DNA replication initiator proteins facilitate CENPA loading on early replicating compact chromatin

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Supplementary Information

All the strains and primers are listed in Supplemental tables S6 and S7, respectively.

Construction of URA3 integration strains

To construct the individual *URA3* integration cassettes, long primer pairs were designed (Supplementary table S7). Briefly, 70 bp regions both upstream and downstream to the site of integration were incorporated in the primers as overhangs. The 1.4 kb *URA3* gene was amplified from the plasmid *pUC19-URA3* (Mitra, Gomez-Raja et al. 2014) using the aforementioned primers. The PCR products were independently transformed in the *C. albicans* J200 (Thakur and Sanyal 2013). The transformants were selected on CM-Uri and confirmed by PCR (Supplemental Table S7). Three independent transformants of each integration type was taken ahead for the assays. All the distances of individual *URA3* insertions are indicated with respect to the mid-point of *CEN7* which has been taken as Ca21Chr7_427262.

Construction of MTW1-Protein A tagged strains

To tag an endogenous copy of *MTW1* with Protein-A, the MTW1-TAP fragment was amplified from CAKS13 (Roy, Burrack et al. 2011) using primers listed in Supplemental table S7. This fragment was then cloned as a *Not*I/*Spe*I fragment in pBS-NAT to obtain the plasmid *pMTW1-TAP(NAT).* This plasmid was linearized by using *Pac*I and the resulting cassette was transformed in strain RM1000AH to obtain LSK436 *(MTW1/MTW1-TAP(NAT)).* Subsequently, the *URA3* cassettes for the 4L and 4R insertions were transformed in LSK436. The neocentromere strains: LSK446, LSK459 (5'FOA sensitive) and LSK450, LSK465 (5'FOA resistant), were transformed with *pMTW1-TAP(NAT)* fragment to obtain the strains LSK469/ LSK470/ LSK473/ LSK474 (5'FOA sensitive) and LSK471/ LSK472/ LSK475/ LSK476 (5'FOA resistant). All strains were confirmed by western blot using anti-Protein A antibodies (Sigma cat. no P3775).

Construction of a conditional orc4 mutant

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 of *ORF19.4221* was amplified using the primers ORC413/ORC414 from the genomic DNA of SC5314 and cloned as a *Kpn*I/*Xho*I fragment into pSFS2a (Reuss, Vik et al. 2004) to create pORC4US. A 490 bp fragment (Ca21Chr5 478025-477535) downstream to Orf19.4221was amplified using ORC4 15/ORC416 and cloned as *Sac*II/*Sac*I fragment into pORC4US to generate pORC4DEL (Supplemental table S7 for primer list). The resulting plasmid was linearized using *Kpn*I and *Sac*I, and used to transform *C. albicans* 8675 (Joglekar, Bouck et al. 2008) and selected for nourseothricin resistance to obtain the strain LSK328. The marker was recycled to obtain the nourseothricin sensitive strain LSK329. To conditionally inactivate the remaining allele, a conditional mutant was constructed by cloning the N-terminus of

Orf19.4221(Ca21Chr5 479720-479221) as a *Bam*HI/*Pst*I fragment in pCaDIS (Care, Trevethick et al. 1999). The resulting plasmid (pMET3ORC4) was linearized using *Bgl*II and transformed in LSK329 to obtain independent transformants of the conditional mutant LSK330, LSK331. Similar deletions were performed in SN148 background.

Construction of a conditional mcm2 mutant

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 of *ORF19.4354* was amplified using the primers MCM213/MCM214 from the genomic DNA of SC5314 and cloned as a *Kpn*I/*Xho*I fragment in pSFS2a to create pMCM2US. A 468 bp fragment (Ca21ChrR 853962-853494) downstream to Orf19.4354 was amplified using MCM215/MCM216 and cloned as *Sac*II/*Sac*I fragment in pMCM2US to generate pMCM2DEL (See Supplemental table S7 for primer list). The plasmid was digested using *Kpn*I and *Sac*I, used to transform *C. albicans* 8675 and selected for nourseothricin resistance to obtain the strain LSK309. The marker was recycled to obtain the nourseothricin sensitive strain LSK310. To inactivate the remaining allele, a conditional mutant was constructed by cloning the N-terminus of Orf19.4354 (Ca21ChrR 856674-856164) as a *Bam*HI/*Pst*I fragment in pCaDIS (47). The resulting plasmid (pMET3MCM2) was linearized using *Bgl*II and used to transform LSK310 to obtain independent transformants of the conditional mutant LSK311, LSK312 and LSK313. Similar deletions were performed in SN148 background.

Construction of the CEN7 deletion strains (CaCEN7)

To delete one copy of *CEN7*, a cassette was constructed as follows. A 1.4 kb fragment containing a 66 bp upstream sequence (Ca21Chr7 424413-424472) and a 70 bp downstream sequence (Ca21Chr7 428994-429053) of *CEN7* and a marker gene (*CaHIS1*) were amplified from pBS-HIS using the primers mentioned in Supplementary Table S7. The PCR product was used to transform the 5’FOA resistant isolates from the strains LSK443 and LSK456 and their corresponding 5’FOA sensitive isolates. The transformants were selected on complete media lacking histidine (CM-His) and screened by PCR. Transformants in *cis*-orientation (for *URA3* and *HIS1*) were screened on the basis of Southern hybridisation (Southern 1975) (see Supplemental table S3 for Southern strategy).

Generation of Orc4 antibodies

The peptide sequence from *C. albicans* Orc4 (YLPKRKIDKEESSI) was chemically synthesized and conjugated with Keyhole Limpet Hemocyanin (KLH) (GeneMed Synthesis, USA). The conjugated peptide (1 mg/ml) was mixed with equal volumes of Freund’s complete adjuvant (Sigma, Cat no. F5881) and used as an antigen to inject non-immunized rabbits as the priming dose. Three subsequent booster doses at an interval of two weeks (per immunization) were given using Freund’s incomplete adjuvant (Sigma, Cat no. F5506). Following antibody detection using ELISA, major bleed was

performed. The anti-serum was collected, IgG fractionated and affinity purified against the free peptide (AbGenex, India). The specificity of the purified antibody preparation was confirmed by western blot and immunolocalization experiments.

Media and growth conditions

All strains of *C. albicans* where *URA3* was integrated on Chr7 and Chr5 were propagated in YPD (1% yeast extract, 2% peptone, 2% dextrose) with uridine, unless otherwise specified. All transformations were done in YPDU. The auxotrophs were selected on appropriate selection media, as mentioned previously. For the 5'FOA plating assays, complete media with 2 % agar were supplemented with 1 mg/ml 5'FOA. ChIP experiments for the silenced colonies were done in a) complete media supplemented with 10 mg/ml uridine and 1mg/ml 5'FOA (CM+5'FOA) and b) CM-Uri. Strains with neocentromeres were grown in YPDU. *ORC4* and *MCM2* mutants were grown either in CM-methionine-cysteine or in CM + 5mM methionine +5mM cysteine for the indicated number of hours. CAKS3b (Sanyal and Carbon 2002) was grown in YP with succinate (2%) for expressing CENPA and YP with dextrose (2%) for depleting CENPA for 6 and 8 h for the ChIP experiments.

Silencing assay

Each of the *URA3* integrant was grown in YPDU overnight. Approximately, one million cells from three independent transformants of each kind of integration were plated on CM with 1 mg/ml 5'FOA. The plates were incubated at 30ºC up to 72 h. One hundred colonies from each plate were patched on CM-Uri and YPDU. These were simultaneously patched on CM-His and CM-Arg plates to detect events such as loss of the marker gene *URA3* or gene conversion. The colonies showing growth in CM-Uri were counted and the percentage of reversible silencing was determined. These colonies were then taken from the corresponding YPD patch and streaked on CM+5'FOA plates to obtain single 5'FOA resistant colonies for the subsequent ChIP assays.

Chromatin Immunoprecipitation (ChIP)

ChIP experiments for the reversibly silenced colonies were performed as follows. Each colony that was isolated from CM+5'FOA media was inoculated simultaneously in liquid media of CM + 5'FOA and CM-Uri and grown till log phase. Crosslinking was done for 15 min (for CENPA) or 30 min (for Mtw1) using formaldehyde to a final concentration of 1% and cells were quenched using 0.135 mM glycine for 5 min at room temperature. For Orc4 ChIP, cultures were grown in YPDU and crosslinked for 1 h, and processed similarly. Quenched cells were incubated in a reducing environment in presence of 9.5 ml distilled water and 0.5 ml of beta mercapto-ethanol (HiMedia cat no. MB041). Rest of the protocol was followed from (Yadav, Sun et al. 2018). The DNA pellet was finally resuspended in 20 µl of MilliQ water. All three samples (I, +, -) were subjected to PCR reactions.

Serial passaging of 5'FOA resistant strains in YPDU

5'FOA resistant colonies obtained from two independent transformants of LSK404 and LSK425 were inoculated from their respective glycerol stocks in CM+5'FOA. These cells were harvested, washed and reinoculated into YPDU. Cultures were monitored for their growth and samples were withdrawn after every 4 doublings, for ChIP, with F_0 being the initial culture grown in 5'FOA. Y_4, Y_8, Y_{12}, Y_{20}, Y_{24} correspond to these reversibly silenced 5'FOA colonies grown on non-selective media for the indicated number of generations. Approximately 50 O.D. cells were harvested from each time point and ChIP was performed using anti-Protein A antibodies to examine the CENPA occupancy in these colonies. The cells from the last time point (Y_{24}) were washed and resuspended in fresh CM+ 5'FOA. Cells from the indicated time points (Y_{12}, Y_{24}) were washed and resuspend in sterile water. Serial dilutions of these along with the parental *URA3* insertion and 5'FOA resistant colony were made and spotted on CM-Uri and CM + 5'FOA. Plates were incubated for 72 h at 30ºC and photographed.

Antibodies used

For western blot analysis, we used rabbit anti-Protein A in 1:5000 dilution, anti-Orc4 (1:1000) and anti-PSTAIRE (Abcam, cat No. 9866) in the dilution of 1: 5000. For ChIP, anti-Prot A (3ug/ ml), rabbit anti-Orc4 antibodies (10 µg/ml) and mouse anti-GFP (Roche, cat no. 11814460001) (4 ug/ml) were used.

Western blotting

Approximately 3 O.D. equivalent cells 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 40 min at 25V. Following protein transfer, the blot was blocked with 5% skimmed milk for an hour. The blot was incubated with primary antibodies (see *Antibodies used*). The blot was washed thrice in PBST (1X PBS + 0.05% Tween) and incubated with goat anti-rabbit IgG-HRP (1:10,000 Bangalore Genei cat No. 105499). Following three PBST washes, the blot was developed using chemi-luminescence method (Super Signal West Pico Chemiluminescent substrate, Thermo scientific, cat No. 34080)

Indirect immuno-fluorescence

Exponentially grown cultures of SC5314 was fixed with 37% formaldehyde. Spheroplasts were made using lysing enzyme and cells were fixed on poly-lysine coated slides using methanol and acetone. Cells were then incubated with 2% skimmed milk to block non-specific binding. Following ten PBS washes, cells were incubated with anti-Orc4 antibodies (1:100) for 1 h in a humid chamber. Post PBS washing, cells were incubated with the Alexa Fluor goat anti-rabbit IgG 568 (Invitrogen, cat. No. 11011) in the dilution of 1:500 for one hour. The slide was mounted on a coverslip using DAPI (10

ng/ul) Sigma cat no. 10236276001. Microscopic images were captured by a laser confocal microscope (Carl Zeiss, Germany) using LSM 510 META software with He/Ne laser (bandpass 565-615 nm) for Alexafluor 568 and a 2-photon laser near IR (bandpass~780 nm) for DAPI. Z-stacks were collected at 0.4-0.5 µm intervals and stacked projection images were processed in Adobe Photoshop.

