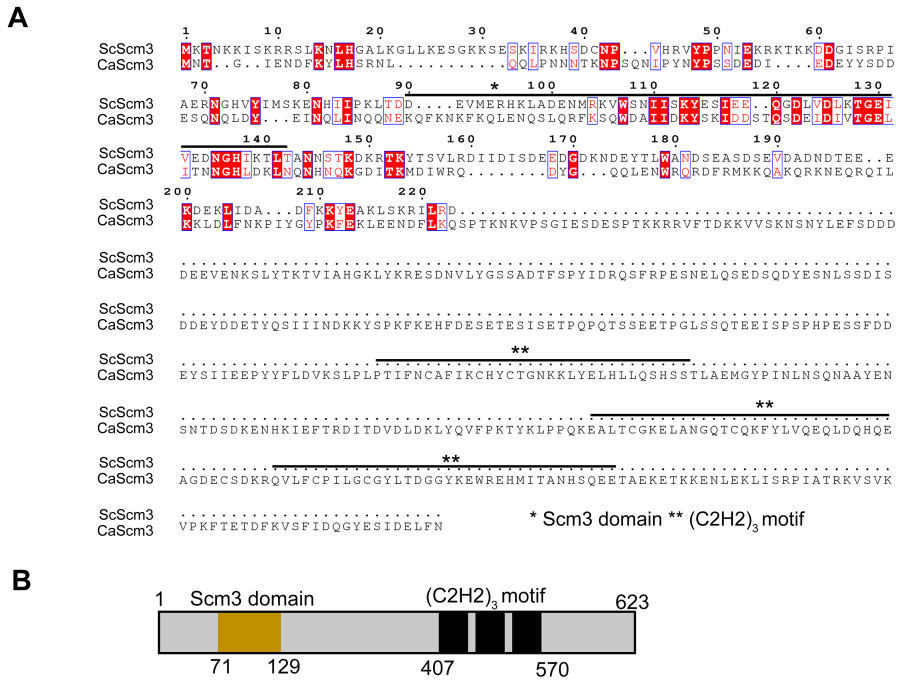

Fig. S6. Identification of Scm3, the homolog of HJURP, in C. *albicans*. (A) A comparison of the Scm3 proteins from *S. cerevisiae* and *C. albicans* shows the conserved Scm3 domain (*) at the N-terminus and the C2H2 Zn-fingers. (**B**) Domain architecture of Scm3 in *C. albicans* reveals a 623 aa-long polypeptide consisting of the conserved Scm3 domain (aa 71-129) and three separate C2H2 zinc finger clusters (aa 407-570) (Aravind et al. 2007).

