Evolution of DNA Replication Origin Specification and Gene Silencing Mechanisms

Hu et al.

Supplementary Information

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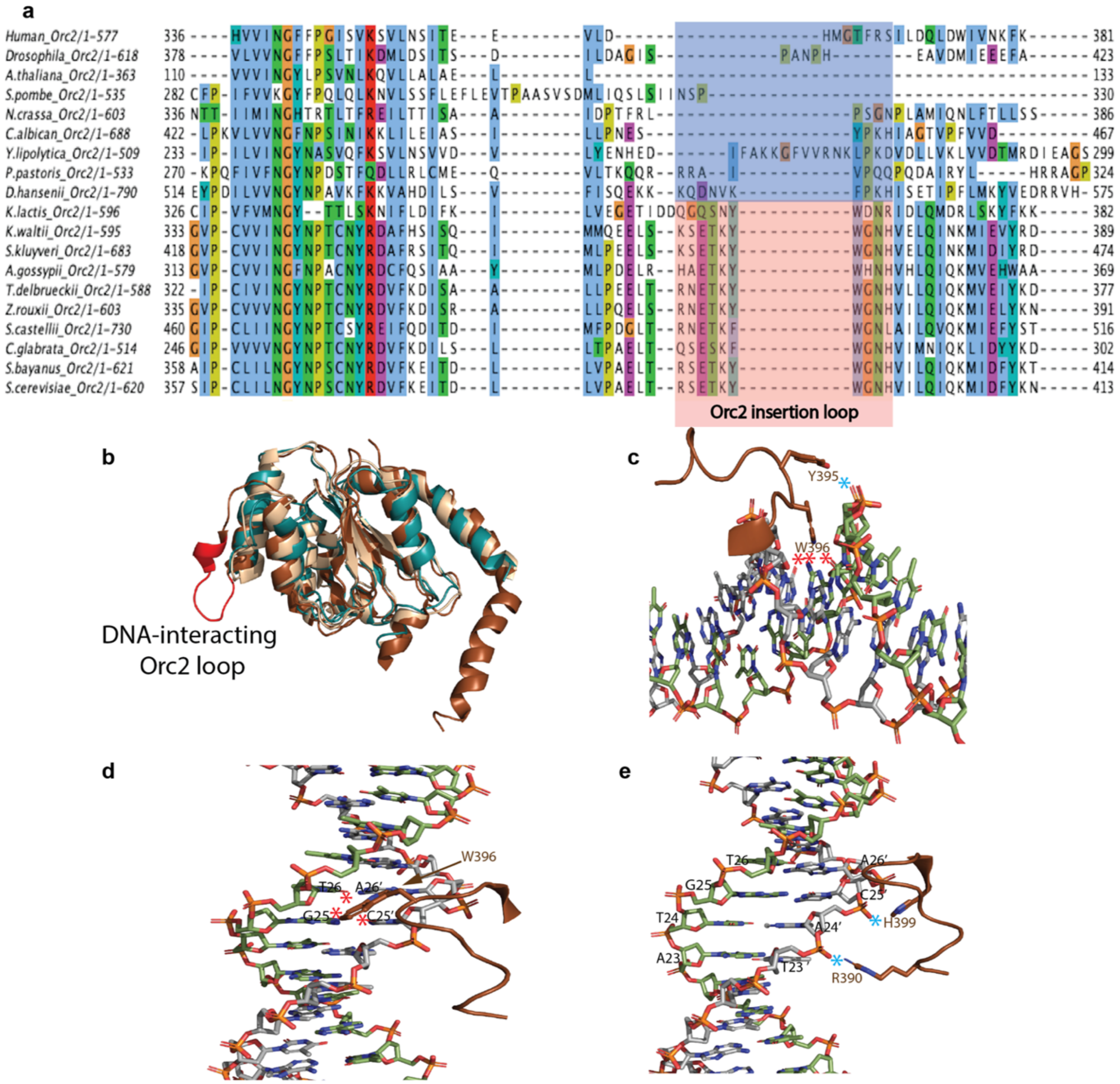
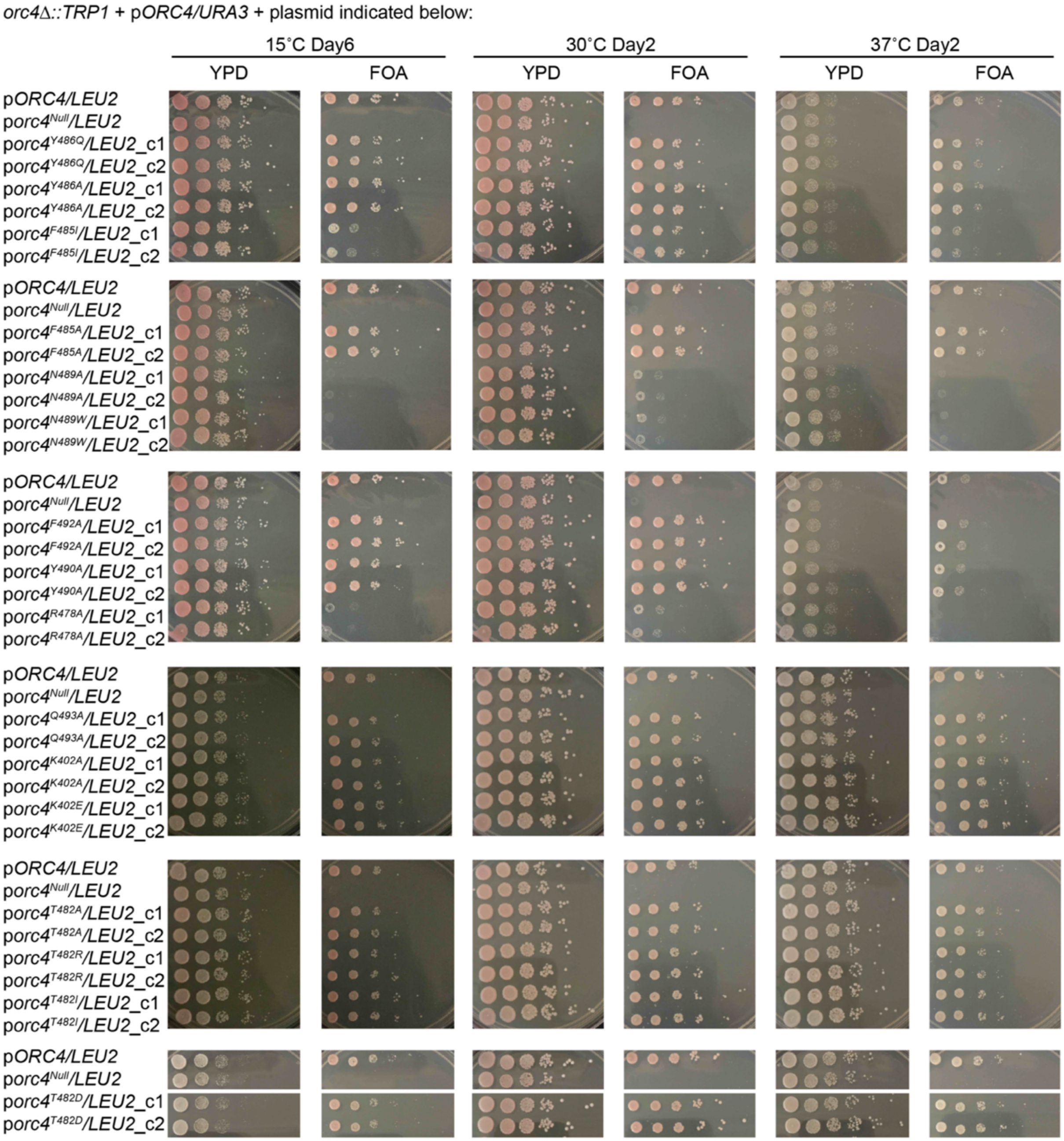
Supplementary Figures



Supplementary Fig. 1 | Orc2 loop DNA interaction exists in species with origin sequence specificity. a, Multiple sequence alignment of Orc2 among representing eukaryotic species as indicated. Orc2 DNA interacting loop region indicated with species that don't have sequence specific origins shadowed in blue and species that sequence specific origins exist shadowed in pink. **b,** Orc2 AAA+ domain structure superposition among human Orc2 in teal color (from PDB code 5uj7), *Drosophila* Orc2 in wheat color (from PDB code 4xgc) and *S. cerevisiae* Orc2 in brown color (from PDB code 5udb). Orc2 loop that interact with DNA is colored in red. **c-e,** R390, Y395, W396, and H399 interact with DNA in base-specific (specificity) and base-nonspecific (affinity) manner in ORC-DNA structure at 3Å (PDB code 5zr1). Red asterisks denote the base interaction between amino acid and DNA base. Blue asterisks denote the base-nonspecific interaction between amino acid and DNA phosphate backbone. Prime symbols denote bases on the opposite strand. Bases numbering denote as the positions in logo (see Fig. 2b). **c** shows Orc2 loop origin DNA minor groove insertion with base-specific interaction between W396 and G25, C25’ and T26, and base-nonspecific interaction between Y395 and phosphate backbone of T27. **d-e,** same as in **c**, but view in different angles. **d** shows the base-specific interaction between W396 and G25, C25’ and T26. **e** shows the base-nonspecific interaction between R390 and phosphate backbone of T23’ and base-nonspecific interaction between H399 and phosphate backbone of A24’.