Microscopy

For conditional expression of genes under the *MET3* promoter, cells were grown in permissive media (CM -met-cys) overnight. They were then grown in presence of CM + 5 mM met+5mM cys for the indicated time point, corresponding to the repressive phenotype. In each case, the cells were washed twice with water and resuspended in distilled water which was placed on a 2% agarose bed on a glass slide. Images were captured in 100x using Zeiss Axio Observer 7 and processed using ImageJ and Adobe photoshop.

ChIP-qPCR analysis

The input and IP DNA were diluted appropriately and qPCR reactions were set up using primers listed in Supplemental table S7. The CENPA/ Mtw1/Orc4 enrichment was determined by the percentage input method. Two- way ANOVA and Bonferonni post tests were performed to determine statistical significance. All the percent IP values represented in the graphs comparing enrichment values in CM-Uri and CM+5'FOA are the ratio of percent IP of the regions indicated to the corresponding values of *CEN1*, which was used as an internal control to estimate the efficiency of the pulldown. For the ChIP experiments with the neocentromere strains, these values have not been normalised to *CEN1*.

ChIP-sequencing analysis

For the CENPA ChIP-seq, immunoprecipitated DNA and the corresponding DNA from whole cell extracts from strains LSK450 and LSK465 were quantified using Qubit before proceeding for library preparation. Around 5 ng ChIP and total DNA were used to prepare sequencing libraries using NEBNext Ultra DNA library preparation kit for Illumina (NEB, USA). The library quality and quantity were checked using Qubit HS DNA (Thermo Fisher Scientific, USA) and Bioanalyzer DNA high sensitivity kits (Agilent Technologies, USA) respectively. The QC passed libraries were sequenced on Illumina HiSeq 2500 (Illumina Inc., USA). HiSeq rapid cluster and SBS kits v2 were to generate 50 bp single end reads. The reads were independently aligned onto the *C. albicans* SC5314 reference genome (v. 21) and a genome with an altered version of Chr7 using bowtie2 (v. 2.3.2) aligner. For the Orc4 ChIP-seq, subtracted reads were aligned onto the *C. albicans* SC5314 reference genome (v. 21) using bowtie2 (v. 2.3.2) aligner (Langmead, Trapnell et al. 2009). More than 95% of the reads mapped onto the reference genome (Control:97.74%; IP:96.13%). All the alignment files (BAM) were processed to remove PCR duplicate reads using Mark Duplicates module of Picard tools.

These processed BAM files were further taken for identification of peaks by MACS2. These peaks were annotated with the *C. albicans* SC5314 reference and altered assembly annotation files. Visualisation of the aligned reads (BAM files) on the reference genome was performed using Integrative Genome Viewer (IGV) (https://software.broadinstitute.org/software/igv/).

Hi-C analysis

Wild-type *C. albicans* Hi-C data was downloaded from PRJNA308106 (Burrack, Hutton et al. 2016). FASTQ files containing 2 X 80bp paired-end (PE) reads were analyzed using hiclib package (http://mirnylab.bitbucket.org/hiclib/) (Imakaev, Fudenberg et al. 2012). First, each side of the PE reads was aligned separately to *C. albicans* reference genome (Ca21) using Bowtie 2 (Langmead and Salzberg 2012) with default parameters except for --very-sensitive option. This step was executed iteratively (iterative mapping) in which 3´ truncated reads was aligned to reference genome, starting from first 20 bases with increment of 5 bases in subsequent iteration till it reached to the end of read length. The reads which were uniquely mapped with MAPQ score ≥ 1 were saved at each iteration and rest were subsequently analyzed in next iteration. The alignment results from both sides were paired, keeping those reads which had at least one side aligned and were assigned to restriction fragments. The output read pairs and their alignment information as well as the assigned restriction fragments were saved in HDF5 file format. The fragment filter then removed reads those have: 1) only one side aligned; 2) both sides aligned to same restriction fragment; 3) two sides which were too close to each other. PCR redundancies (duplicates) were also removed and all the unique valid pairs were binned into genomic intervals of 2 kb-5 kb (bin size). The resulted symmetric matrix was processed for bin filtering step, including removal of bins with <50% sequence information in reference genome and removal of 1% bins with low read coverage. Diagonal bins were excluded from further downstream analysis. The genome-wide interaction matrix was generated following bin bias correction as described (Imakaev, Fudenberg et al. 2012). The interaction matrix was then converted to a contact probability matrix where the sum of values in each row/column approached 1. The 3C profile anchored on a bin was generated using single row/column containing the anchor from the matrix. To plot distance-dependent contact probability curves, the mean *cis* contact probabilities (excluding the bins with 0 values) were calculated for each distance (bin size=2kb) for pericentric and non-pericentric regions. Mann-Whitney U test was performed for pericentric and all *cis* interactions, as well as for pericentric and non-pericentric interactions.

For the Orc4 binding regions, Hi-C interactions were analyzed according to the chromosome coordinates, different modes identified by DIVERSITY and also based on replication timing (early and late). 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 origins, using the same scaling and colour 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

For motif analysis, the *de novo* motif discovery tool DIVERSITY (Mitra, Biswas 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 ORC binding regions, fully processed timing data available in GSE17963_final_data.txt (Koren, Tsai et al. 2010) was used. A larger replication time value implies earlier replication. All the 414 genomic origins were aligned according to their timing scores, and categorized as early (first 207) and late (last 207) origins.

Supplementary figures

Figure S1. Mapping the inter and intra-chromosomal interactions in *C. albicans*.

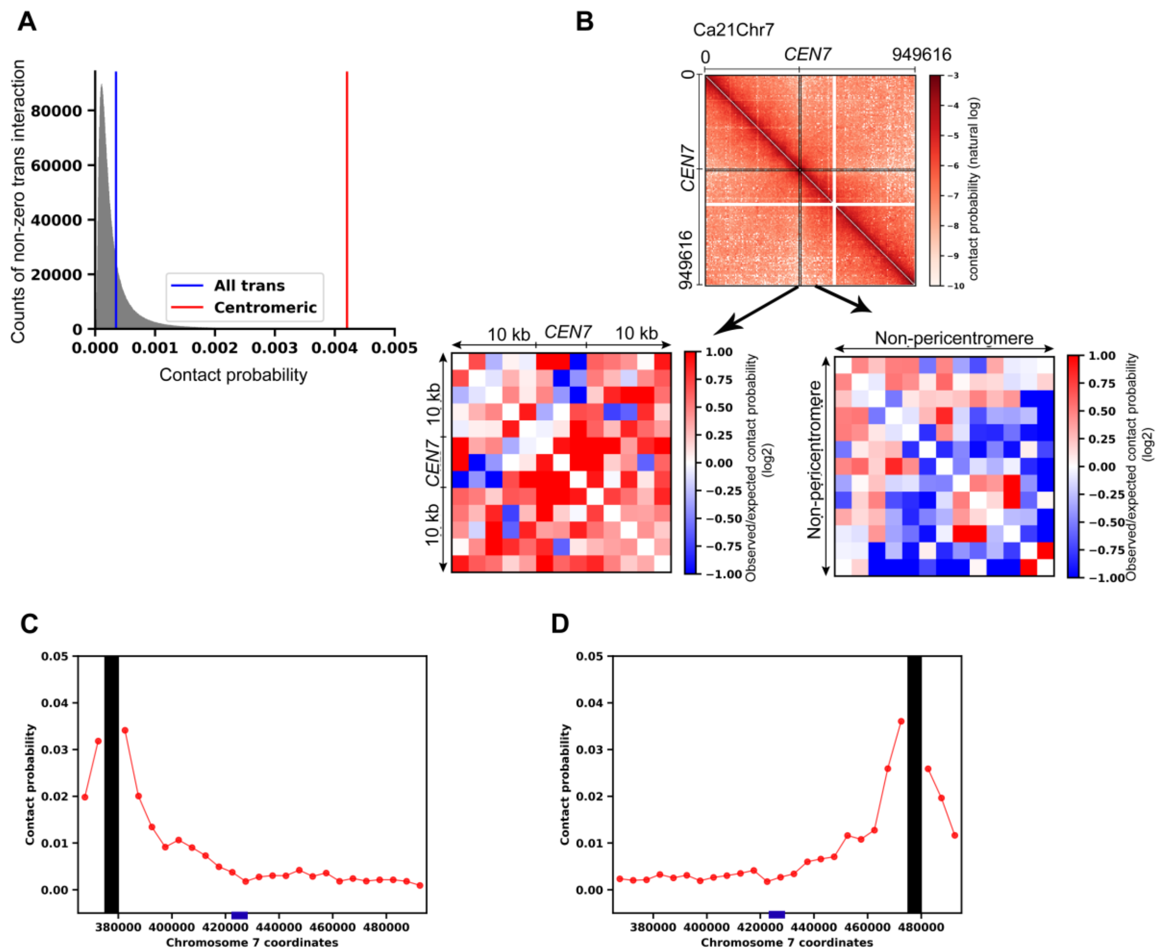
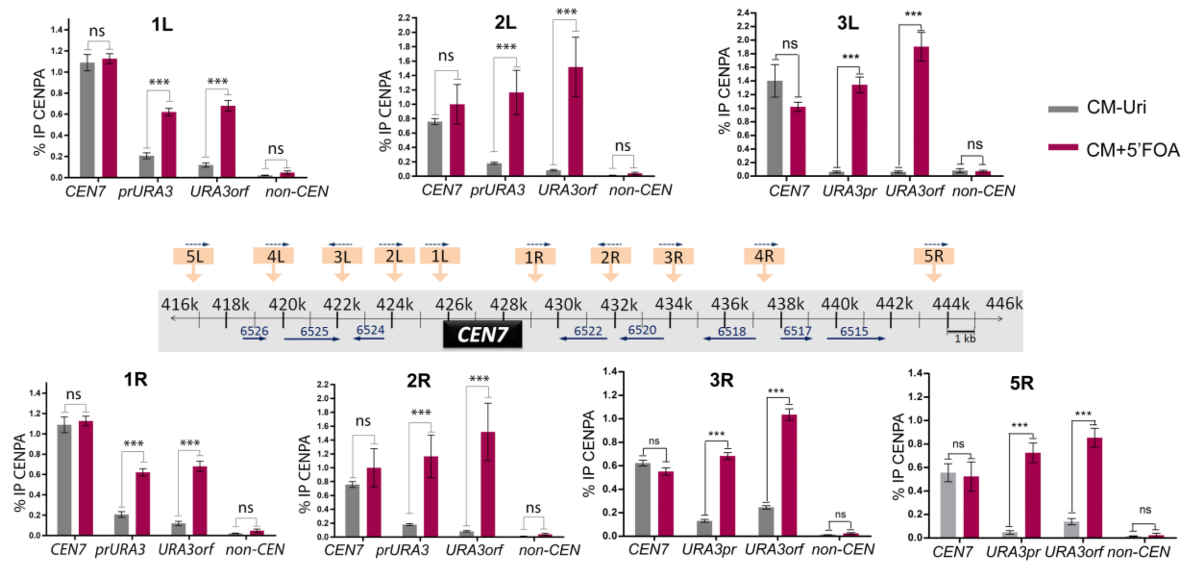
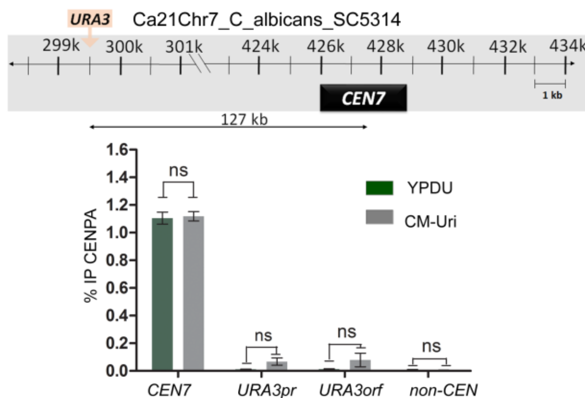


Figure S1. The inter and intra-chromosomal interactions in *C. albicans*. (A) A histogram of non-zero *trans* contact probabilities (grey) from the genome-wide interaction matrix depicts that the mean contact probability of all *trans* (black line) is much lower than interactions among centromeric bins (red) (bin size=2kb). (**B**) Heatmaps of observed/expected contact probabilities (bin size=2kb) at Chr7 zoomed into a pericentromeric region (left) and a non-pericentric region (Chr7:440000-466000) with same size (right). The expected matrix was obtained from mean contact probabilities of all *cis* interactions at each distance. (**C**) and (**D**) The 3C profile (bin size=5kb) anchored on a bin 50 kb upstream (Chr7:375000-380000) (black) (B) and another bin 50kb downstream (Chr7:475000-480000) (black) (C) of *CEN7* (blue box). The red dots represent contact probabilities.