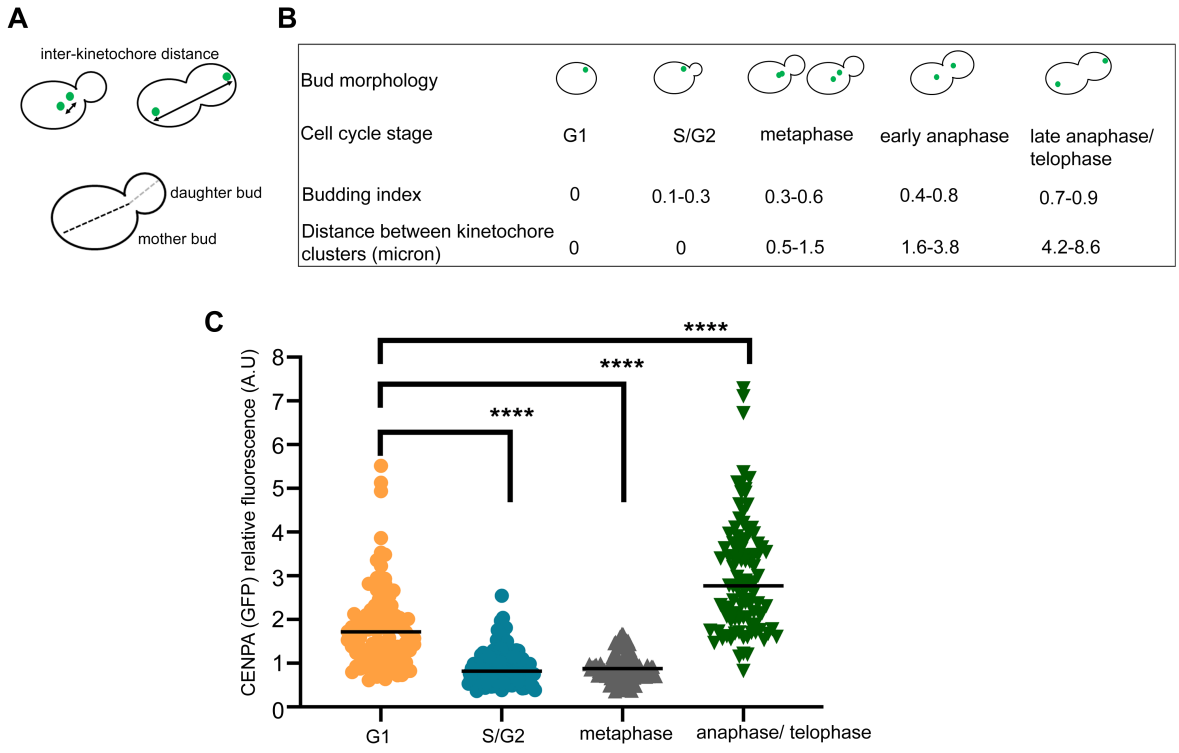

Fig. S7. Centromeric localization of Scm3 coincides with CENPA loading time in *C. albicans* (**A**) Schematic showing calculation for the distance between two kinetochores clusters (green circle) or the inter-kinetochore distance in a metaphase cell and an anaphase cell. The budding index is calculated as the ratio of diameters of a daughter bud (gray dashed line) and its mother bud (black dashed line). (**B**) Schematic depicting bud morphology and cell cycle stages in *C. albicans* based on budding indices and inter-kinetochore distance calculations described in Fig. S7A. Here, a metaphase cell is considered to have two kinetochore clusters in the same bud, whereas, an anaphase cell is considered to have one kinetochore cluster per bud in a large budded cell. (**C**) Graphical representation of CENPA (GFP) intensities in a population of cells in various stages of the cell cycle measured using ratiometric analysis (*t*-test, **** *p*-value<0.0005; n=100 for each category).

Movie S1. Polymer modeling of *C. albicans* chromosome reveals spatiotemporal localization of Orc4 within the nucleus. The early (red), mid (yellow), and late (blue) replicating Orc4-bound sites have been superimposed on the individual beads (gray) of the coarse-grained polymer generated from the simulation. The movie depicts one of the 1,000 configurations.

Dataset S1: Excel file containing all the 417 Orc4 binding sites in *C. albicans* identified by ChIP-sequencing analysis, with their corresponding genomic coordinates, fold enrichment values, *p*- and *q*-values.

Supplemental table S1. Parameters used in simulation for polymer modeling of chromosomes in *C. albicans*

Simulation parameter	Values
Number of beads, N	1432
Spring stiffness, k_s	100 k_BT/l_0^2
Spring stiffness, k_c	500 k_BT/l_0^2
Interaction strength, E_{ij}	1 k_BT/l_0^2
Natural length l_0	1 l_0
Radius of sphere, R_s	12 l_0

Supplemental table S2. Chromosomal coordinates representing Orc4 binding regions at centromeres based on *C. albicans* assembly 21