orc4Δ::TRP1 + pORC4/URA3 + plasmid indicated below:
15˚C Day6
30˚C Day2
37˚C Day2
YPD
FOA
YPD
FOA
YPD
FOA
pORC4/LEU2
porc4^{Null}/LEU2
porc4^{Y486Q}/LEU2_c1
porc4^{Y486Q}/LEU2_c2
porc4^{Y486A}/LEU2_c1
porc4^{Y486A}/LEU2_c2
porc4^{F485I}/LEU2_c1
porc4^{F485I}/LEU2_c2
pORC4/LEU2
porc4^{Null}/LEU2
porc4^{F485A}/LEU2_c1
porc4^{F485A}/LEU2_c2
porc4^{N489A}/LEU2_c1
porc4^{N489A}/LEU2_c2
porc4^{N489W}/LEU2_c1
porc4^{N489W}/LEU2_c2
pORC4/LEU2
porc4^{Null}/LEU2
porc4^{F492A}/LEU2_c1
porc4^{F492A}/LEU2_c2
porc4^{Y490A}/LEU2_c1
porc4^{Y490A}/LEU2_c2
porc4^{R478A}/LEU2_c1
porc4^{R478A}/LEU2_c2
pORC4/LEU2
porc4^{Null}/LEU2
porc4^{Q493A}/LEU2_c1
porc4^{Q493A}/LEU2_c2
porc4^{K402A}/LEU2_c1
porc4^{K402A}/LEU2_c2
porc4^{K402E}/LEU2_c1
porc4^{K402E}/LEU2_c2
pORC4/LEU2
porc4^{Null}/LEU2
porc4^{T482A}/LEU2_c1
porc4^{T482A}/LEU2_c2
porc4^{T482R}/LEU2_c1
porc4^{T482R}/LEU2_c2
porc4^{T482I}/LEU2_c1
porc4^{T482I}/LEU2_c2
pORC4/LEU2
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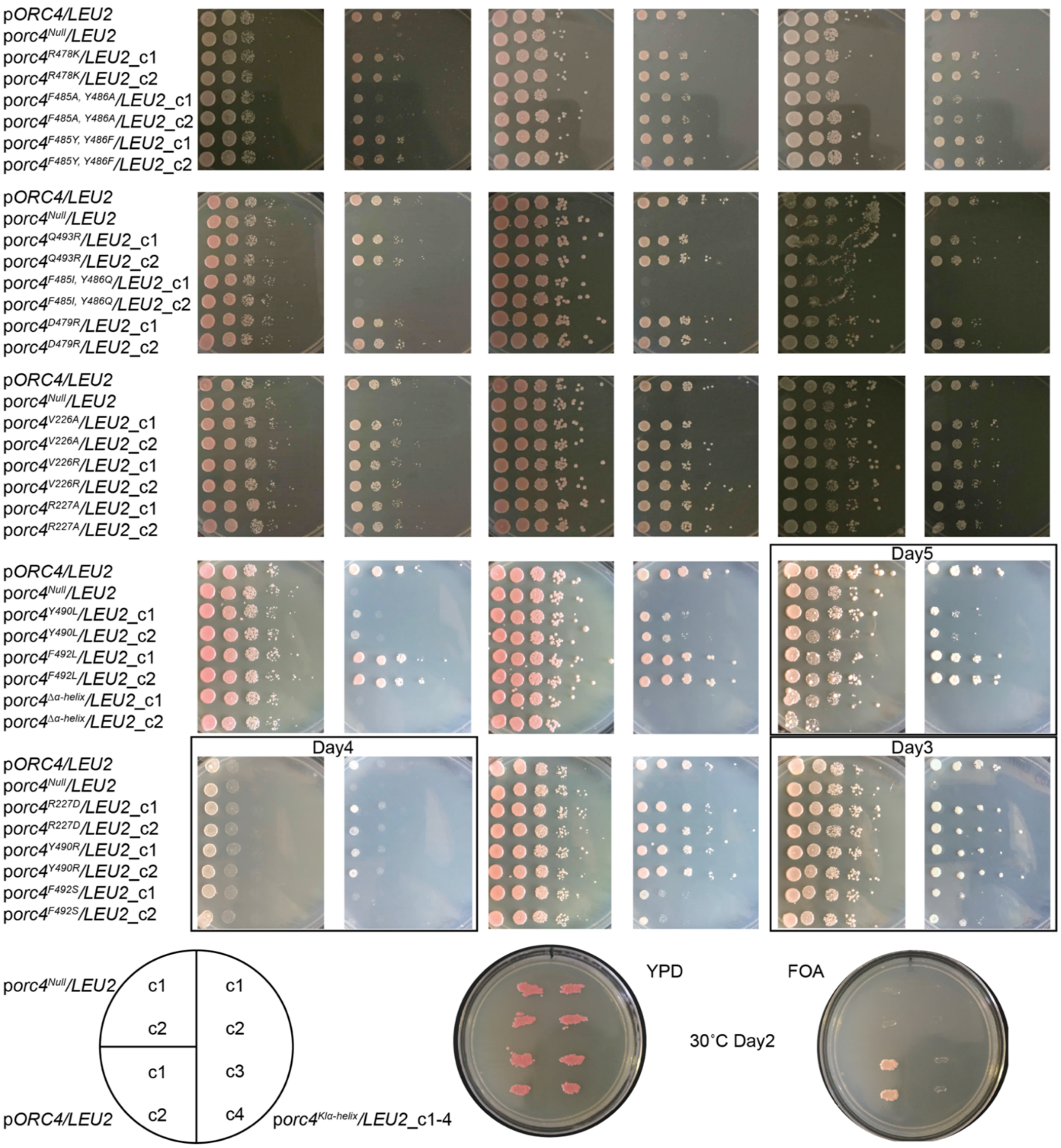
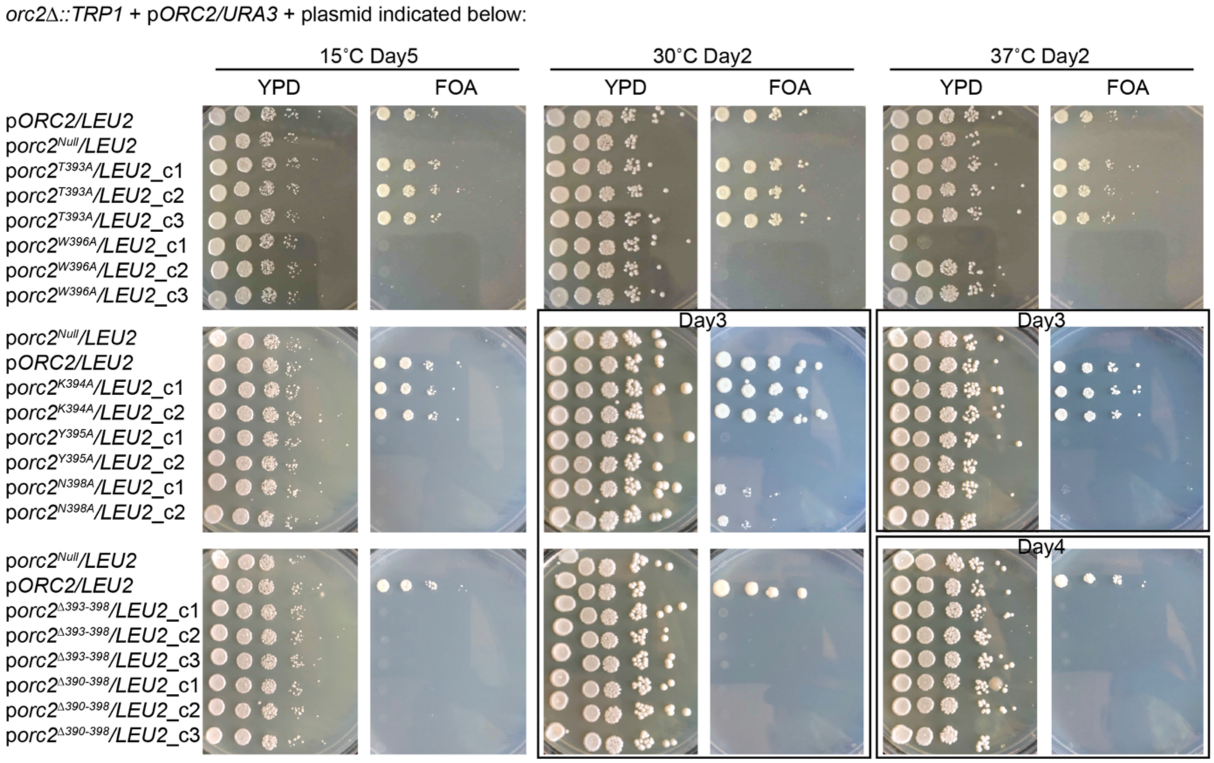
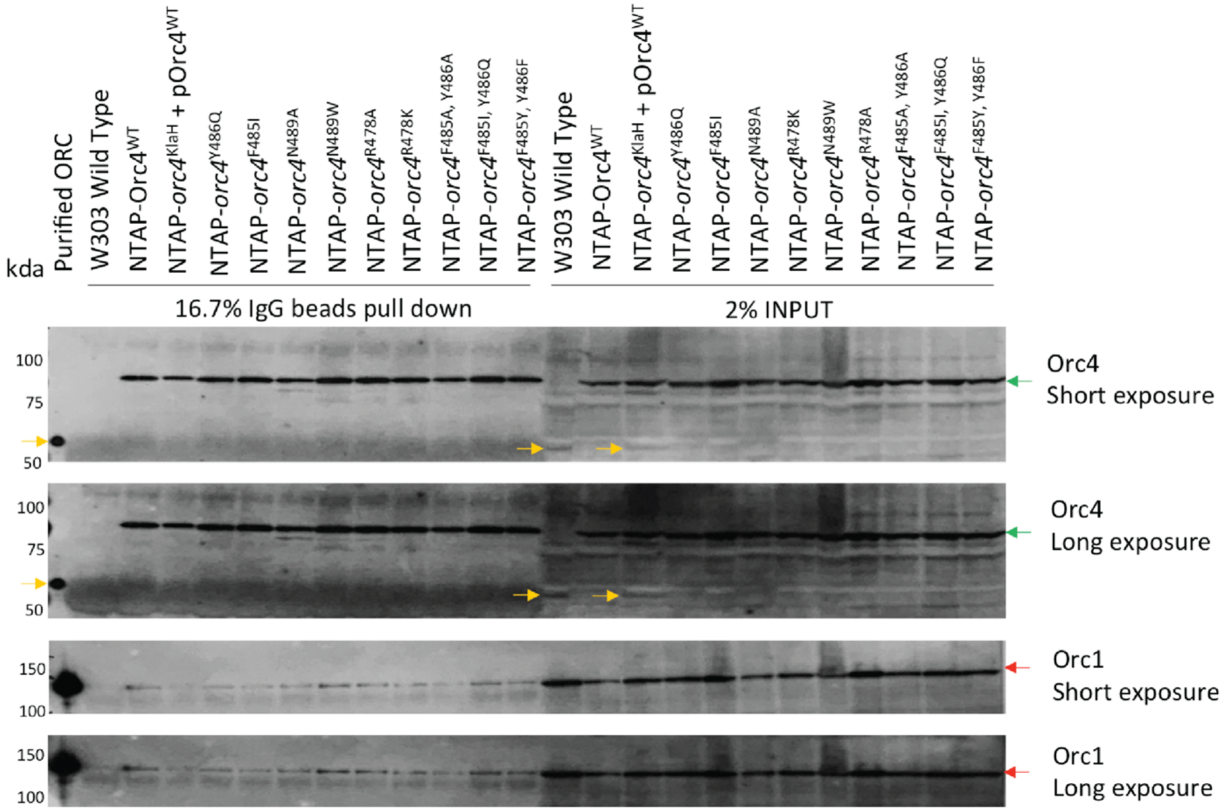
porc4^{T482D}/LEU2_c1
porc4^{T482D}/LEU2_c2