Figure S2





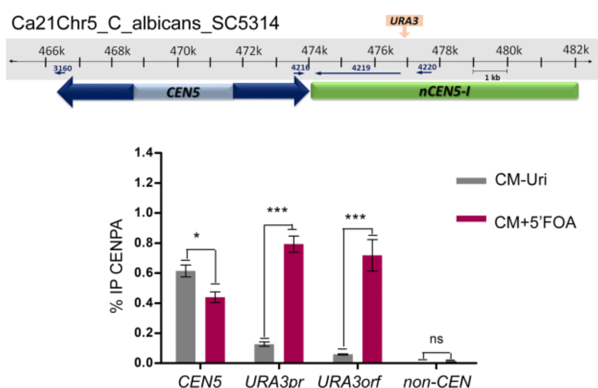


Figure S2. Ectopic centromeres are formed at pericentromeric regions of *C. albicans*. (A) Schematic of *URA3* (orange boxes) integrated at pericentromeres of Chr7 is shown (middle panel). Arrowheads and numbers indicate positions and identities of the ORFs. Corresponding sites (1L,2L…5R) are mentioned as graph titles (see Supplemental table S1 for integration coordinates). Standard ChIP-qPCR analysis (using anti-protein A antibodies) of the 5'FOA resistant colonies obtained from these strains was used to compare CENPA enrichment on the indicated loci (*CEN7*, *URA3pr*, *URA3orf*, non-*CEN* region) in CM-Uri (grey) and CM+5'FOA (red). **(B)** Schematic of *URA3* integration at a far-CEN locus, 127 kb away from *CEN7*. ChIP q-PCR results of this strain in YPDU (green bar) and CM-Uri (grey bar) show no significant enrichment of CENPA at the *URA3* locus. (**C**) *CEN5* of *C. albicans*, contains a mid-core (light blue) flanked by inverted repeats (dark blue arrows). *URA3* was integrated at the indicated location, at one of the neocentromere hotspots (*nCEN5-II*). ChIP qPCR results for the 5'FOA resistant colonies obtained from the integrant was grown in CM + 5'FOA (red bar) and CM- Uri (grey bar). Percent input values were normalised to corresponding values on *CEN1*. Statistical significance was determined by two-way ANOVA followed by Bonferroni post-tests (*** p<0.001, ** p<0.01, ns: p>0.05).

Figure S3

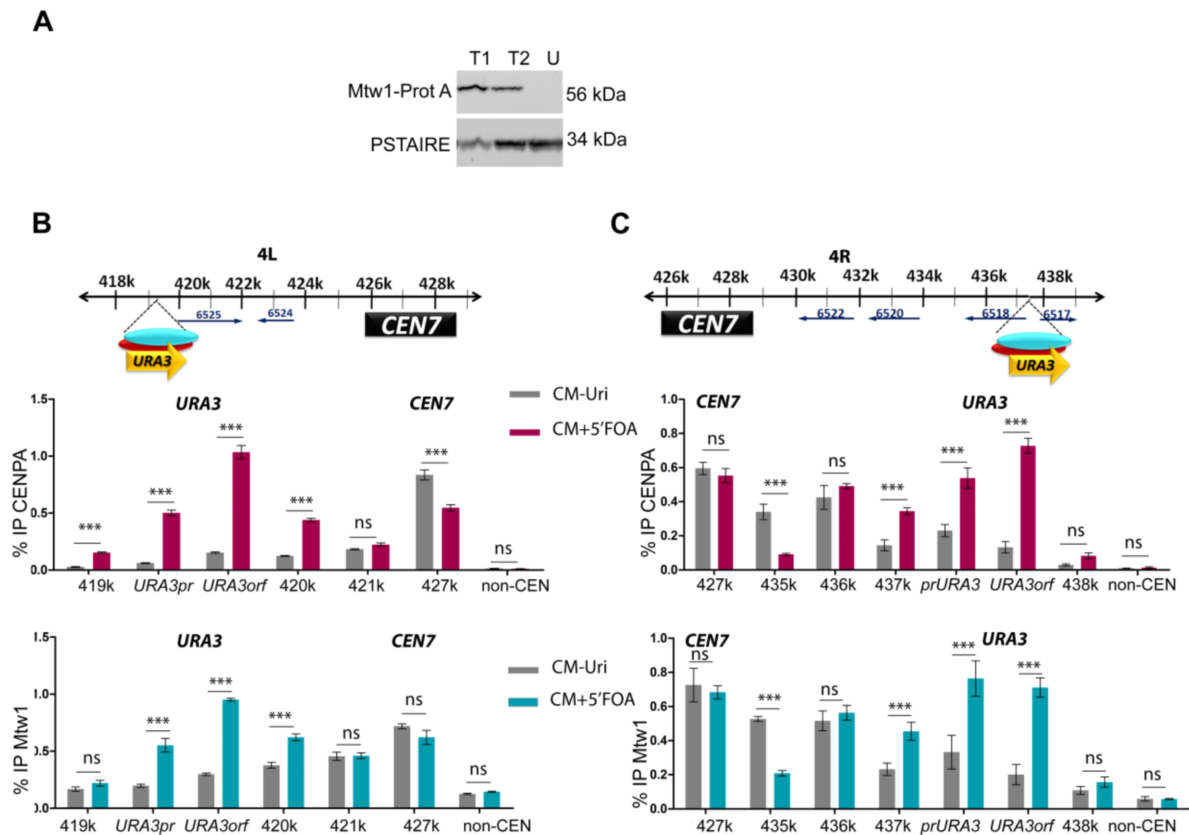


Figure S3. Kinetochore binding to ectopic centromeres is restricted to the silent *URA3* locus. (A) Western blot analysis determines the expression level of the endogenous copy of *MTW1* tagged with Protein A (Prot A) in the strain RM1000AH. Mtw1-Prot A could be detected as a 56 kDa band (T_1, T_2) which was absent in the untagged control (U). PSTAIRE was used as the loading control. **(B)** Both CENPA and Mtw1 bind to the ectopic centromere at *URA3* when the 5'FOA resistant colonies from LSK404 (*4L/4L::URA3 CSE4/CSE4-TAP)* (top panels) and LSK437 (*4L/4L::URA3 CEN7 MTW1/MTW1-TAP)* (bottom panels), are grown in CM +5'FOA (red/ blue bar) or CM-Uri (grey bar). Primers flanking the *URA3* locus (Supplementary table S7) were used to check for the extended binding of CENPA and Mtw1 beyond *URA3*. **(C)** Similar ChIP-qPCR assays were done for 5'FOA resistant colonies from LSK425 (*4R/4R::URA3 CEN7 CSE4/CSE4-TAP)* and LSK440 (*4R/4R::URA3 MTW1/MTW1-TAP).* Percent input values were normalised to *CEN1*. ChIP q-PCR was performed in three independent transformants and technical triplicates for each transformant. Statistical significance was determined by two-way ANOVA followed by Bonferroni post-tests (*** $p<0.001$, ** $p<0.01$, ns: $p>0.05$).

Figure S4

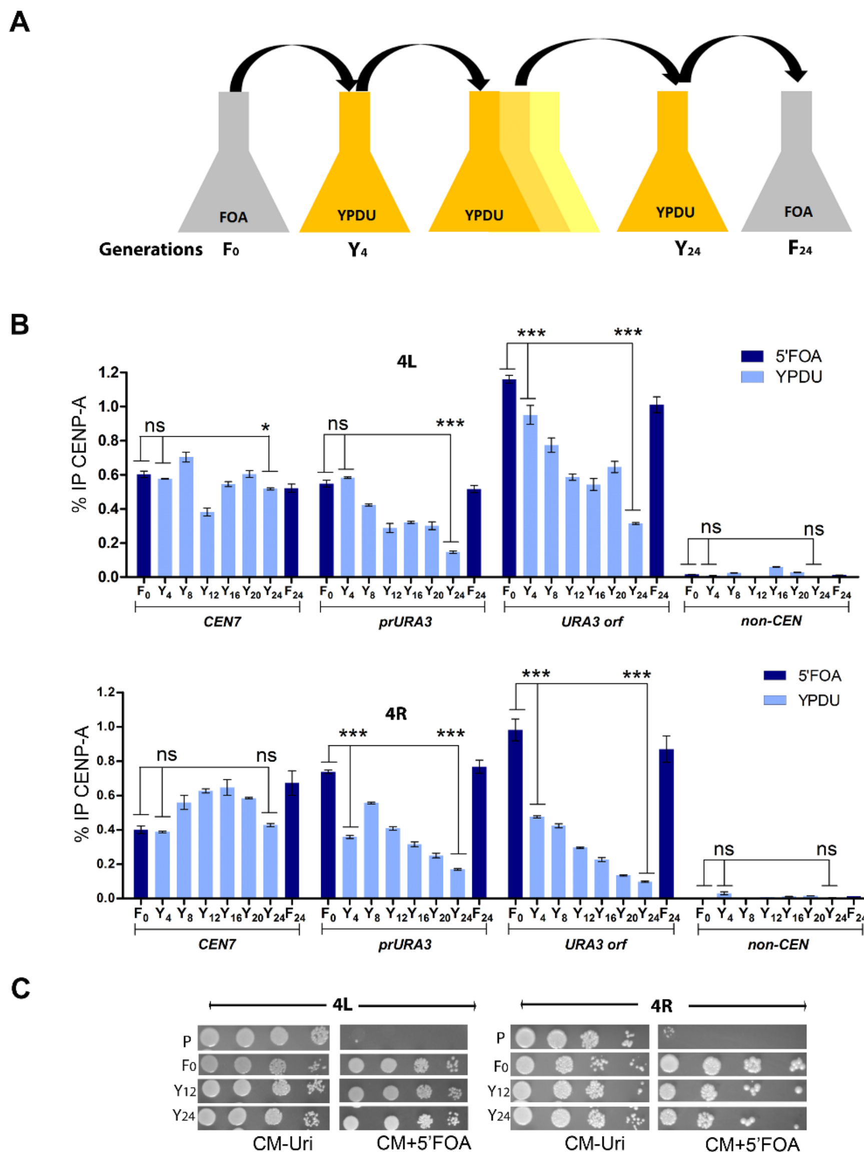


Figure S4. Ectopic kinetochore formed at *URA3* is transient and unstable. (A) Schematic of the experiment showing serial passaging of 5'FOA resistant colonies obtained from the strains LSK404 (*4L/4L::URA3 CSE4/CSE4-TAP*) and LSK425 (*4R/4R::URA3 CSE4/CSE4-TAP*) in non-selective media (YPDU). The primary 5'FOA resistant colony was grown in YPDU for the indicated number of generations and then regrown in CM+5'FOA. **(B)** ChIP using anti-Protein A antibodies followed by qPCR analysis reveals a steady decline in enrichment at *URA3* when cells were passaged in the non-selective media (light blue) Percent input values were normalised to *CEN1*. ChIP-qPCR was performed in two independent transformants of 4L (top) and 4R (bottom) with technical triplicates for each transformant. Statistical significance was determined by one-way ANOVA followed by Bonferroni post-tests (*** $p<0.001$, ** $p<0.01$, ns: $p>0.05$). **(C)** A spotting assay showing the frequency of reversible silencing of the 5'FOA resistant colonies from strains 4L (left) and 4R (right) after they were grown in non-selective media for the indicated number of generations. Individual panels show serially diluted cultures of the 5'FOA sensitive strain (P), primary 5'FOA resistant colony (F_0) and 5'FOA resistant colony grown in YPD for 12 (Y_{12}) and 24 (Y_{24}) generations, spotted on CM+5'FOA and CM-Uri plates. Plates were incubated for 48 h at 30^{0}C and then photographed.