Chromosome	CENPA binding region coordinates (length)	Orc4 binding region coordinates (length)
1	Ca21Chr1 15662315-1566930 (4616 bp)	Ca21Chr1 1562748- 1566244 (3497 bp)
2	Ca21Chr2 1925206- 1929688 (4483 bp)	Ca21Chr2 1926183- 1929443 (3261 bp)
3	Ca21Chr3 822762-827727 (4966 bp)	Ca21Chr3 823057- 826863 (3807 bp)
4	Ca21Chr4 991382-996030 (4649 bp)	Ca21Chr4 992010- 995522 (3513 bp)
5	Ca21Chr5 4673814-472497 (5114 bp)	Ca21Chr5 468552- 471618 (3067 bp)
6	Ca21Chr6 979686-984007 (4322 bp)	Ca21Chr6 980541- 983910 (3370 bp)
7	Ca21Chr7 425129- 431652 (6524 bp)	Ca21Chr7 425910- 429297 (3388 bp)
R	Ca21ChrR 1742833- 1748598 (5766 bp)	Ca21ChrR 1743951- 1747274 (3324 bp)

Supplemental table S3. Quantification of the relative fluorescence intensity of CENPA (GFP) from photobleaching experiments

Cell no.	Pre-bleach intensity (A.U.)	Post-bleach intensity (A.U.)	Max. intensity at recovery (A.U.)	% recovery
1	16.1	2.03	16.83	105.20

2	20.68	7.814	23.823	124.4
3	12.949	8.955	36.708	283.4
4	16.96	0.586	11.11	64.27
5	6.083	0.421	5.309	86.32
6	5.023	0.263	8.232	167.4

Supplemental table S4. Chemicals and antibodies used in the study

Reagent	Source	Identifier
Chemicals		
CaOrc4 peptide	GeneMed Synthesis, USA	Custom synthesized
Freund's complete adjuvant	Sigma	Cat. no. F5881
Freund's incomplete adjuvant	Sigma	Cat. no. F5506
2-mercaptoethanol	HiMedia	Cat. no. MB041
Hydroxyurea	HiMedia	Cat no. 6487
Dimethyl sulfoxide (DMSO)	Sigma	Cat. no. D2650
Nocodazole	Sigma	CAS 31430-18-9
Doxycycline hyclate	Sigma	Cat. no. D9891
Super Signal West Pico chemiluminescent substrate	Thermo Scientific	Cat. no. 34080
DAPI	Sigma	Cat. no. 10236276001
Hoechst	Sigma	Cat no. H6024
Lysing enzyme	Sigma	Cat. no. L1412
Protein A-Sepharose beads	Sigma	Cat. no. P3391
Nourseothricin	Werner Bioagents	CAS 96736-11-7
SensiFast SYBR kit	Bioline	Cat. no. BIO-98020
Antibodies		
Polyclonal anti-Protein A	Sigma	Cat. no. P2921
Monoclonal anti-PSTAIRE	Abcam	Cat. no. 9866
Monoclonal anti-GFP	Roche	Cat. no. 11814460001
Polyclonal anti-H3	Abcam	Cat. no. ab1791
Polyclonal anti-H4	Abcam	Cat. no. ab10158
Goat anti-rabbit IgG-HRP	Bangalore Genei	Cat. no. 105499
Goat anti-mouse IgG-HRP	Bangalore Genei	Cat. no. HP06
Alexa Fluor goat anti-rabbit IgG 568	Invitrogen	Cat. no. 11011
Alexa Fluor goat anti-rabbit IgG 488	ThermoFischer	Cat no. A27034

Supplemental table S5. Strains used in the study

Name (Description)	Genotype	Reference
SC5314	Wild type	(Aszalos et al. 1968)
BWP17	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG*	(Wilson et al. 1999)
SN148	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG*	(Noble and Johnson 2005)