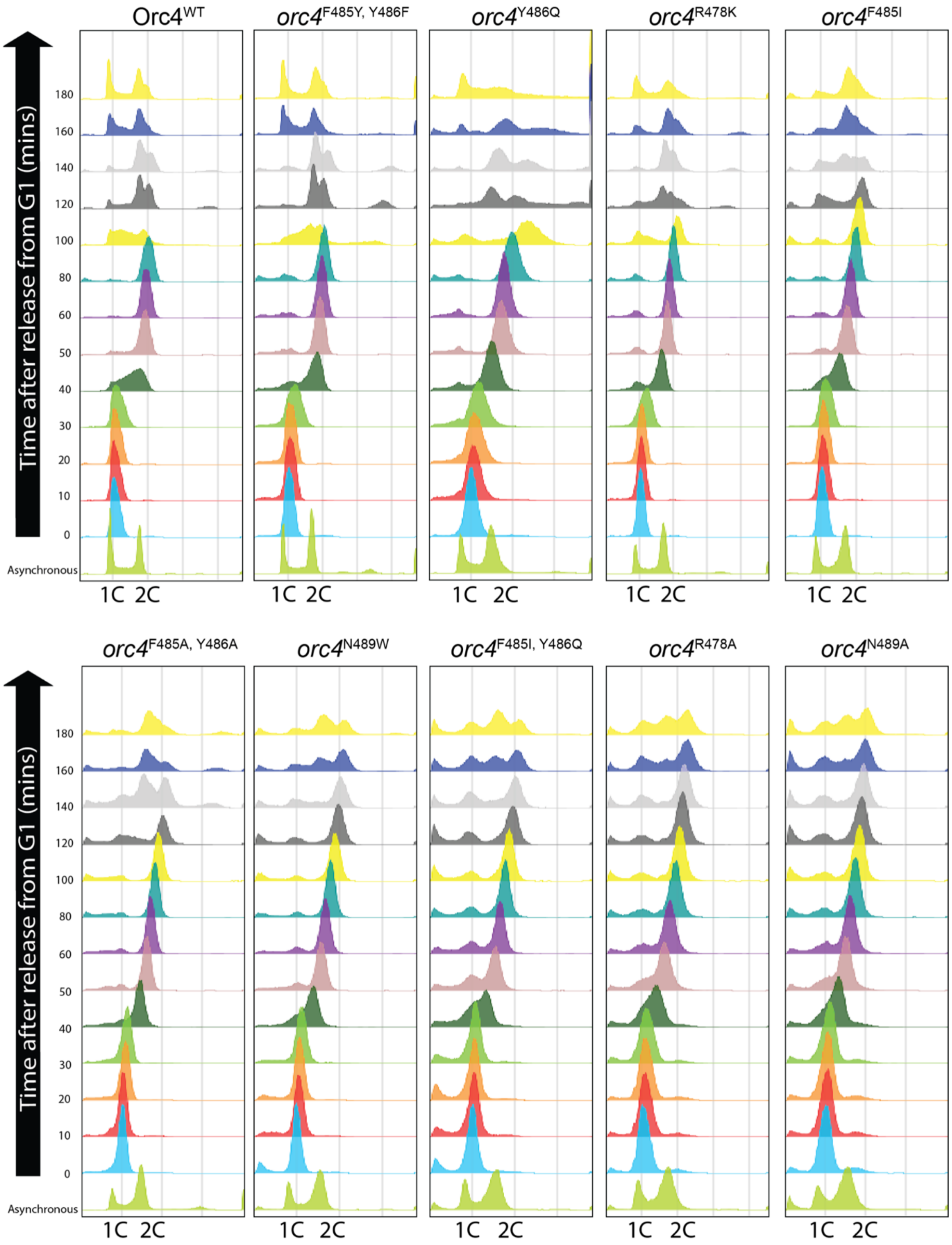
Supplementary Fig. 2 | Orc4 mutants viability phenotypes in plasmid shuffle assay. Detailed procedure of plasmid shuffle assay is described in Method, Plasmid shuffle assay. Briefly, strains (*orc4*Δ*::TRP1* + p*ORC4/URA3* + p*orc4*$^{tested\ allele}$*/LEU2*) were grown overnight in YPD and spotted onto 5-FOA plates with 10-fold serial dilutions starting from 1.5x10^{7} cells and spotted onto YPD plates as control. Mutations were indicated. Strain (*orc4*Δ*::TRP1* + p*ORC4/URA3* + p*ORC4/LEU2*) and strain (*orc4*Δ*::TRP1* + p*ORC4/URA3* + p*orc4*^{null}*/LEU2*) were spotted as controls. Biological duplicates are denoted as c1 and c2. Plates were cultured under 25˚C, 30˚C, or 37˚C for different days (as indicated) to test their cold or temperature sensitivity. *Orc4* mutant phenotypes summarized in Supplementary Table 1.