Figure S5

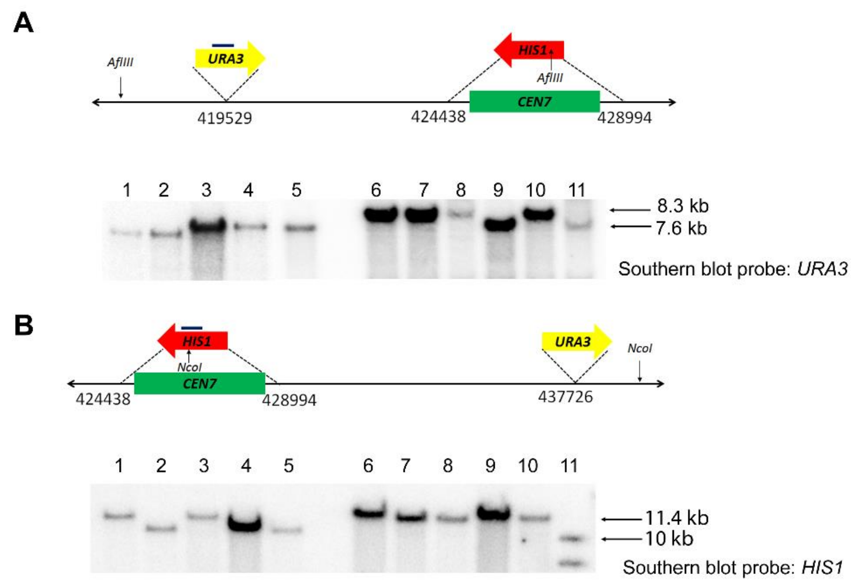


Figure S5. Southern analysis of *CEN7* deletion strains. (A) A line diagram showing restriction sites of pericentromeres of Chr7 when *URA3* (yellow arrow) is located at 4L (7.7 kb left of *CEN7)*. *CEN7* (CaChr7 424475-428993) (green) has been replaced with *HIS1* (red). Genomic DNA from strains LSK445, LSK446, LSK 447, LSK 448, LSK449, LSK450, LSK451, LSK452, LSK453, LSK454 and LSK455 (lanes 1-11) were digested with *Afl*III, Southern hybridized and probed with a *URA3* fragment. The desired band of 8.3 kb suggests the presence of *URA3* and *HIS1* on the same homolog of Chr7. **(B)** A line diagram showing restriction digestion of pericentromeres of Chr7 when *URA3* is located at 4R (10.4 kb right of *CEN7)*. *CEN7* (CaChr7 424475-428993) has been replaced with *HIS1*. Genomic DNA from strains LSK459, LSK460, LSK461, LSK462, LSK463, LSK464, LSK465, LSK466, LSK467 and LSK468 (lanes 1-10) were digested with *Nco*I, Southern hybridized and probed with a *HIS1* fragment. The desired band of 11.4 kb suggests the presence of *URA3* and *HIS1* on the same homolog of Chr7.

Figure S6

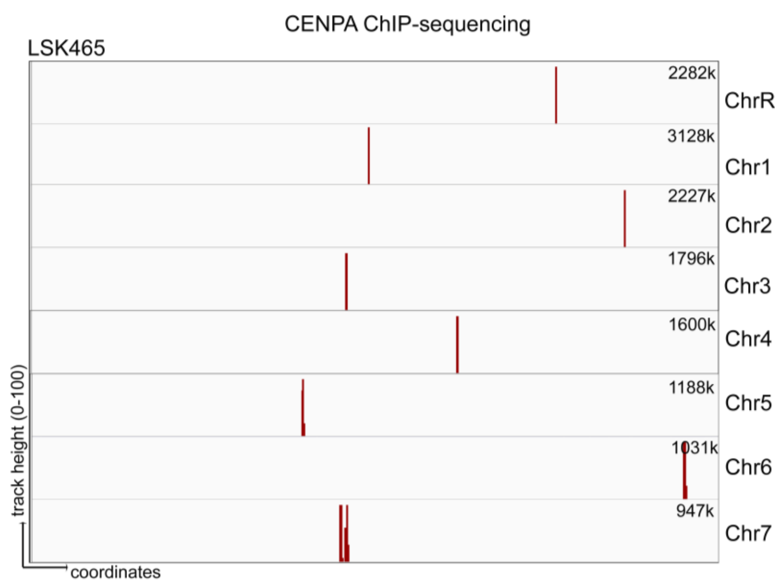


Figure S6. CENPA ChIP sequencing of a *CEN7* deletion strain. ChIP-sequencing using anti-GFP antibodies in the strain LSK465 (*CSE4/CSE4-GFP-CSE4 4R/4R::URA3 CEN7/CEN7::HIS1)* reveals a single peak on all chromosomes, except Chr7 that shows two closely spaced CENPA peaks, centromere (*CEN7*) and neocentromere (*URA3nCEN7-II).*

Figure S7

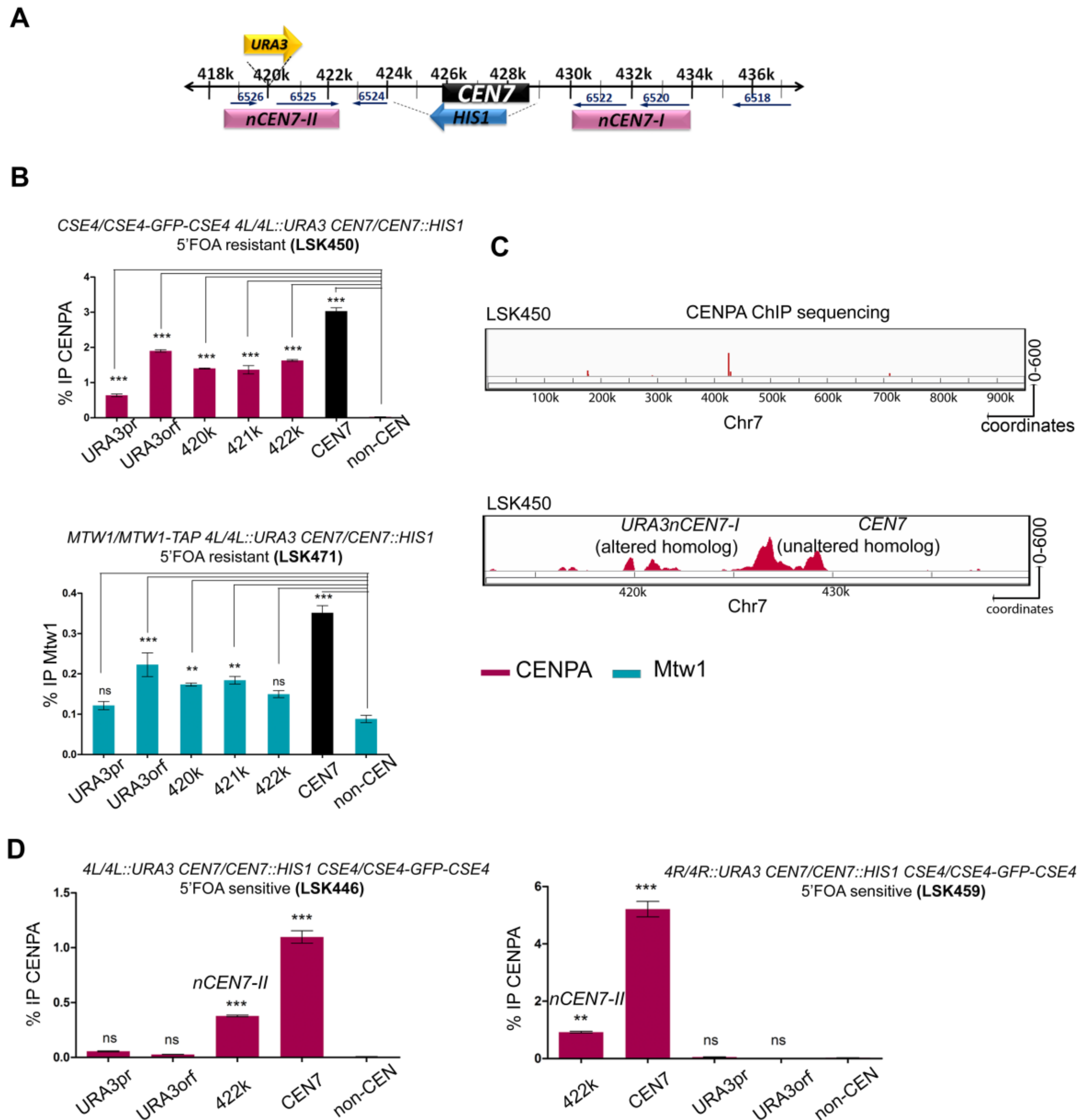


Figure S7. The number of CENPA molecules at a CEN proximal region determines the site of neocentromere formation. (A) In the diploid *C. albicans*, only one homolog of Chr7 where *CEN7* (CaChr7 424475-428994) has been replaced with *HIS1* in a *URA3* integrant (CaChr7 419529-419530) is shown. **(B)** Top panel indicates relative enrichment of CENPA at native *CEN7* from the unaltered homolog (black) and the neocentromere locus *URA3nCEN7-I* (red) in the 5'FOA resistant strain LSK450 (*CSE4/CSE4-GFP-CSE4 4L/4L::URA3 CEN7/CEN7::HIS1)*. Bottom panel indicates relative enrichment of Mtw1 at *CEN7* (black bar) and *URA3nCEN7-I* (blue) at the native centromere (427k) in LSK471 *(CSE4/CSE4-GFP-CSE4 4L/4L::URA3 CEN7/CEN7::HIS1 MTW1/MTW1-TAP)*. Relative enrichment of CENPA and Mtw1 indicate that neocentromere formed on the altered homolog

(*URA3nCEN7-II*) was mapped to a region surrounding the integration locus (CaChr7 435078-440387) (***p<0.001, ** p<0.01, ns p>0.05). **(C)** CENPA ChIP-sequencing confirmed the presence of neocentromere in the strain LSK450, where the profile is a combination of two peaks, the one at *CEN7* is on the unaltered homolog the one at *URA3nCEN7-I* is on the altered homolog. A 30 kb region harbouring *CEN7* depicts the track height (as on IGV) on *y*-axis and coordinates on the *x*-axis. **(D)** CENPA ChIP followed by qPCR in the 5'FOA sensitive strains LSK446 (*CSE4/CSE4-GFP-CSE4 4L/4L::URA3 CEN7/CEN7::HIS1)* (left panel) and LSK459 (*CSE4/CSE4-GFP-CSE4 4R/4R::URA3 CEN7/CEN7::HIS1)* (right panel) indicates that neocentromeres are activated at the previously identified hotspot *nCEN7-II*. There was no CENPA enrichment seen on *URA3*. The experiment was performed in two independent transformants for each type of neocentric strain. Statistical significance was determined by one-way ANOVA followed by Bonferroni post-test (*** p<0.001, ** p<0.01, ns: p>0.05).