YJB8675	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, CSE4-GFP-CSE4/CSE4*	(Joglekar et al. 2008)
CaLS301 (*MCM2* heterozygous null in SN148)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, mcm2::SAT1/MCM2*	This study
CaLS302 (*MCM2* heterozygous null in SN148)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, mcm2::FRT/MCM2*	This study
CaLS303 (*mcm2* conditional mutant in SN148)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG,Δarg4::hisG/Δarg4::hisG,Δleu2::hisG/ Δleu2::hisG MET3pr(URA3)MCM2/mcm2 ::FRT*	This study
CaLS304 (*mcm2* conditional mutant in SN148)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MET3pr(URA3)MCM2/mcm2 ::FRT*	This study
CaLS305 (*mcm2* conditional mutant in SN148)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MET3pr(URA3)MCM2/mcm2::FRT*	This study
CaLS306 (*mcm2* conditional mutant (SN148) CENPA-Prot A)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MET3pr(URA3)MCM2/mcm2::FRT CSE4-TAP(HIS)/CSE4*	This study
CaLS307 (*mcm2* conditional mutant (SN148) CENPA-Prot A)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MET3pr(URA3)MCM2/mcm2::FRT CSE4-TAP(HIS)/CSE4*	This study
CaLS308 (*mcm2* conditional mutant (SN148) CENPA-Prot A)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MET3pr(URA3)MCM2/mcm2::FRT CSE4-TAP(HIS)/CSE4*	This study
CaLS309 (*MCM2* heterozygous null in 8675)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, mcm2::SAT1/MCM2 CSE4-GFP-CSE4/CSE4*	This study
CaLS310 (*MCM2* heterozygous null in 8675)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, mcm2::FRT/MCM2 CSE4-GFP-CSE4/CSE4*	This study
CaLS311 (*mcm2* conditional mutant in 8675)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MET3pr(URA3)MCM2/mcm2::FRT CSE4-GFP CSE4/CSE4*	This study
CaLS312 (*mcm2* conditional mutant in 8675)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG,*	This study

	MET3pr(URA3)MCM2/mcm2::FRT CSE4-GFP-CSE4/CSE4	
CaLS313 (*mcm2* conditional mutant in 8675)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MET3pr(URA3)MCM2/mcm2::FRT CSE4-GFP-CSE4/CSE4*	This study
CaLS320 (*ORC4* heterozygous null in SN148)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, orc4::SAT1/ORC4*	This study
CaLS321 (*ORC4* heterozygous null in SN148)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, orc4::FRT/ORC4*	This study
CaLS322 (*orc4* conditional mutant in SN148)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MET3prORC4(URA3)/ orc4::FRT*	This study
CaLS323 (*orc4* conditional mutant in SN148)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MET3prORC4(URA3)/ orc4::FRT*	This study
CaLS324 (*orc4* conditional mutant in SN148)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MET3prORC4(URA3)/ orc4::FRT*	This study
CaLS325 (*orc4* conditional mutant (SN148) CENPA-Prot A)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MET3prORC4(URA3)/ orc4::FRT CSE4 TAP(HIS)/CSE4*	This study
CaLS326 (*orc4* conditional mutant (SN148) CENPA-Prot A)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MET3prORC4(URA3)/ orc4::FRT CSE4 TAP(HIS)/CSE4*	This study
CaLS327 (*orc4* conditional mutant (SN148) CENPA-Prot A)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MET3prORC4(URA3)/ orc4::FRT CSE4 TAP(HIS)/CSE4*	This study
CaLS328 (*ORC4* heterozygous null in 8675)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, orc4:SAT1/ORC4 CSE4-GFP-CSE4/CSE4*	This study
CaLS329 (*ORC4* heterozygous null in 8675)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, orc4:FRT/ORC4 CSE4-GFP-CSE4/CSE4*	This study
CaLS330 (*orc4* conditional mutant in 8675)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MET3prORC4(URA3)/ orc4::FRT CSE4-GFP-CSE4/CSE4*	This study