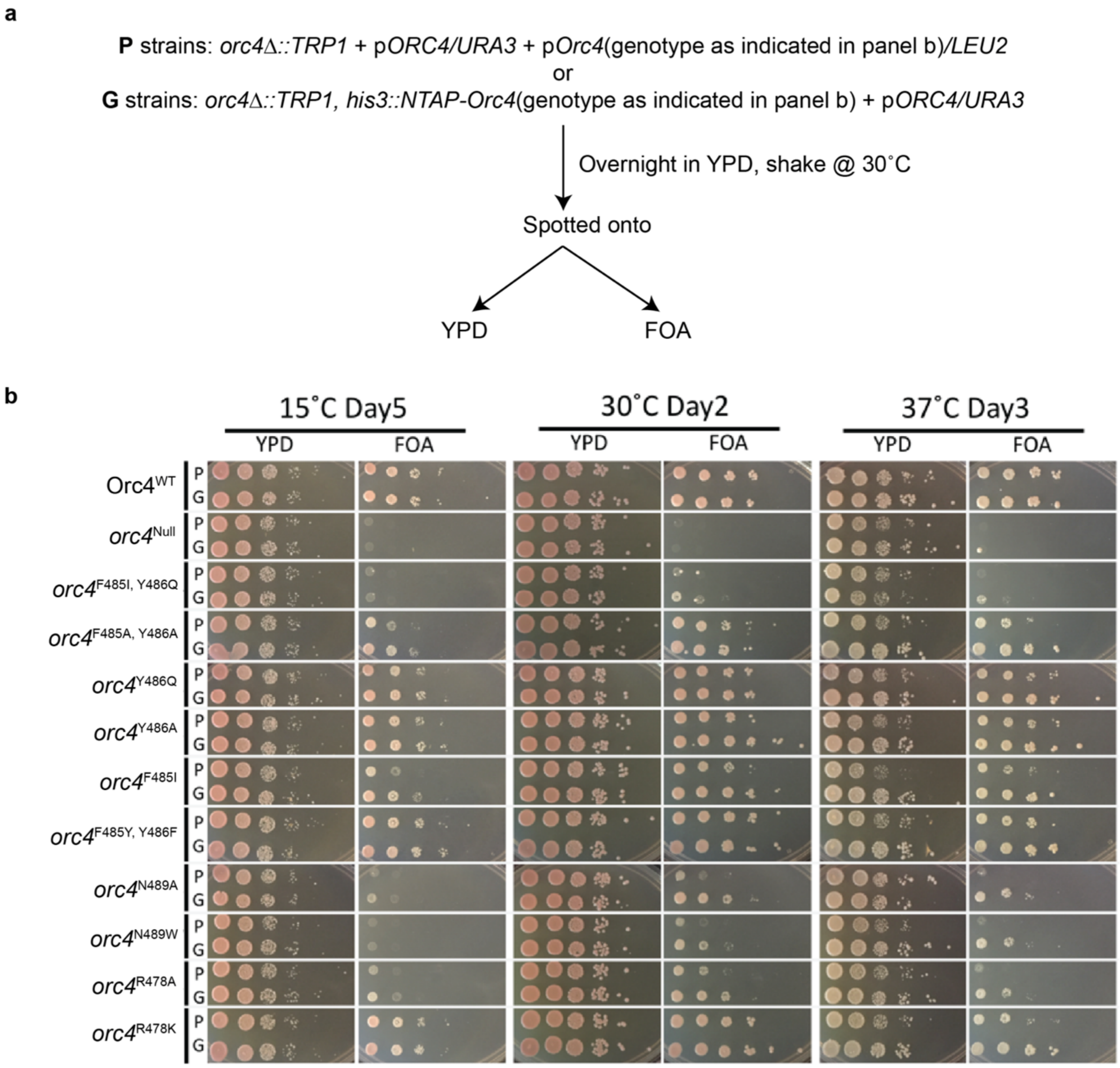
Supplementary Fig. 3 | *Orc2* mutants viability phenotypes in plasmid shuffle assay. Detailed procedure of plasmid shuffle assay is described in Method, Plasmid shuffle assay. Briefly, strains (*orc2*Δ*::TRP1* + p*ORC2/URA3* + p*orc2*$^{tested\ allele}$/*LEU2*) were grown overnight in YPD and spotted onto 5-FOA plates with 10-fold serial dilutions starting from $1.5x10^7$ cells and spotted onto YPD plates as control. Mutations were indicated. Strain (*orc2*Δ*::TRP1* + p*ORC2/URA3* + p*ORC2/LEU2*) and strain (*orc2*Δ*::TRP1* + p*ORC2/URA3* + p*orc2*^{null}/*LEU2*) were spotted as controls. Biological duplicates are denoted as c1 and c2. Plates were cultured under 25˚C, 30˚C, or 37˚C for different days (as indicated) to test their cold or temperature sensitivity. *Orc2* mutant phenotype summarized in Supplementary Table 2.



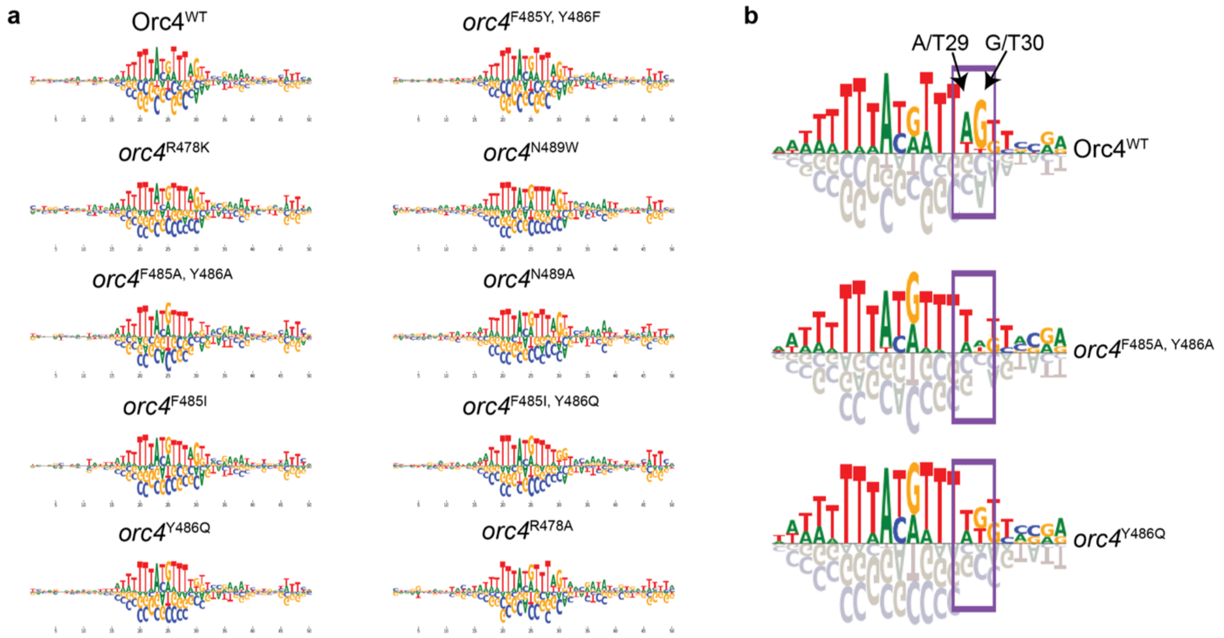
Supplementary Fig. 4 | Orc4 protein expression detection and ORC complex formation detection. Details of method is described in Method, Cell extract preparation, immunoprecipitation, immunoblot analysis and antibodies. NTAP-tagged Orc4 were immunoprecipitated via incubation with IgG beads. Wild type W303 strain, which contains non-tagged Orc4, is used parallelly as a control of pulldown assay. 2% of input and 16.7% of pulled-down lysate were loaded and subsequently immunoblot with anti-Orc4 (SB12) and anti-Orc1 (SB13). Purified ORC complex (including Orc1 and non-tagged Orc4) was also loaded as control for immunoblotting of Orc1 and Orc4. NTAP-tagged Orc4 is around 83kDa (indicated with green arrows) and non-tagged Orc4 is around 56kDa (indicated with yellow arrows), while Orc1 is around 120kDa (indicated with red arrows). Both short and long exposure of blots are indicated. Source data are provided as a Source Data file.