Figure S8

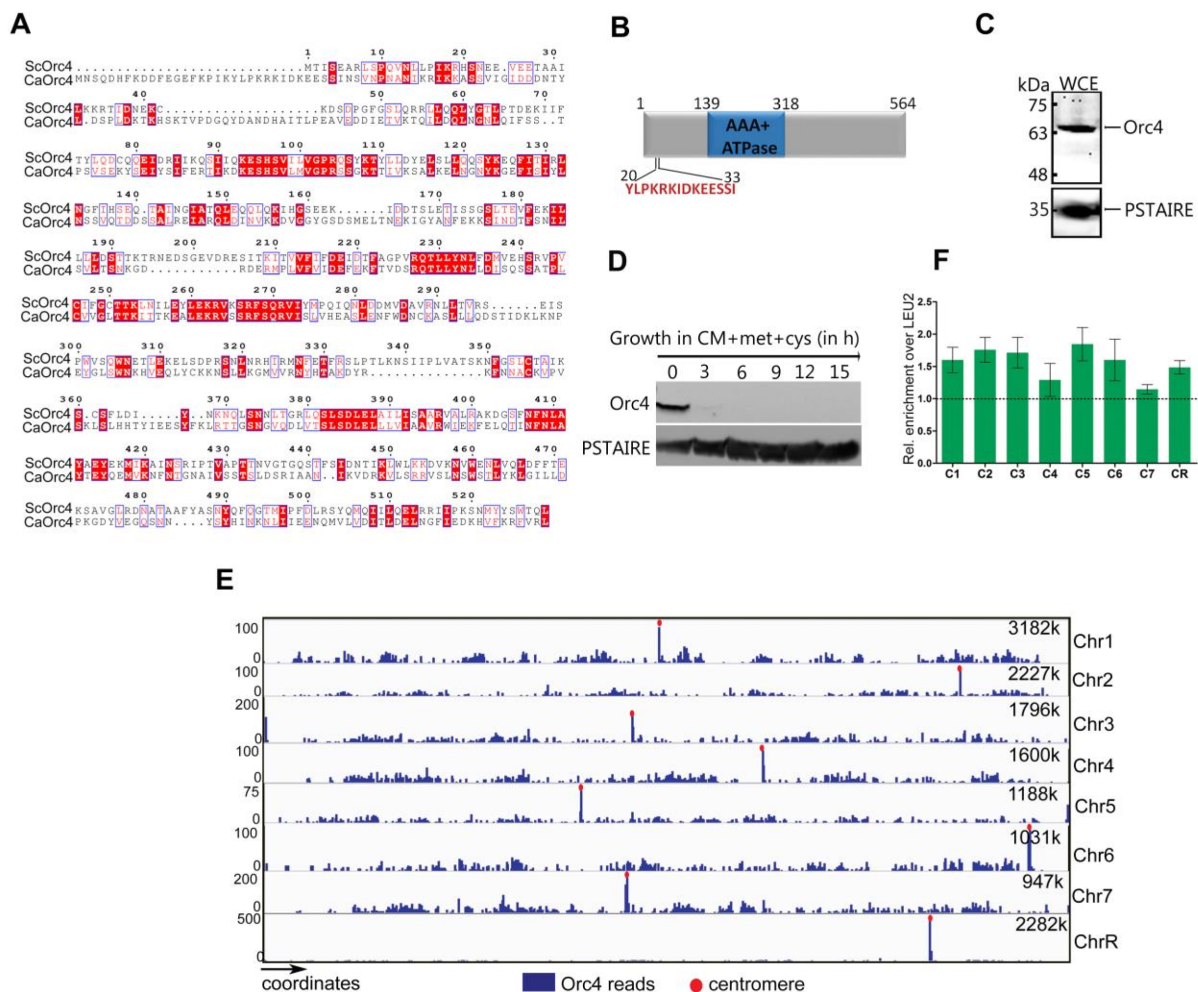


Figure S8. Expression and *in vivo* localisation of CaOrc4. (**A**) Multiple sequence alignment of ScOrc4 (query sequence) with the *C. albicans* homolog, CaOrc4 reveals conserved stretched of amino acids from constituting the AAA+ ATPase domain (aa 139-318). (**B**) Domain architecture of Orc4 reveals a 564 aa long polypeptide consisting of a central AAA+ ATPase domain. The peptide sequence chosen to raise the antibodies has been highlighted in red letters (residues 20-33). (**C**)

Expression of Orc4 was verified by western blot with anti-Orc4 antibodies using whole cell extract (WCE) from *C. albicans* SC5314. Orc4 yielded a band at the expected molecular weight at ~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 LSK330 when the strain was grown for the indicated time (0, 3h, 6h, 9h, 12h, 15h) in presence of 5mM met +5mM cys. PSTAIRE was used as the loading control. (**E**) ChIP-sequencing analysis revealed that Orc4 binds to discrete genomic sites in *C. albicans*. The total Orc4 reads (blue histogram) were obtained by subtracting the relative number of sequencing reads from the whole cell lysate from the Orc4 ChIP sequence reads and aligning them to the reference genome *C. albicans* SC5314 Assembly 21. The *x*-axis represents chromosome coordinates while the *y*-axis represents track height as visualised in IGV. (**F**) Orc4 ChIP followed by standard qPCR assays were used to validate the binding of Orc4 on one region in each of the eight *C. albicans* chromosomes. Primers corresponding to an Orc4-enriched region on each chromosome (C1- C7, CR) were used to amplify the Orc4 ChIP DNA. qPCR analysis was performed to calculate the relative enrichment of Orc4 at each of these chromosomal regions over a control region (*LEU2*). Relative enrichment values were plotted as mean of three technical replicates ± SD.

Figure S9

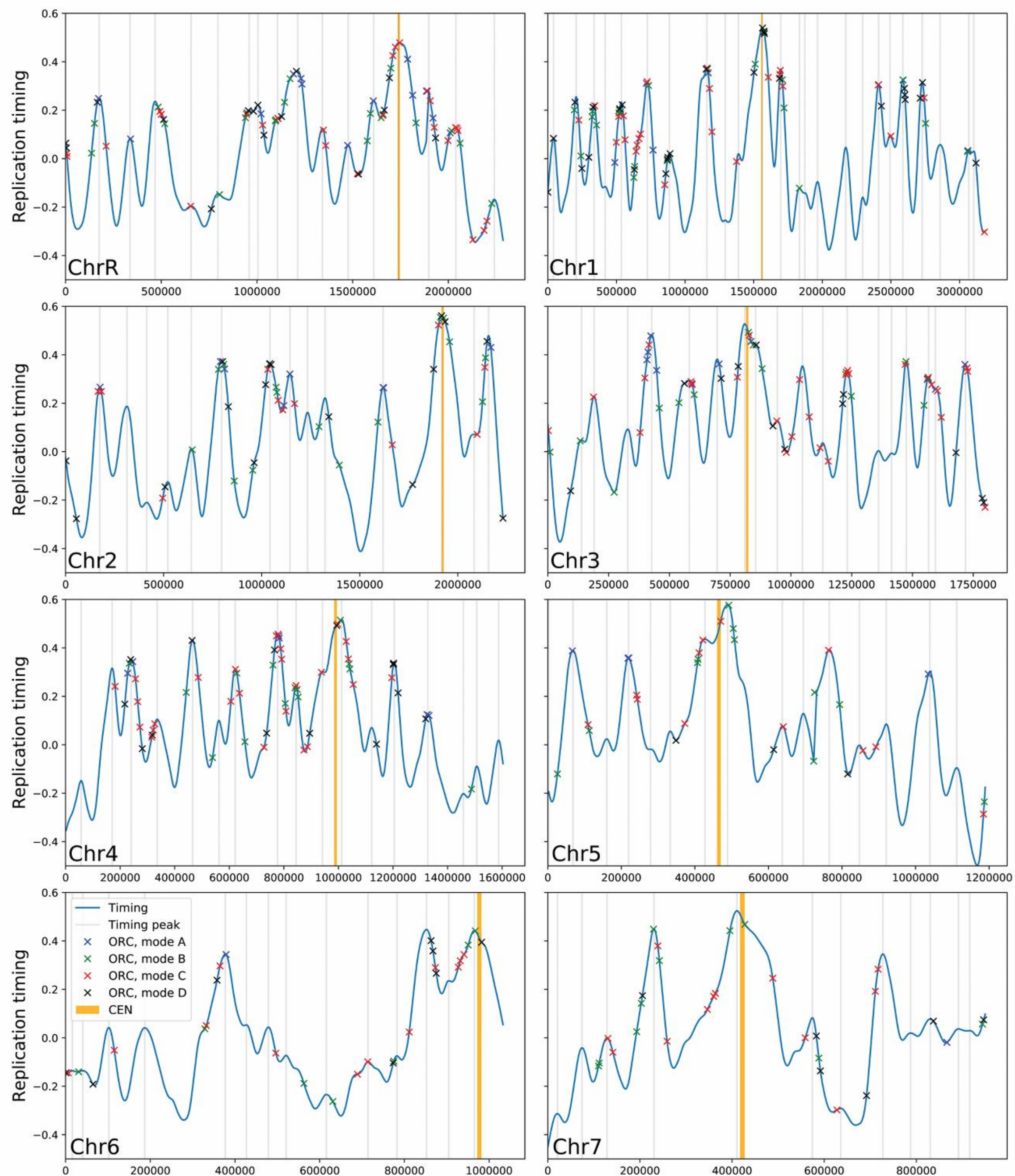


Figure S9. Replication timing profile of various modes associated with Orc4 binding. Orc4 ChIP-seq peaks were aligned to the replication timing profile obtained from *C. albicans* from a previous report (Koren, Tsai et al. 2010). Color-coded stars indicate each of the four motifs identified by DIVERSITY which covers all the 414 chromosomal origins. Peaks represent early replicating regions, including the centromere (yellow lines). A significant fraction of the modes cluster towards the local maxima of the peaks. The *x*-axis represents chromosomal coordinates and *y*-axis shows replication timing scores.

Figure S10

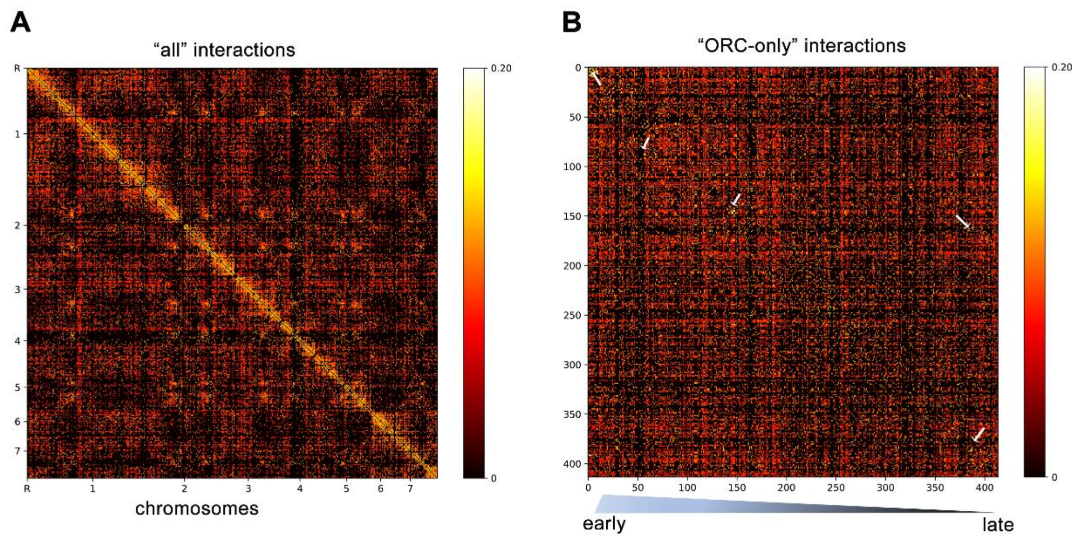


Figure S9. Early replicating regions interact among themselves to form clusters/ replication factories. (A) The Hi-C heatmap shows a whole-genome "all" heatmap representation of the Hi-C data (Burrack, Hutton et al. 2016) as a 7145x7145 matrix. The maximum value in the data was 0.2015 and the minimum was zero. For plotting, the values were log-transformed with a pseudocount of 0.0001. **(B)** The Hi-C "ORC-only" heatmap shows interactions between the 414 chromosomal ORC binding regions, ordered by timing (early to late), to the same colour scale as in (A). White arrows directing towards the yellow pixels indicate clustered/ strongly interacting origins. The analysis was performed at a resolution of 2 kb.