CaLS331 (*orc4* conditional mutant in 8675)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MET3prORC4(URA3)/ orc4::FRT CSE4-GFP-CSE4/CSE4*	This study
CaLS332 (*orc4* conditional mutant in 8675)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MET3prORC4(URA3)/ orc4::FRT CSE4-GFP-CSE4/CSE4*	This study
CAKS3b (*CENPA* under *PCK1* promoter)	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG Δarg4::hisG/ Δarg4::hisG CSE4::PCK1prCSE4/ cse4::hisG:URA3:hisG*	(Sanyal and Carbon 2002)
LSK446 (*CEN7* deletion)	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	(Sreekumar et al. 2019)
J161 (*CEN7* deletion)	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 CEN7::URA3/ CEN7*	(Thakur and Sanyal 2013)
J200 (CENPA-TAP)	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 CSE4/CSE4TAP(NAT)*	(Thakur and Sanyal 2013)
SBC189 (*cdc15* mutant)	*ura3Δ::imm434/ura3Δ::imm434 ade2Δ::hisG/ade2Δ::hisG ENO1/eno1::ENO1-tetR-ScHAP4AD-3xHA-ADE2 URA3-TETp-CDC15/cdc15Δ::dpl200*	(Bates 2018)
CaKS107 (Mcm2-TAP)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MCM2-TAP(NAT)/MCM2*	This study
CaLS334 (Mcm2-TAP)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MCM2-TAP(NAT)/ mcm2::URA3*	This study
CaLS335	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MCM2-TAP(NAT)/ mcm2::FRT*	This study
CaLS336	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MCM2-TAP(NAT)/ mcm2::FRT*	This study
CaLS337	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MCM2-TAP(NAT)/ mcm2::FRT*	This study
CaLS338 (*mcm2* conditional mutant)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MET3pr(URA3)MCM2-TAP(NAT)/ mcm2::FRT*	This study
CaLS339 (*mcm2* conditional mutant)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MET3pr(URA3)MCM2-TAP(NAT)/mcm2::FRT*	This study
CaLS340 (*mcm2* conditional mutant)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG,*	This study

	MET3pr(URA3)MCM2-TAP(NAT)/mcm2::FRT	
CALS341 (*MCM2-GFP/MCM2*)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MCM2-GFP(URA3)/MCM2*	This study
CaAB1(*SCM3* heterozygous in SN148)	*Δura3::imm434/Δura3::imm434 Δhis1::hisG/ Δhis1::hisG Δarg4::hisG/ Δarg4::hisG Δleu2::hisG/ Δleu2::hisG SCM3/scm3:SAT1*	This study
CaAB2 (*SCM3* heterozygous in SN148)	*Δura3::imm434/Δura3::imm434 Δhis1::hisG/ Δhis1::hisG Δarg4::hisG/ Δarg4::hisG Δleu2::hisG/ Δleu2::hisG SCM3/scm3::FRT*	This study
CaAB3 (*scm3* conditional mutant in SN148)	*Δura3::imm434/Δura3::imm434 Δhis1::hisG/ Δhis1::hisG Δarg4::hisG/ Δarg4::hisG Δleu2::hisG/ Δleu2::hisG MET3p(URA3)SCM3 /scm3::FRT*	This study
CaAB4 (*scm3* conditional mutant in SN148)	*Δura3::imm434/Δura3::imm434 Δhis1::hisG/ Δhis1::hisG Δarg4::hisG/ Δarg4::hisG Δleu2::hisG/ Δleu2::hisG MET3p(URA3)SCM3 /scm3::FRT*	This study
CaAB5 (*SCM3* heterozygous in 8675)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, CSE4/CSE4-GFP-CSE4 scm3::SAT1 /SCM3*	This study
CaAB6 (*SCM3* heterozygous in 8675)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, CSE4/CSE4-GFP-CSE4 scm3::FRT/SCM3*	This study
CaAB7 (*scm3* conditional mutant in 8675)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, CSE4/CSE4-GFP-CSE4 MET3pSCM3(URA3)/scm3::FRT*	This study
CaKS102 (CENPA-ProtA)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG, Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG CENP-A/CENP-A-TAP(URA3)*	(Mitra et al. 2014)
CaAB8 (*SCM3* heterozygous)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG, Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG CENP-A/CENP-A-TAP(URA3) scm3::FRT/SCM3*	This study
CaAB9 (*SCM3* heterozygous)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG, Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG CENP-A/CENP-A-TAP(URA3) scm3::FRT/SCM3*	This study
CaNV52 (*scm3* conditional mutant in Cse4-ProtA)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG, Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG CENP-A/CENP-A-TAP(URA3) MET3pSCM3(HIS1)/ scm3::FRT*	This study
CaNV50 (Scm3-2×GFP)	*Δura3::imm434/Δura3::imm434 Δhis1::hisG/ Δhis1::hisG Δarg4::hisG/ Δarg4::hisG Δleu2::hisG/ Δleu2::hisG SCM3-2×GFP(URA3)/SCM3*	This study