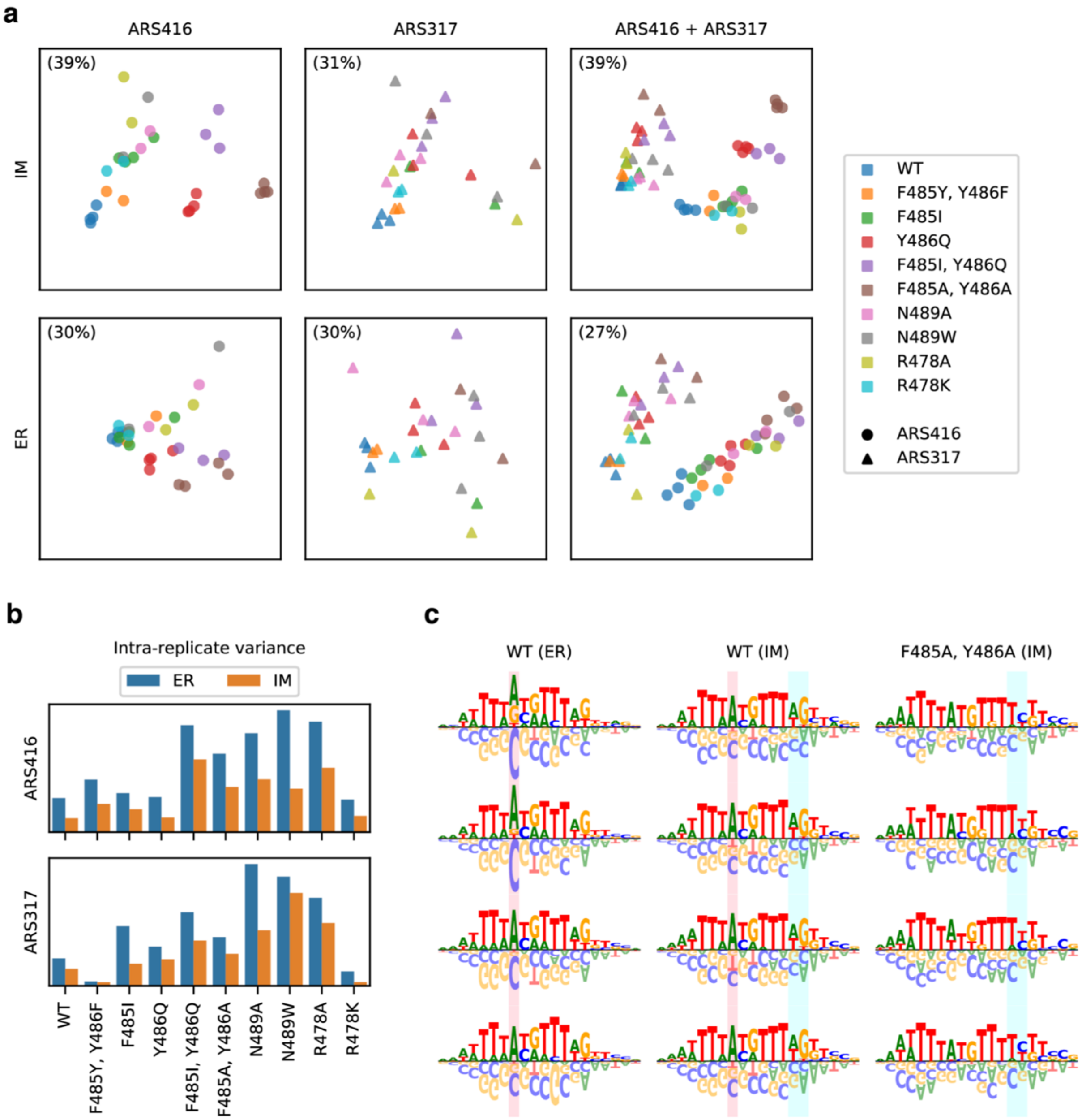
Supplementary Fig. 5 | Cell cycle of NTAP-Orc4 integrated strains. Flow cytometry was done by growing cells into log phase, arresting at G1 phase with α-factor block for 3 hours (around 1~2 cell cycle time length) and then releasing into S phase for different time point (as indicated above on the left). Different time points were harvest and prepared for flow cytometry with method previously described[50]. DNA strained with SYBR green. *Orc4* mutants seemed to have hard time going through S phase and progression through mitosis.



Supplementary Fig. 6 | Analysis of plasmid borne and genome integrated *Orc4* mutants. a, Schematic diagram of viability comparison assay between strains surviving dependent on single episomal origin (*Orc4* on plasmid, denoted as [P]) or multiple chromosomal origins (*Orc4* integrated into genome, denoted as [G]). The [P] strain relies on a CEN-based plasmid with a single replication origin to carry the tested Orc4 mutation and is therefore stringent. **b,** [P] strains (*orc4::TRP1* + p*ORC4/URA3* + p*orc4/LEU2*) and [G] strains (*his3::NTAP-orc4*^{mut}, *orc4::TRP1*, *bar1*Δ*::TRP1*, *LEU2::BrdU-Inc* + p*Orc4/URA3*) were grown overnight in YPD and spotted onto 5-FOA plates with 10-fold serial dilutions starting from 1.5x10^7 cells and spotted onto YPD plates as control. As controls for [P] strains, strain (*orc4::TRP1* + p*ORC4/URA3* + p*ORC4/LEU2*) and strain (*orc4::TRP1* + p*ORC4/URA3* + p*orc4*^{null}*/LEU2*) were spotted. As controls for [G] strains, strain (*his3::NTAP-Orc4*^{WT}, *orc4::TRP1*, *bar1*Δ*::TRP1*, *LEU2::BrdU-Inc* + p*Orc4/URA3*) and strain (*orc4::TRP1*, *bar1*Δ*::TRP1*, *LEU2::BrdU-Inc* + p*Orc4/URA3*) were spotted. Plates were cultured under 25˚C, 30˚C, or 37˚C for different days (as indicated) to test their temperature sensitivity. The strain lacking a NTAP-tagged Orc4 did not grow on FOA. The viability deficient phenotype of *Orc4* mutants on single-origin plasmid seemed to be partially rescued when the mutants are integrated into the genome and survive on multiple origins.

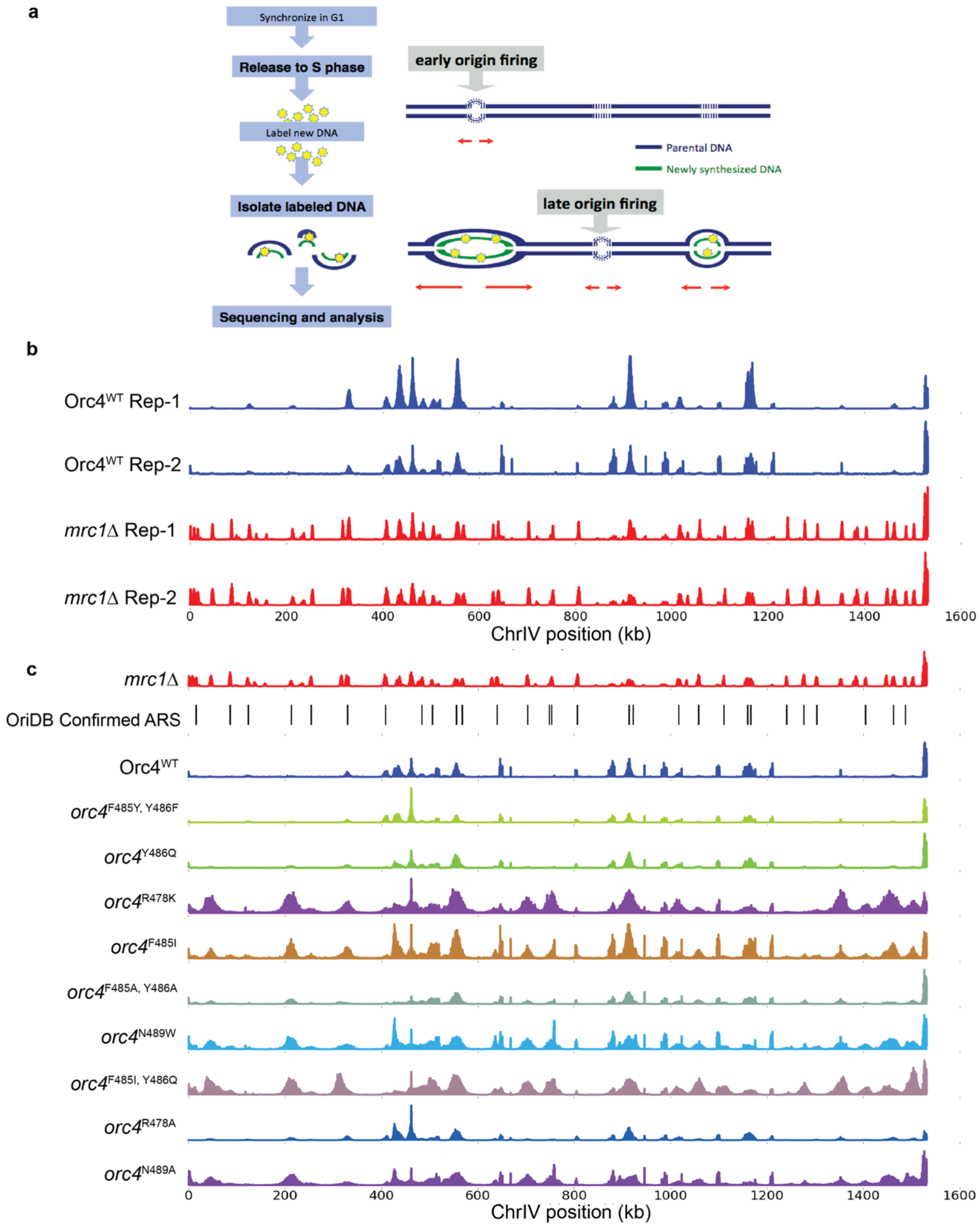


Supplementary Fig. 7 | ARS motif logos generated from MPOS assay using *ARS317* mutations. a, ARS motif logos for *Orc4* integrated strains at A and B1 elements generated using mutation library with *ARS317* sequence backbone. Same as Fig 3, top-half of logos representing the origin sequences that were selected-for in MPOS assay and bottom-half of logos representing the origin sequences that were selected-against in MPOS assay. **b,** Magnified view of A element region in a logo from *Orc4*^{WT}, *orc4*$^{\mathrm{F485A,\ Y486A}}$, *orc4*$^{\mathrm{Y486Q}}$ strains with bottom-half of logo faded. Dark purple circles indicate the major changes at A/T29, G/T30 logo positions in the *Orc4* mutant strains.