Figure S11

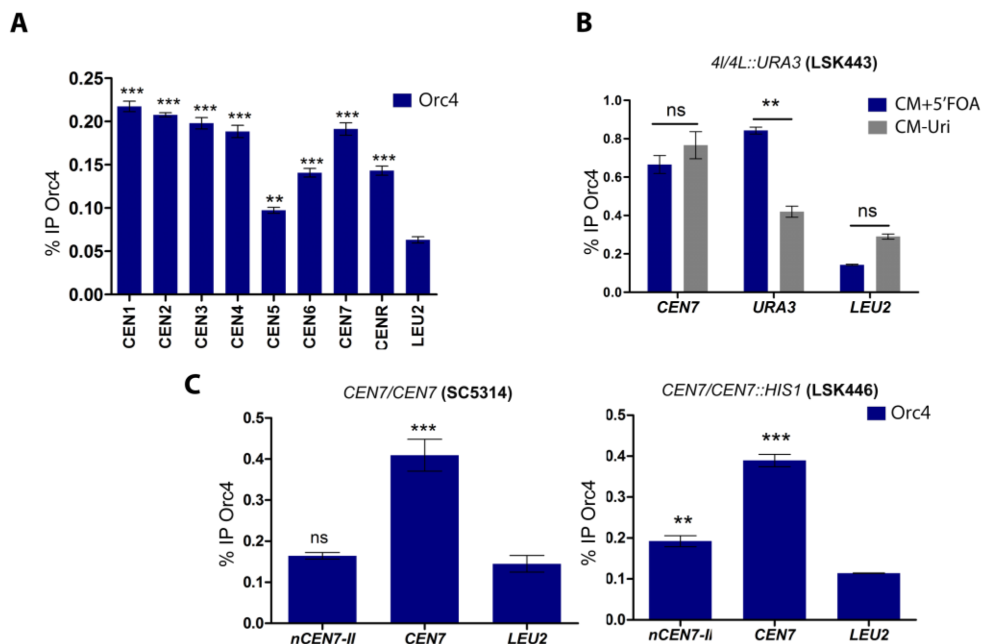


Figure S11. Orc4 binds to ectopic and neocentromeres in *C. albicans*. (A) Orc4 ChIP followed by standard qPCR assays was used to validate the enrichment of Orc4 at all *C. albicans* centromeres. Primers corresponding to the central core of each CEN was used to amplify IP DNA (Supplementary table S7). *LEU2* was used as a control region. The experiment was performed in two replicates of SC5314. Statistical significance was determined by one-way ANOVA followed by Bonferroni post-tests (*** $p<0.001$, ** $p<0.01$, ns: $p>0.05$). **(B)** Orc4 shows significant enrichment in the conditional ectopic centromere formed on *URA3*. Orc4 ChIP followed by qPCR analysis in the strain LSK443 *(4L/4L::URA3)* revealed the significant enrichment of Orc4 on *URA3orf* in CM+5'FOA over CM-Uri. ChIP was performed in two independent transformants and q-PCR was performed with three technical replicates for each transformant. Statistical significance was determined by two-way ANOVA followed by Bonferroni post-tests (*** $p<0.001$, ** $p<0.01$, ns: $p>0.05$). **(C)** Orc4 binds to neocentromeres in *C. albicans*. Orc4 ChIP qPCR in the wild type (*CEN7/CEN7*) (left side) and *CEN7* deletion strain LSK446 (*CEN7/CEN7::HIS1)* (right panel) indicates significant enrichment of Orc4 at *nCEN7-II,* the neocentromere hotspot over the control region (*LEU2*). ChIP qPCR was performed in three independent transformants with three technical replicates for each transformant. Statistical significance was determined by one-way ANOVA followed by Bonferroni post-tests (*** $p<0.001$, ** $p<0.01$, ns: $p>0.05$).

Figure S12

Figure S12. Mcm2 is a highly conserved protein in *C. albicans*. A multiple sequence alignment showing the protein sequences of *S. cerevisiae* Mcm2, ScMcm2 (query sequence) and *C. albicans* Orf19.4354 (CaMcm2) displays the conserved MCM box containing the Walker A, Walker B and the R finger motifs, indicated as stars.

Figure S13

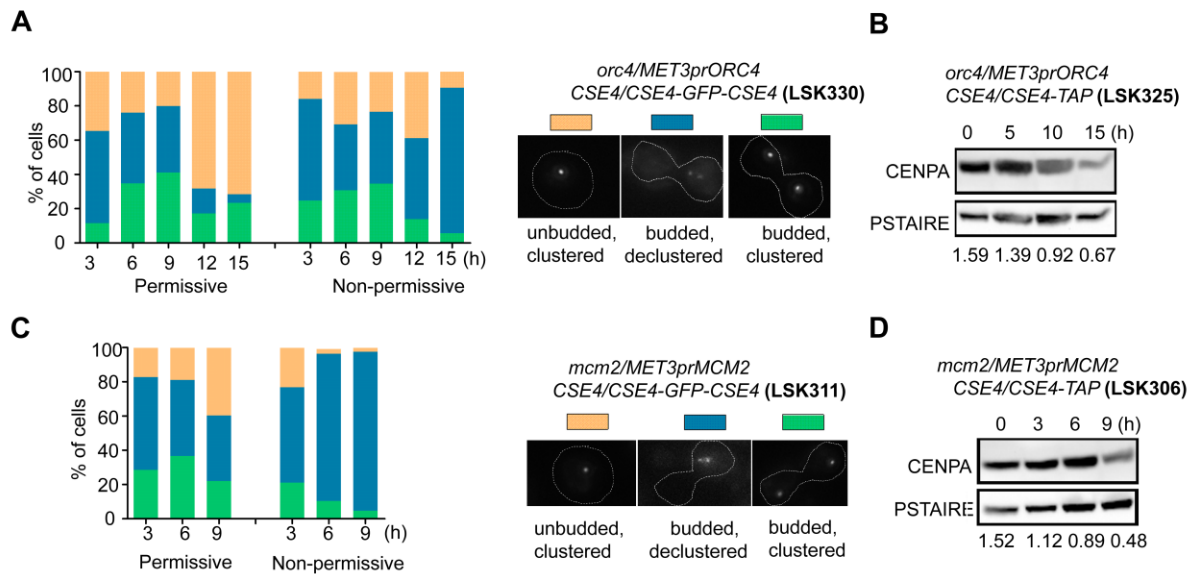


Figure S13. Orc4 and Mcm2 affect chromosome segregation and CENPA stability. (A) CENPA (clustered kinetochore) segregation pattern was examined in an *orc4* conditional mutant LSK330. The strain was grown either in CM-met-cys or CM+5mM met +5mM cys for 3,6,9,12 and 15h to shut down the expression of *ORC4* and the percentage of cells showing a specific segregation phenotype of clustered kinetochores in small budded (yellow), unsegregated budded (blue) and segregated budded (green) was counted. Approximately 100 cells from three independent transformants of *orc4* mutant were analyzed for this assay, where 80% of the *orc4* mutants displayed abrogated kinetochore segregation. **(B)** Western blot using anti-Protein A antibodies shows time dependant decrease in CENPA levels when Orc4 is depleted for 0,5,10,15 h, when normalized with the loading control, PSTAIRE. (**C**) CENPA (clustered kinetochore) segregation pattern was examined in an *mcm2* conditional mutant LSK311. The strain was grown either in CM-met-cys or CM+ 5mM met+ 5mM cys for 3,6,9 h to shut down the expression of *MCM2*. Approximately 100 cells from three independent transformants of *orc4* mutant were analyzed for this assay, where 80% of the *mcm2* mutants displayed unsegregated kinetochore. (**D**) Western blot showing protein levels of CENPA upon depletion of Mcm2 for 3,6,9 h showed dramatic reduction in CENPA after 6 h of Mcm2 depletion. Normalization was performed using PSTAIRE.

Supplemental tables:

Supplemental table S1. Coordinates for *URA3* insertion in *C. albicans*

Type of insertion	Coordinate of insertion	Distance from mid-CEN
5L	Ca21Chr7 417202-417203	10 kb (left of CEN7)
4L	Ca21Chr7 419529-419530	7.7 kb (left of CEN7)
3L	Ca21Chr7 422037-422038	5.2 kb (left of CEN7)
2L	Ca21Chr7 423682-423683	3.5 kb (left of CEN7)
1L	Ca21Chr7 425563-425564	1.7 kb (left of CEN7)

1R	Ca21Chr7 429198-429199	1.9 kb (right of CEN7)
2R	Ca21Chr7 432145-432146	4.9 kb (right of CEN7)
3R	Ca21Chr7 434069-434070	6.8 kb (right of CEN7)
4R	Ca21Chr7 437729-437730	10.4 kb (right of CEN7)
5R	Ca21Chr7 443546-443547	16.2 kb (right of CEN7)
Far-CEN	Ca21Chr7 299510-299511	127 kb (left of CEN7)
CEN5int	Ca21Chr5 477918-477919	7.5 kb (right of CEN5)

Supplemental table S2. Frequency of reversible silencing of *URA3* integration strains

Integration type	**Transformant no.**	**No. of 5'FOA resistant colonies analyzed**	**% reversible silencing (% 5'FOA^r *URI*$^+$ *HIS*$^+$*ARG*$^+$)**
5L	1	107	ND
	2	73	ND
	3	95	ND
4L	1	116	0.862
	2	103	ND
	3	117	0.854
3L	1	86	1.162
	2	96	2.083
	3	98	2.04
2L	1	97	1.03
	2	117	2.564
	3	100	ND
1L	1	110	10
	2	158	14.556
	3	160	3.125
CEN7::URA3/CEN7	J151	74	97.297
	J153	61	78.688
	J154	79	94.936
1R	1	101	98.019
	2	58	100
	3	78	91.025
2R	1	118	1.694
	2	111	0.9
	3	100	1
3R	1	111	0.9
	2	108	0.925
	3	88	1.136
4R	1	97	3.09
	2	96	1.04
	3	101	1.98

5R	1	138	0.724
	2	157	1.273
	3	100	ND
far-CEN	1	200	ND
	2	200	ND
	3	205	ND
CEN5 int	1	98	4.08
	2	114	1.75
	3	89	ND

ND= Not determined

Supplemental table S3. Southern blot strategy for *CEN7* deletion strains

Strain	**Restriction enzyme**	**Primers used to amplify probe (length of probe)**	**Size of the expected band/wild type**
4.5 kb *CEN7* deletion in URA3 at 4L locus	*Afl*III	URA3RT1, URA3 RT4 (870 bp)	8.3/7.6 kb
4.5 kb *CEN7* deletion in *URA3* at 4R locus	*Nco*I	HIS ORF_2, HIS ORF_1 (480 bp)	11.4/10 kb

Supplemental table S4. Neocentromere coordinates of *CEN7* deletion strains (from CENPA ChIP-sequencing analysis)

Strain	**Description**	**Coordinates for neocentromere**
LSK450	4.5 kb *CEN7* deleted in 5'FOA resistant *URA3* integrant (4R)	Ca21Chr7 419629-422084
LSK465	4.5 kb *CEN7* deleted in 5'FOA resistant *URA3* integrant (4L)	Ca21Chr7 435078-440387

Supplemental table S5. Chromosomal coordinates for Orc4 binding at centromeres based on *C. albicans* Assembly 21

Centromere	**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)

Supplementary table S6. Strains used in the study. (FOA^{r}, 5'FOA resistant; FOA^{s}, 5'FOA sensitive)

Name	**Genotype**	**Description**	**Reference**
J200	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 CSE4/CSE4-TAP(NAT)*	*CSE4-TAP(NAT)* in RM1000AH	(Thakur and Sanyal 2013)
8675	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, CSE4-GFP-CSE4/CSE4*	*CSE4-GFP-CSE4/CSE4*	(Joglekar, Bouck et al. 2008)
LSK401	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 5L/5L::URA3 CSE4/CSE4-TAP(NAT)*	5L_T1	This study
LSK402	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 5L/5L::URA3 CSE4/CSE4-TAP(NAT)*	5L_T2	This study
LSK403	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 5L/5L::URA3 CSE4/CSE4-TAP(NAT)*	5L_T3	This study
LSK404	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4L/4L::URA3 CSE4/CSE4-TAP(NAT)*	4L_T1	This study
LSK405	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4L/4L::URA3 CSE4/CSE4-TAP(NAT)*	4L_T2	This study