CaNV51 (Scm3-2×GFP/Ndc80-RFP)	*Δura3::imm434/Δura3::imm434 Δhis1::hisG/ Δhis1::hisG Δarg4::hisG/ Δarg4::hisG Δleu2::hisG/ Δleu2::hisG SCM3-2×GFP(URA3)/SCM3 NDC80-RFP(ARG4)/NDC80*	This study
CaLS342 (SCM3-2×GFP/scm3/ Ndc80-RFP	*Δura3::imm434/Δura3::imm434 Δhis1::hisG/ Δhis1::hisG Δarg4::hisG/ Δarg4::hisG Δleu2::hisG/ Δleu2::hisG SCM3-2×GFP-URA3/scm3::SAT1 NDC80-RFP(ARG4)/NDC80*	This study
CaLS343 (SCM3-2×GFP/scm3/ Ndc80-RFP	*Δura3::imm434/Δura3::imm434 Δhis1::hisG/ Δhis1::hisG Δarg4::hisG/ Δarg4::hisG Δleu2::hisG/ Δleu2::hisG SCM3-2×GFP-URA3/SAT1 NDC80-RFP(ARG4)/NDC80*	This study
CaLS344 (CSE4-GFP-CSE4/CSE4 NDC80-RFP/NDC80)	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, CSE4-GFP-CSE4/CSE4 NDC80-RFP(ARG4)/NDC80*	This study
JBY254	*MATa ADE2 can1-100 his3-11,15 leu2-3, 112::LEU2-Δ80ura3p-TetR-GFP-TAP-ADHt trp1-1 ura3-1::pRS306-112× tetO RAD5*	(Wisniewski et al. 2014)

Supplemental table S6. Plasmids used in the study

Name	Description	Reference
pSFS2a	Recyclable *SAT1*-flipper cassette	(Reuss et al. 2004)
pLSK1	*ORC4* upstream sequence cloned in pSFS2a	This study
pLSK2	Deletion cassette for ORC4 in pSFS2a	This study
pCaDIS	Plasmid for promoter replacement with *MET3pr*	(Care et al. 1999)
pLSK3	*ORC4* N-terminus cloned in pCaDIS	This study
pLSK4	*MCM2* upstream sequence cloned in pSFS2a	This study
pLSK5	Deletion cassette for *MCM2* in pSFS2a	This study
pLSK6	Deletion cassette for *MCM2* using recyclable *URA3*	
pLSK7	*MCM2* N-terminus cloned in pCaDIS	This study
pASB1	*SCM3* upstream sequence cloned in pSFS2a	This study
pASB2	Deletion cassette for *SCM3* in pSFS2a	This study
pASB3	N-terminus of Scm3 cloned in pCaDIS	This study
pGFP-URA3	*GFP* ORF cloned in pBS-URA3	(Chatterjee et al. 2016)
pNV31	GFP tagging plasmid for Scm3	This study
pNV32	2×GFP tagging plasmid for Scm3	This study
pNdc80-RFP-ARG4	RFP tagging plasmid for Ndc80	(Varshney and Sanyal 2019)