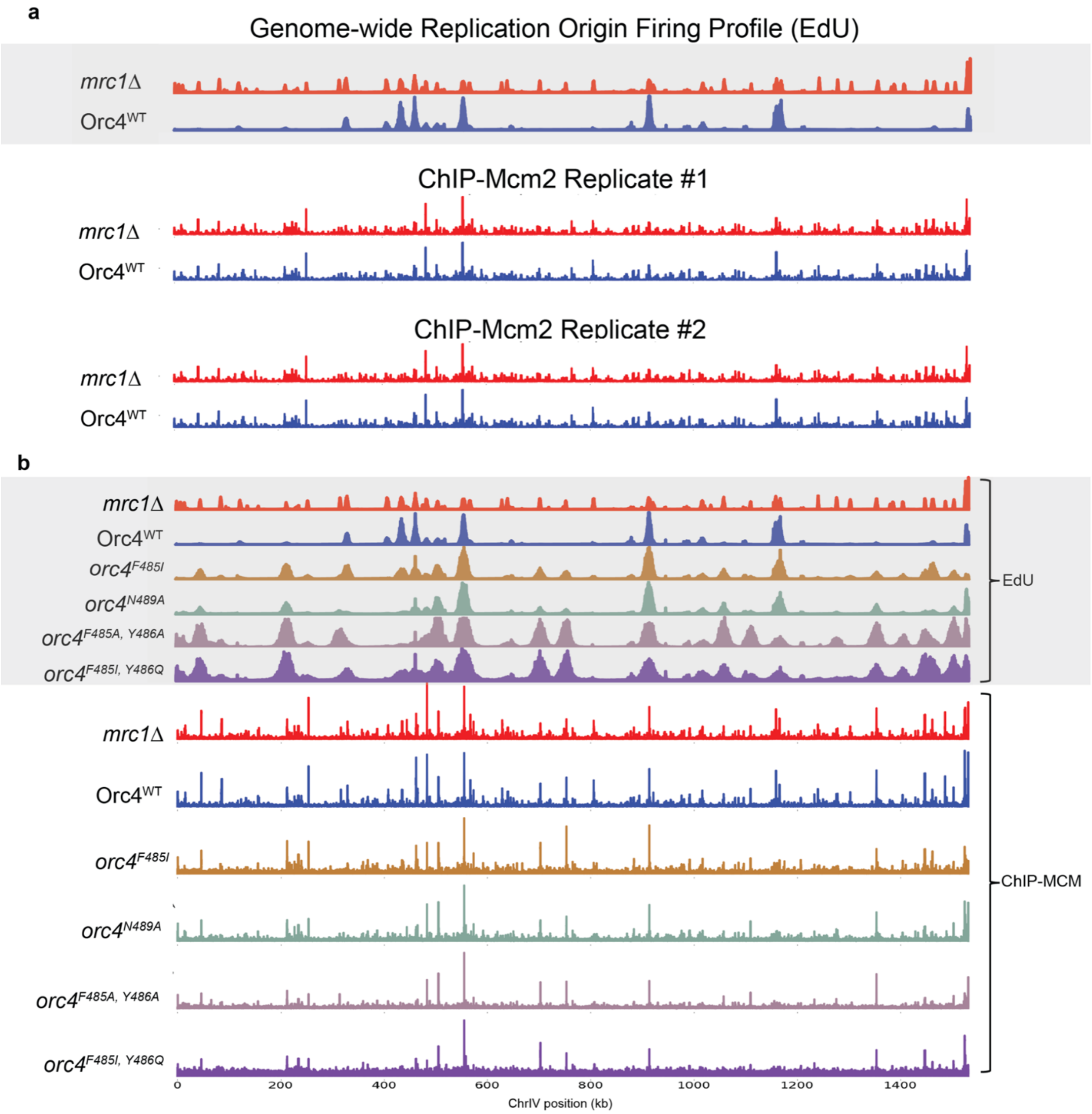


Supplementary Fig. 8 | Principal component analysis and comparison of motif inference methods. a, PCA analyses of motifs (performed on the *ARS416* library MPOS data, the *ARS317* library MPOS data, or both libraries), and inferred using either information maximization (IM) or enrichment ratios (ER). The variance explained by the first two principal components, corresponding to the x- and y-axes of each plot, is indicated in the upper left corner. The dots within each plot represent biologically independent MPOS experiments, dot color indicates the *Orc4* variant assayed, and dot shape indicates the library of mutated ARSs used as input. Note that motifs cluster according to the *Orc4* variant assayed, and that this clustering is stronger for information maximization (IM)-inferred motifs compared to enrichment ratio (ER)-inferred motifs. **b,** Total variance across the motifs inferred for each *Orc4* variant using either ER or IM inference. IM inference consistently yielded less intra-replicate variance than ER inference (48.3%

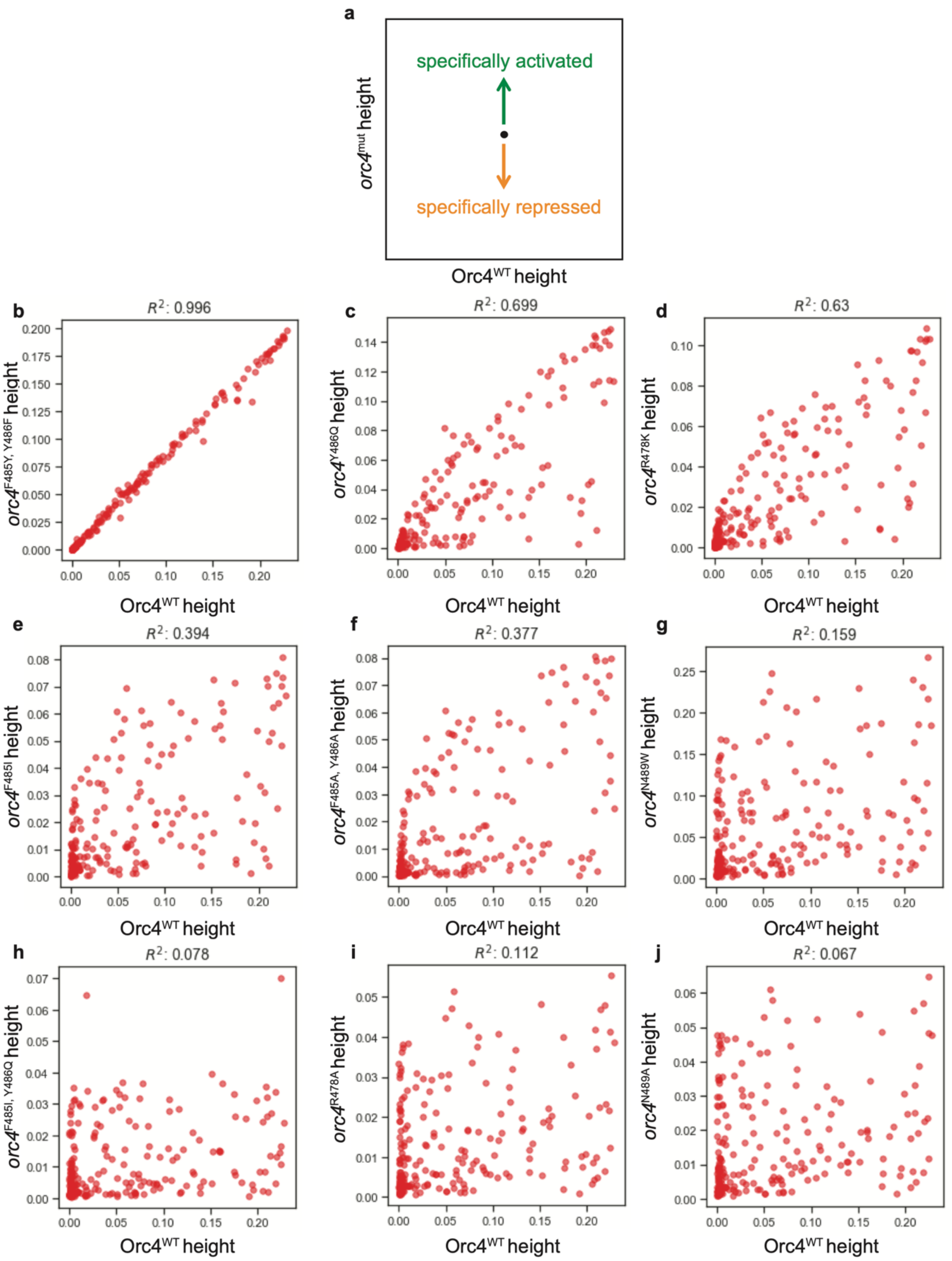
less on average for *ARS416* motifs and 39.9% less for *ARS317* motifs). This again reflects the robustness of IM inference in the face of experiment-to-experiment variation. **c,** Logos showing the *ARS416* motifs for two *Orc4* variants. For clarity, only 20 bp encompassing the essential A element are shown. ER motifs exhibited substantially more variability at key positions than did IM motifs (e.g. rose highlighted positions). *Orc4* mutants resulted in consistently and clearly visible differences in the inferred IM motifs (e.g. cyan positions).