LSK406	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4L/4L::URA3 CSE4/CSE4 -TAP(NAT)*	4L_T3	This study
LSK407	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 3L/3L::URA3 CSE4/CSE4-TAP(NAT)*	3L_T1	This study
LSK408	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 3L/3L::URA3 CSE4/CSE4-TAP(NAT)*	3L_T2	This study
LSK409	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 3L/3L::URA3 CSE4/CSE4-TAP(NAT)*	3L_T3	This study
LSK410	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 2L/2L::URA3 CSE4/CSE4-TAP(NAT)*	2L_T1	This study
LSK411	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 2L/2L::URA3 CSE4/CSE4-TAP(NAT)*	2L_T2	This study
LSK412	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 2L/2L::URA3 CSE4/CSE4-TAP(NAT)*	2L_T3	This study
LSK413	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 1L/1L::URA3 CSE4/CSE4-TAP(NAT)*	1L_T1	This study
LSK414	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 1L/1L::URA3 CSE4/CSE4-TAP(NAT)*	1L_T2	This study
LSK415	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 1L/1L::URA3 CSE4/CSE4TAP(NAT)*	1L_T3	This study
J151	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 CEN7/CEN7::URA3 CSE4/CSE4-TAP(NAT)*	CEN7::URA3_T1	(Thakur and Sanyal 2013)
J153	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 CEN7/CEN7::URA3 CSE4/CSE4-TAP(NAT)*	CEN7::URA3_T2	(Thakur and Sanyal 2013)
J154	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 CEN7/CEN7::URA3 CSE4/CSE4-TAP(NAT)*	CEN7::URA3_T3	(Thakur and Sanyal 2013)
LSK416	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 1R/1R::URA3 CSE4/CSE4-TAP(NAT)*	1R_T1	This study

LSK417	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 1R/1R::URA3 CSE4/CSE4-TAP(NAT)*	1R_T2	This study
LSK418	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 1R/1R::URA3 CSE4/CSE4-TAP(NAT)*	1R_T3	This study
LSK419	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 2R/2R::URA3 CSE4/CSE4-TAP(NAT)*	2R_T1	This study
LSK420	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 2R/2R::URA3 CSE4/CSE4-TAP(NAT)*	2R_T2	This study
LSK421	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 2R/2R::URA3 CSE4/CSE4-TAP(NAT)*	2R_T3	This study
LSK422	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 3R/3R::URA3 CSE4/CSE4-TAP(NAT)*	3R_T1	This study
LSK423	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 3R/3R::URA3 CSE4/CSE4-TAP(NAT)*	3R_T2	This study
LSK424	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 3R/3R::URA3 CSE4/CSE4-TAP(NAT)*	3R_T3	This study
LSK425	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4R/4R::URA3 CSE4/CSE4-TAP(NAT)*	4R_T1	This study
LSK426	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4R/4R::URA3 CSE4/CSE4-TAP(NAT)*	4R_T2	This study
LSK427	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4R/4R::URA3 CSE4/CSE4-TAP(NAT)*	4R_T3	This study
LSK427	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 5R/5R::URA3 CSE4/CSE4-TAP(NAT)*	5R_T1	This study
LSK428	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 5R/5R::URA3 CSE4/CSE4-TAP(NAT)*	5R_T2	This study
LSK430	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 CEN5/CEN5-7 kb_right ::URA3 CSE4/CSE4TAP(NAT)*	CEN5_T1	This study
LSK431	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 CEN5/CEN5-7 kb_right ::URA3 CSE4/CSE4-TAP(NAT)*	CEN5_T2	This study

LSK432	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 CEN5/CEN5-7 kb_right ::URA3 CSE4/CSE4-TAP(NAT)*	CEN5_T3	This study
LSK433	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 CEN7::URA3_ 127 kb farCEN/ CEN7 CSE4/CSE4-TAP(NAT)*	FAR URA_T1	This study
LSK434	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 CEN7::URA3_ 127 kb farCEN/ CEN7 CSE4/CSE4-TAP(NAT)*	FAR URA_T2	This study
LSK435	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 CEN7::URA3_ 127 kb farCEN/ CEN7 CSE4/CSE4-TAP(NAT)*	FAR URA_T3	This study
LSK436	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 MTW1/MTW1-TAP(NAT)*	MTW1-TAP IN RM1000AH	This study
LSK437	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4L/4L::URA3 MTW1/MTW1-TAP(NAT)*	MTW1-TAP IN 4L_T1	This study
LSK438	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4L/4L::URA3 MTW1/MTW1-TAP(NAT)*	MTW1-TAP IN 4L_T2	This study
LSK439	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4L/4L::URA3 MTW1/MTW1-TAP(NAT)*	MTW1-TAP IN 4L_T3	This study
LSK440	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4R/4R::URA3 MTW1/MTW1-TAP(NAT)*	MTW1-TAP IN 4R_T1	This study
LSK441	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4R/4R::URA3 MTW1/MTW1-TAP(NAT)*	MTW1-TAP IN 4R_T2	This study
LSK442	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG arg4::HIS1/ARG4 4R/4R::URA3 MTW1/MTW- TAP(NAT)*	MTW1-TAP IN 4R_T3	This study
YJB8675	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, CSE4/CSE4-GFP-CSE4*	Cse4-GFP	This study
LSK443	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4*	4L in Cse4-GFP_T1	This study
LSK444	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4*	4L in Cse4-GFP_T2	This study
LSK445	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4*	4L in Cse4-GFP_T3	This study
LSK446	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG,*	CEN7 del in 4L (FOA^{s}, in *cis)*	This study

	4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1		
LSK447	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4L (FOA^{s}, in *cis)*	This study
LSK448	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4L (FOA^{s}, in *trans)*	This study
LSK449	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4L (FOA^{s}, in *trans)*	This study
LSK450	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4L (FOA^{r}, in *cis)*	This study
LSK451	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4L (FOA^{r}, in *cis)*	This study
LSK452	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4L (FOA^{r}, in *trans)*	This study
LSK453	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, CEN7/CEN7::HIS1::URA3_7.7kb left CSE4-GFP-CSE4/CSE4*	CEN7 del in 4L (FOA^{r}, in *trans)*	This study
LSK454	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4L (FOA^{r}, in *trans)*	This study
LSK455	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4L (FOA^{r}, in *trans)*	This study
LSK456	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4*	4R in Cse4-GFP_T1	This study
LSK457	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4*	4R in Cse4-GFP_T2	This study
LSK458	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4*	4R in Cse4-GFP_T3	This study

LSK459	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4R (FOA^{s}, in *cis)*	This study
LSK460	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4R (FOA^{s}, in *cis)*	This study
LSK461	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4R (FOA^{s}, in *cis)*	This study
LSK462	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4R (FOA^{s}, in *trans)*	This study
LSK463	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4R (FOA^{s}, in *trans)*	This study
LSK464	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4R (FOA^{r}, in *cis)*	This study
LSK465	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4R (FOA^{r}, in *cis)*	This study
LSK466	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4R (FOA^{r}, in *cis)*	This study
LSK467	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4R (FOA^{r}, in *trans)*	This study
LSK468	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1*	CEN7 del in 4R (FOA^{r}, in *trans)*	This study
LSK471	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1MTW1/MTW1-TAP(NAT)*	CEN7 del in 4L (FOA^{r}, in *cis)* MTW1-TAP	This study
LSK472	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4L/4L::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1MTW1/MTW1-TAP(NAT)*	CEN7 del in 4L (FOA^{r}, in *cis)* MTW1-TAP	This study

LSK475	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1MTW1/MTW1-TAP(NAT)*	CEN7 del in 4R (FOA^r, in *cis)* MTW1-TAP	This study
LSK476	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, 4R/4R::URA3 CSE4/CSE4-GFP-CSE4 CEN7/CEN7::HIS1MTW1/MTW1-TAP(NAT)*	CEN7 del in 4R (FOA^r, in *cis)* MTW1-TAP	This study
LSK301	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MCM2::NAT/MCM2*	*MCM2* heterozygous null (SN148)	This study
LSK302	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MCM2::FRT/MCM2*	*MCM2* heterozygous null (SN148)	This study
LSK303	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MCM2:: FRT/MET3prMCM2*	*mcm2* conditional mutant (SN148)	This study
LSK304	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MCM2:: FRT/MET3prMCM2*	*mcm2* conditional mutant (SN148)	This study
LSK305	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MCM2:: FRT/MET3prMCM2*	*mcm2* conditional mutant (SN148)	This study
LSK306	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MCM2:: FRT/MET3prMCM2 CSE4 TAP(HIS)/CSE4*	*mcm2* conditional mutant (SN148) CENPA-Prot A	This study
LSK307	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MCM2:: FRT/MET3prMCM2 CSE4 TAP(HIS)/CSE4*	*mcm2* conditional mutant (SN148) CENPA-Prot A	This study
LSK308	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, MCM2:: FRT/MET3prMCM2 CSE4 TAP(HIS)/CSE4*	*mcm2* conditional mutant (SN148) CENPA-Prot A	This study
LSK309	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MCM2::NAT/MCM2 CSE4-GFP-CSE4/CSE4*	*MCM2* heterozygous null (10118)	This study
LSK310	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MCM2::NAT/MCM2 CSE4-GFP-CSE4/CSE4*	*MCM2* heterozygous null (10118)	This study
LSK311	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MCM2::FRT/MET3prMCM2 CSE4-GFP-CSE4/CSE4*	*mcm2* conditional mutant (10118)	This study

LSK312	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MCM2::FRT/MET3prMCM2 CSE4-GFP-CSE4/CSE4*	*mcm2* conditional mutant (10118)	This study
LSK313	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, MCM2::FRT/MET3prMCM2 CSE4-GFP-CSE4/CSE4*	*mcm2* conditional mutant (10118)	This study
LSK320	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, ORC4::NAT/ORC4*	*ORC4* heterozygous null (SN148)	This study
LSK321	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, ORC4::NAT/ORC4*	*ORC4* heterozygous null (SN148)	This study
LSK322	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, ORC4::FRT/MET3prORC4*	*orc4* conditional mutant (SN148)	This study
LSK323	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, ORC4::FRT/MET3prORC4*	*orc4* conditional mutant (SN148)	This study
LSK324	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, ORC4::FRT/MET3prORC4*	*orc4* conditional mutant (SN148)	This study
LSK325	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, ORC4:: FRT/MET3prORC4 CSE4 TAP(HIS)/CSE4*	*orc4* conditional mutant (SN148) CENPA-Prot A	This study
LSK326	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, ORC4:: FRT/MET3prORC4 CSE4 TAP(HIS)/CSE4*	*orc4* conditional mutant (SN148) CENPA-Prot A	This study
LSK327	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG, ORC4:: FRT/MET3prORC4 CSE4 TAP(HIS)/CSE4*	*orc4* conditional mutant (SN148) CENPA-Prot A	This study
LSK328	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, ORC4:NAT/ORC4 CSE4-GFP-CSE4/CSE4*	*ORC4* heterozygous null (10118)	This study
LSK329	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, ORC4:NAT/ORC4 CSE4-GFP-CSE4/CSE4*	*ORC4* heterozygous null (10118)	This study
LSK330	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, ORC4::FRT/MET3prORC4 CSE4-GFP-CSE4/CSE4*	*orc4* conditional mutant (10118)	This study

LSK331	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, ORC4::FRT/MET3prORC4 CSE4-GFP-CSE4/CSE4*	*orc4* conditional mutant (10118)	This study
LSK332	*Δura3::imm434/Δura3::imm434, Δhis1::hisG/Δhis1::hisG ,Δarg4::hisG/Δarg4::hisG, ORC4::FRT/MET3prORC4 CSE4-GFP-CSE4/CSE4*	*orc4* conditional mutant (10118)	This study
CAKS3b	*Δura3::imm434/ Δura3::imm434 Δhis1::hisG/ Δhis1::hisG Δarg4::hisG/ Δarg4::hisG CSE4::PCK1prCSE4/ cse4::hisG:URA:hisG*	CENPA depletion	(Sanyal and Carbon 2002)

Supplemental table S7. Primers used in the study.