Supplemental table S7. Oligonucleotide primers used in the study

Name	Sequence	Description

ORC4_13	CGGGGTACCTTGGTTTGTAAAAATGTTGTTTC	Deletion cassette for *ORC4*
ORC4_14	CCGCTCGAGAAATAGTTTTACTCTTGAGTTAGC	
ORC4_15	TCCCCGCGGGTTATAGGTTGCTTTTAGTGC	
ORC4_16	TCCCCGCGGGTTATAGGTTGCTTTTAGTGC	
ORC4_11	CGCGGATCCATGAATTCACAGGACC	N-term of Orc4 (For *MET3pr* cloning)
ORC4_12	AACTGCAGTGCCATTTAACTCTTTTAAGGCG	
MCM2_13	CGGGGTACCCTAATCCCATTTTGTTATGAATAT	Deletion cassette for *MCM2*
MCM2_14	CCGCTCGAGGGTTGATTAAATAGTAATGTAATTAATAAAG	
MCM2_15	TCCCCGCGGGTGATTAGTGGGTTATGG	
MCM2_16	CGGAGCTCTGCATTCCAGATTATTTTCTG	
MCM2_11	CGCGGATCCATGTCAAGTCCACCAGCTG	N-term of Mcm2 (For *MET3pr* cloning)
MCM2_12	AACTGCAGGCGTCTTCATCTTCATCATCGTC	
M1	AATTTTATCCAGATTTGATATTATG	TAP tagging of Mcm2 by overlap PCR
M2	TTCCATCTTCTCTTTTCCATCAAAGTATATTTCATAAATTTACTTC	
M3	GAAGTAAATTTATGAAATATACTTTGATGGAAAAGAGAAGATGGAA	
M4	TACAAACAATAACAATAACTATAACGATATCAAGCTTGCCTCGTC	
M5	GACGAGGCAAGCTTGATATCGTTATAGTTATTGTTATTGTTTGTA	
M6	TTCAAGATATTATAAAATAGTCGAA	
CEN1 CORE RT1	CAATCTAGCATTTCCTTCACACA	qPCR for CEN1
CEN1 CORE RT2	TGACGCAATGAAGTAGGTGAT	
CEN2 FP RT	CTCATTCGGAAGATTATAGTACTTGG	qPCR for CEN2
CEN2 RP RT	CATAGTCAATACAATACGTCTTCTG	
CEN3 FP RT	CCTGTGTTGTAAATCAGATCAG	qPCR for CEN3
CEN3 RP RT	CATCCCTTGCTCTATCTTATTCAC	
CEN4 FP RT	GCATTAACGTTCTGCTGTTCTTAG	qPCR for CEN4
CEN4 RP RT	CTCACCGGAACAGACTGAAC	
CACH5F1	CCCGCAAATAAGCAAACACT	qPCR for CEN5
CACH5R1	TTCATGGAAGAGGGGTTTCA	
CEN6 FP RT	CGATTGATCCATCACGATGG	qPCR for CEN6
CEN6 RP RT	CTTTTAGTGAGGATGTATGGGATGC	
nCEN7-3	GCATACCTGACACTGTCGTT	qPCR for CEN7
nCEN7-4	AACGGTGCTACGTTTTTTTA	
CENR FP RT	GGAGCCGCCTAAAACTTTTG	qPCR for CENR
CENR RP RT	CTATTGCCATCCAGGCTG	
7S14 RTF	GGATGTTGAGTTCAAAGCCTG	qPCR of nCEN7-II (neocen at Chr7)
7S14 RTR	CCAGCCAAATAATCTAGCTGC	