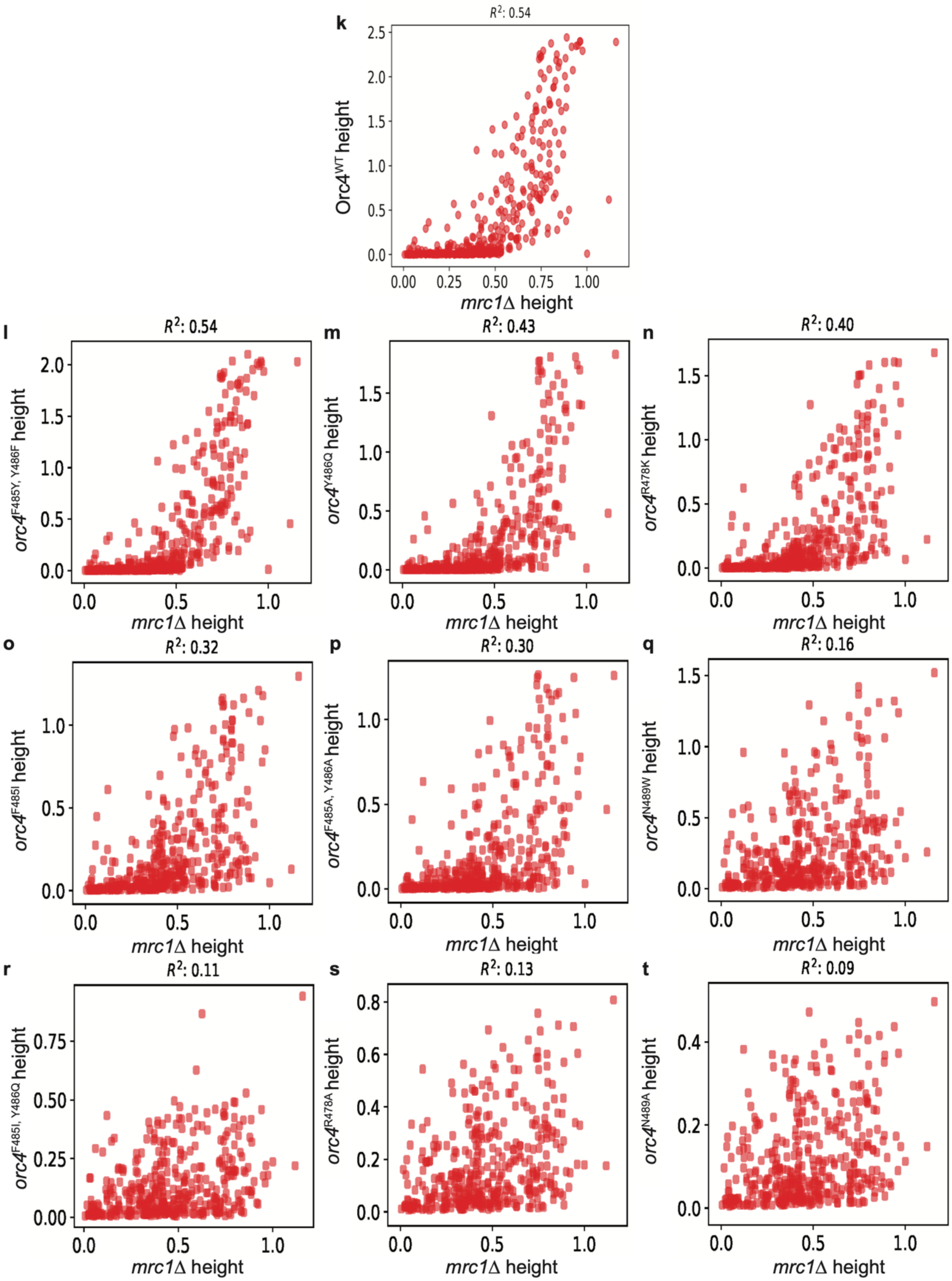


Supplementary Fig. 9 | Replicates of genome-wide replication origin profile. a, Schematic diagram for genome-wide replication origin profile analysis. Details of method is described in Methods, Genome-wide replication origin profile analysis. Briefly, Yeast cells were α-factor blocked in G1 phase for 3 hours and then released into the growth medium (YPD with 200mM HU, 500uM EdU and 0.2mg/ml pronase E) for 90mins before harvest. Flow cytometry was done to check the stage of the cells. DNA is isolated from the harvested cells and sonicated using Bioruptor. EdU labeled newly synthesized DNA is pulldown by Click-iT chemistry with biotinylated azide and Streptavidin T1 magnetic beads. Then Illumina TruSeq Kit is used to establish and amplify the sequencing library. Sequencing data is then analyzed to show peaks on newly synthesized DNA with detailed computational method in Method, Computational analyses of replication origin profile and ChIP-seq data. **b-c,** Replicates of origin firing profiles in Fig. 3. Chromosome IV(ChrIV) is used as representation and replicates are from two independent experiments. **b** shows the direct comparison of two replicates with profiles from *Orc4*^{WT} and *mrc1*Δ strains shown as examples. **c** shows the genome-wide replication origin firing profiles from the all strains in replicate experiment.

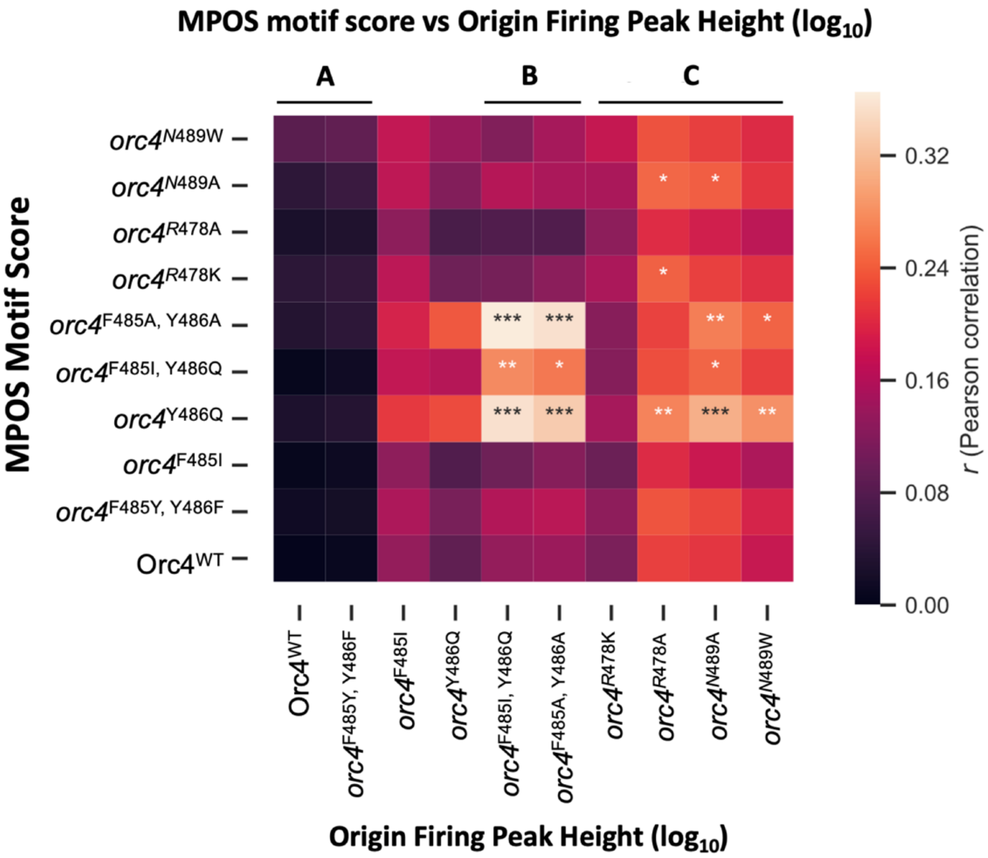


Supplementary Fig. 10 | Chromatin immuno-precipitation (ChIP) of MCM in *Orc4* strains. **a-b,** ChIP profile of MCM (anti-Mcm2) in G1 phase (*Orc4*^{WT}, *orc4*$^{\text{F485I, Y486Q}}$ and *mrc1*Δ profiles in Fig. 3). Chromosome IV(ChrIV) is used as representation. Genome-wide replication origin firing profiles from Fig. 3 is attached for better reference of origin firing pattern and are shadowed in grey. **a** shows the direct comparison of two independent replicates with Mcm2-ChIP profiles from *Orc4*^{WT} and *mrc1*Δ strains shown as examples. **b** shows the Mcm2-ChIP profiles of the all the strains from one of the two replicates.