Name	**Sequence**	**Description**
URA3 EXT HSP2_FP	GTTTCAGAATCCGAAAAAGTGACGAAACTTATCAT AATTGTACGAATATTCTTATCAAACACACCCTGAG CTTCCGGATAATAGGAATTG	Cassette primers for *URA3* integrated 10 kb left of CEN7
URA3 EXT HSP2_RP	GTTGCTCGAGGTTAGAGTCTATCTTGAAAAATTTT GTACATACAAACTGATATAACTCGACAATGGTCTT AGAAGGACCACCTTTGATTG	
URA3 AT HSP2_FP	CTCAAAAATACTTTAACAAACGGGTATATTGCTGA TATTCTGATTAAAACATTTGATCGTTTTATGTGAGC TTCCGGATAATAGGAATTG	Cassette primers for *URA3* integrated 7.7 kb left of CEN7
URA3 AT HSP2_RP	CTTAACCCCAGACAGTTTTAACAATTTAGACACTA CTACTAATTGCAACGTACTAACTAGTGAAACCCTT AGAAGGACCACCTTTGATTG	
19.6520_AvrII _F	AAACCCCCTAGGTTGCGAATATCTATTG	Construction of pFA-URA3-I-SceI-TS-Orf 19.6520/65
19.6520_HindIII_MluI_R	AAACCCAAGCTTACGCGTAATGGTCCCATCAGCAG TGCA	
19.6522_HpaI _MluI_F	CCCAAAGTTAACACGCGTCTGCCAACAAGAATGC AACT	
19.6522_SacII _R	CCCAAACCGCGGTATATTTTTGTTGTATCAGAATC CTACGCC	
L1_URA INT_FP	CACATATTTTTACTTTCTGTATTATTCAGATCTTTA CTCGTTGAAAAAAAATTTTTTTTTCAAAAGCTTCC GGATAATAGGAATTG	Cassette primers for *URA3* integrated 3.5 kb left of CEN7
L1_URA INT_RP	GATGTAGTTGTATCTTTAATATCACAGTTATGATA AGGGTCGTGTATATGTGAACATGGATTTGCTTAGA AGGACCACCTTTGATTG	

PJ71	TGCTTACCATAATAGATGCTTAAAGCAACTAAAATTAAGCTACTGGAAAGCTCCAGTGGTCCTAGATCCCGACTAATAGG	Cassette primers for *URA3* integrated 1.7 kb left of CEN7
PJ72	ATTCGGGCAATTGTGTTCGTTATTGGTGGTAAATAATGGTAAGACTACTTGGCACATGTATAGAAGGACCACCTTTGATTG	
PJ67	ATTGATTGAATTTATAGCGGAAAATGGATGACAATTAAAGGTTACGTGACGCTTTTTGCTCCTAGATCCCGACTAATAGG	Cassette primers for *URA3* integrated 1.9 kb right of CEN7
PJ68	CTACATTTTCATGGACCAAACCCACTACAACACATGCACCACACTGCACCTCCCCTAAAATAGAAGGACCACCTTTGATTG	
19.6525_HpaI_MluI_F	CCCAAAGTTAACACGCGTGTCAATGCAGTCGTTGAATAC	Construction of pFA-URA3-I-SceI-TS-Orf 19.6524/65
19.6525_SacII_R	CCAAACCGCGGTTTCAATATCGCAGAGATGGGAT	
19.6524_AvrII_F	AAACCCCCTAGGGAGTGATGATGAGATTAACCAG	
19.6524_HindIII_MluI_R	AAACCCAAGCTTACGCGTGCCTTATATGCCACCGATGA	
R1_URA INT_FP	GTCAGAAATTGATTTATGGACGAGATAAGACTAAAATATGATTCTTCTAAAATCACATAATTAATTAGAGCTTCCGGATAATAGGAATTG	Cassette primers for *URA3* integrated 6.5 kb right of CEN7
R1_URA INT_RP	GTGTAACAAAAATTTGCAATCACATCATTGACAGCCACCACAGTTTTTTATAATAAGTGATATTGTTAGAAGGACCACCTTTGATTG	
PJ70	TTGCTTTAAATGTTTCAAACCATAGGTATGAGTTTGGGTAGTATTTGGCGGAATTAATGTCCTAGATCCCGACTAATAGG	Cassette primers for *URA3* integrated 10.4 kb right of CEN7
PJ71	ATCACTCTTGTCGTTTATTGTAGATCACTAAAAGTAATGGTTGTGTGAATAACTCCTGCTTAGAAGGACCACCTTTGATTG	
URA3 AT HSP3_FP	CAGTTTTAAGAAGGTTTACATTATTAGCCTACGAACAAAGACAGGTTATGATAGGAAACAGAGCTCCTGTTTTTATTCAGCTTCCGGATAATAGGAATTG	Cassette primers for *URA3* integrated 16.3 kb right of CEN7
URA3 AT HSP3_RP	GCAATCGATCGTAAACGCCACTCAAGCTAAACTGAAAACTACTACGCCTAGAAGGCTAATCGGTACCAATTAGAAGGACCACCTTTGATTG	

URA3 AT CRTL7_FP	GATCACATATGATTCTAGTACCACTAAACATTATCAACAACTATCATCAATTAGTAGAATTACTCTGAGAGCTTCCGGATAATAGGAATTG	Cassette primers for *URA3* integrated 100 kb left of CEN7
URA3 AT CRTL7_RP	CCACGTGGATTTTTAAAATCTCAATAGTTTCTATAGTGGTGGTATACCACTACTACGACTGTGGATTCATTAGAAGGACCACCTTTGATTG	
URA3 at nCEN5-II_FP	CAATTCCTATTATCCGGAAGCTGTCGTGTAAGGCGGTAAATGGTTTTGGTGGGTTTATTTTTCTTTAAAAATCCAGACATGTCTTGC	Cassette primers for *URA3* integrated 7.5 kb left of CEN5
URA3 at nCEN5-II_RP	CAATCAAAGGTGGTCCTTCTAACACACTATTTACTTGTGGTAAACATACTATTGGTTGATAATGATGTTAGCAATGGGTTTATGCTTATTTAC	
nCEN7-3	GCATACCTGACACTGTCGTT	q-PCR of *CEN7*
nCEN7-4	AACGGTGCTACGTTTTTTTA	
URA3 RT1	TGTTGAAAGTTGCTGTAGTG	q-PCR for *URA3* promoter
URA3 RT2	TGCAGGAAATAAGATTGC	
URA3 RT3	TCATCAGTGGGATCATTAGCA	q-PCR for *URA3* ORF
URA3 RT4	CACGTTGGGCAATAAATCCA	
CEN1 core RT1	CAATCTAGCATTTCCTTCACACA	q-PCR for *CEN1*
CEN1 core RT2	TGACGCAATGAAGTAGGTGAT	
CACH5F1	CCCGCAAATAAGCAAACACT	qPCR for CEN5
CACH5R1	TTCATGGAAGAGGGGTTTCA	
7S10 RTF	CTTGTAAATTTAATTGTCGCTGAGG	qPCR primers for neocentromere mapping
7S10 RTR	CGGATAATCGTCCAACATATGAC	
7S11 RTF	GTCTTCTGACCTACCCATCAC	
7S11 RTR	GAGGCGGAAGTTGGACC	
7S12 RTF	CGTTGTGGCAATTGTATTTATG	
7S12 RTR	GCCATAGCTTAGCAAATAACC	
7S13 RTF	CATGGCTAATCCAACAACACATG	
7S13 RTR	GCTGGCTCTTGTTCTTGTATC	
421K RT1	CCTATCGCCACAAGGGAGA	
421K RT2	CAACGACTGCATTGACTCTTT	
7S14 RTF	GGATGTTGAGTTCAAAGCCTG	
7S14 RTR	CCAGCCAAATAATCTAGCTGC	
4RTF	ATTTGTCCCATCCGTAATTGATTC	

3RTR	ACGTTTTACCAGCCTATGC	
18RTF	AATAGCTATATCAGTTGTCAGCTTAC	
17RTF	AATGCTTGGCCCTCAGTATAAC	
20RTF	ACTGAAGTCGGCTGTGATC	
19RTR	GATAACTGGACTCATTAGGCGAA	
16RTF	ACCAGGATAATCTAACTGGCAAC	
15RTR	CTATTGCCCCAATCAATAACCTT	
7F1RT	CAGTAAACGTCATCTCTTTTATACCT	
7R1RT	GGAAGTGTAACTATTGAGCTCC	
7F2RT	ATTAAATAGAATGCGGCAATACC	
7R2RT	ATTTTAAGGATGAGAGGTGTGG	
7F3RT	CTGGTATTCACAATGGAACGGT	
7R3RT	GTCACCCCAATTCAAATCACGT	
7F4RT	GGAGCTGGCGATCAATTTGT	
7R4RT	TCACACATGAGAGGACCGTT	
7F4A RTF	CGGATAATTGAAAGCAGCAATG	
7R4A RTR	CCACAACCTGTTGACGAG	
7F4B RT	GTAGGCGCGGATTTAATGTG	
7R4B RT	CCAACTTGTTTAGTTGTTGGATCTG	
7F4C RT	GACAAACACTCAAGGAGCAG	
7R4C RT	CTGCAAATCTATTGGAGGTGG	
7F5 RT	GGACAAAATCAGATACCAAGCC	
7R5 RT	GCTTTGGTCATACCAATACCAG	
7F6 RT	CTCCAAGAACATCAAATTGGG	
7R6 RT	CAAGGAAGTCATTTCTTCAGAAG	
CEN7DHIS_FP	GTAAACTTTTTCGATTCTCAATTTACTTTGAGGGCATTGTCGCAAATGGAGATTCCTTACGATGGGAATTCCGGAATATTTATGAGAAAC	CEN7 DELETION WITH HIS1
CEN7DHIS_RP	CACAAAAATGCCCGCTAACAATACCATTAATTCCTACTCCATGTACAGAATACCCAACATGCTTTGTATCGAATTCCGGGGATCCTGGAG	
HIS ORF_2	GGAGTAATGGTTAAACATTTTGC	
HIS ORF_1	CAAAGAAGCTGAACAATTCGAC	
ORC4 13	CGGGGTACCTTGGTTTGTAAAAATGTTGTTTC	Deletion cassette for ORC4
ORC4 14	CCGCTCGAGAAATAGTTTTACTCTTGAGTTAGC	
ORC4 15	TCCCCGCGGGTTATAGGTTGCTTTTAGTGC	
ORC4 16	TCCCCGCGGGTTATAGGTTGCTTTTAGTGC	
ORC4 11	CGCGGATCCATGAATTCACAGGACC	MET3 cassette cloning for ORC4
ORC4 12	AACTGCAGTGCCATTTAACTCTTTTAAGGCG	
MCM2_13	CGGGGTACCCTAATCCCATTTTGTTATGAATAT	

MCM2_14	CCGCTCGAGGGTTGATTAAATAGTAATGTAATTAATAAAG	Deletion cassette for MCM2
MCM2_15	TCCCCGCGGGTGATTAGTGGGTTATGG	
MCM2_16	CGGAGCTCTGCATTCCAGATTATTTTCTG	
MCM2_11	CGCGGATCCATGTCAAGTCCACCAGCTG	N term of Mcm2 (For *MET3pr* cloning)
MCM2_12	AACTGCAGGCGTCTTCATCTTCATCATCGTC	

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