7F2 RT	ATTAAATAGAATGCGGCAATACC	qPCR of nCEN7-I (neocen at Chr7)
7R2 RT	ATTTTAAGGATGAGAGGTGTGG	
C1_FP	GGCATAGTGAATAATTCTATAAGCAC	qPCR analysis of strong Orc4 binding regions
C1_RP	CAAGCTATGTGATTTGACTTAATGAAC	
C3_FP	CTACATCGGACTTGTTGTGTC	
C3-RP	CATTCTGAAGGTTACAACATATGC	
C5_FP	GTGGCGATCACATCGG	
C5-RP	CATAACATTTGCAAGGCAAG	
C2_FP	GGCAGCAATATTTGGTGC	
C2_RP	GCCAAGAGATACAAATCAAACAAC	
C4_FP	CGGGGAATCGAACCCC	
C4-RP	CAAGTCAGCTAATTAGCTCAG	
C6_FP	CCTTCTTACGGTGTTGCTG	
C6_RP	GTGAGCAAAAGCGTTATATC	
CR_RP	GAAAGCAATGACTTCATAACCTTG	
CR_RP	CAACAGAAACATGTCAAAGGG	
C7 mid FP	CCTCTTCTCCGTCCTTGAC	qPCR analysis of medium Orc4 binding regions
C7 mid RP	CAGGTGATGAGCCAGAAC	
C2 mid FP	CAGTGGCATAAACTGAATATTCG	
C2 mid RP	CAATTACAACTGGGACCATTTG	
C4 weak FP	GCAGCAGCACCATTAGG	
C4 weak RP	CTTGGAATTTTGCTATGCAAGG	
nLeu2-1	GTACCGAAATTGTCAATGAAG	LEU2 ChIP-qPCR
nLeu2-2	GTGGTGTTTGAAATCAAATTG	
Non-CEN7a	ACTCGCCTTCCCCTCCTTTAAATAG	ChIP PCR at non-CEN7
Non-CEN7b	CCACTACTACGACTGTGGATTCACT	
ASB25	AGTGGTACCCCAGACGACATCAGGTGTTTC	Deletion cassette for *SCM3*
ASB26	CAGCTCGAGGCTATGATTTACGGCAAACAC	
ASB27	TCCCCGCGGTTATGCGGTTTCTGGAGCAG	
ASB28	ATCGAGCTCAATGGCTCAACAAATGATCTTG	
ASB34	CGCGGATCCATGAATACCGGTATTGAAAATGAT	N -term cloning of SCM3 (for *MET3* promoter)
ASB35	TGCACTGCAGAGATTGATAAGTTTCATCGTCG	
ASB36	TTGTCACTTTCTTCCTGTTAAC	Confirmation of *MET3pr* integration
ASB37	CTGCTCCAGAAACCGCATAA	
ASB38	TCTATTATCCCTCGTGGTCAAG	confirmation of *scm3* deletion
NAT mid F	TTAGAGACACAAACGAACAATGTACC	
ASB41	ATGTGATGAGGCATTACTGAAATC	confirmation of *scm3* deletion
PJ3 FRT rev	CTATTCTCTAGAAAGTATAGGAACTTC	
NV158	TCCCCGCGGACTTTCATCTCAAACTGAAGAA	

NV159	CGGACTAGTATTGAATAATTCATCTAT TGATTCATA	Construction of *SCM3-GFP* cassette
SR67	CGCACTAGTATGAGTAAGGGAGAAGAAC TTTTCAC	Construction of *SCM3-2×GFP* cassette
NV250	CGCACTAGT TTTGTATAGTTCATCCAT GCC	

Supplemental table S8. Software and algorithms used

Name	Source/ Reference
Candida Genome Database	http://www.candidagenome.org/
Integrative Genomics Viewer	http://software.broadinstitute.org/software/igv/
ESPript 3.0	http://espript.ibcp.fr/ESPript/ESPript/
MACS2	(Feng et al. 2012)
Bowtie	(Langmead et al. 2009; Langmead and Salzberg 2012)
DIVERSITY	(Mitra et al. 2018)
HiCUP	(Wingett et al. 2015)
DryHiC	(Vidal et al. 2018)
LAMMPS	(Plimpton 1995)

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