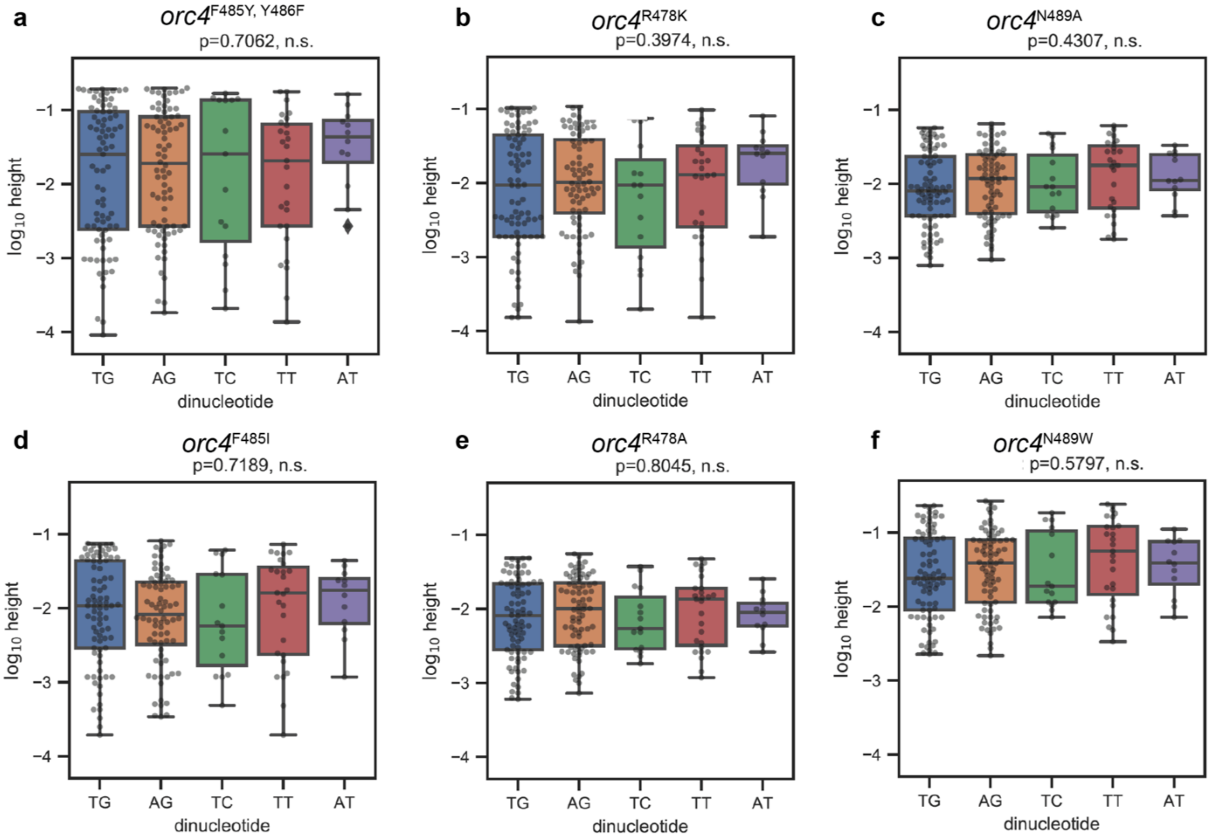

a
specifically activated
specifically repressed
orc4^mut height
Orc4^WT height
b
R²: 0.996
orc4^F485Y, Y486F height
0.000
0.025
0.050
0.075
0.100
0.125
0.150
0.175
0.200
c
R²: 0.699
orc4^Y486Q height
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
d
R²: 0.63
orc4^R478K height
0.00
0.02
0.04
0.06
0.08
0.10
e
R²: 0.394
orc4^F485I height
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
f
R²: 0.377
orc4^F485A, Y486A height
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
g
R²: 0.159
orc4^N489W height
0.00
0.05
0.10
0.15
0.20
0.25
h
R²: 0.078
orc4^F485I, Y486Q height
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
i
R²: 0.112
orc4^R478A height
0.00
0.01
0.02
0.03
0.04
0.05
j
R²: 0.067
orc4^N489A height
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.00
0.05
0.10
0.15
0.20
Orc4^WT height



Supplementary Fig. 11 | Genomic origin firing peak heights scatter plot comparisons. Each dot represent a single replication origin that has its origin firing peak height in *Orc4^{WT}* (in **b-j**) or *mrc1*Δ (in **k-t**) as the x-value and its origin firing peak height in *orc4^{mut}* (in **b-j**, **l-t**) or *Orc4^{WT}* (in **k**) as y-value. Two origins exhibited aberrantly large height values, believed to have arisen from read mapping artefacts, and were removed from this analysis. Height values are normalized by computing the number of reads bounding 99.5% of positions within each profile and divided the entire profile by this number. Coefficient of determination values (R^2) are shown atop each panel. **a**, Illustration diagram for **b**-**j** showing the directions of activation (in green) and repression (in orange) for each replication origin (denote as black dot) in *orc4* mutant strains. **b**-**j**, *orc4*$^{\mathrm{mut}}$ strains direct comparison with *Orc4*^{WT} strain. **k**-**t**, all ten *Orc4* strains direct comparison with *mrc1*Δ strain.

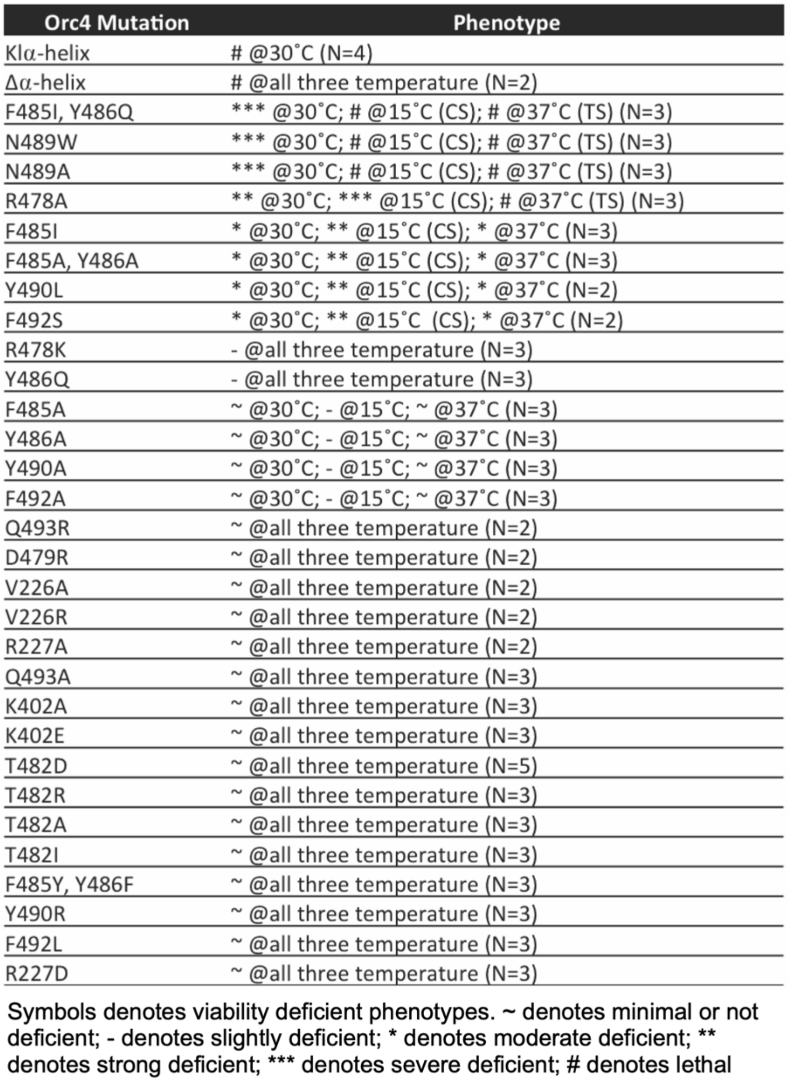


Supplementary Fig. 12 | Correlation of genomic origin peak height and MPOS motif scores. DNA sequences under origin firing peaks that were predicted to be ACS were obtained from OriDB[13,37] and used for analysis. MPOS motif scores were assigned by how good the annotated ACSs matched to the MPOS motifs. Correlations between origin firing peak heights (in $\log_{10}$) and MPOS motif scores were evaluated for each annotated ACS. A section indicates the origin peak heights in wild-type strain or wild-type like *orc4* mutant control strain. We took late vs early origin factors into consideration. However, a large value in origin height does not guarantee a high MPOS motif score. B section indicates the *orc4* F485 and Y486 mutants that were shown to have their origin sequence recognition altered (Fig. 2c and Fig. 4). C section indicates the *orc4* R478 and N489 mutation strains. P-values were also computed to assess the null hypothesis that log EdU heights and motif scores are not correlated; all P-values were Bonferoni corrected (by multiplying by the total number of tests). Significant correlations were indicated: *$p<0.05$, **$p<0.01$, ***$p<0.001$.



Supplementary Fig. 13 | Non-Y486 *orc4* mutants efficiently use origins with the "AG" dinucleotide. Supplemental figure for Fig. 4. Box plots for the 6 *orc4* mutant strains that do not have Y486 changed, except for the *orc4*$^{\mathrm{F485Y,\ Y486F}}$ strain that contains a conserved mutation whose strain grows similar to *Orc4*^{WT} and therefore is an exception of Y486 mutation strain. **a.** *orc4*$^{\mathrm{F485Y,Y486F}}$, **b.** *orc4*$^{\mathrm{R478K}}$, **c.** *orc4*$^{\mathrm{N489A}}$, **d.** *orc4*$^{\mathrm{F485I}}$, **e.** *orc4*$^{\mathrm{R478A}}$, **f.** *orc4*$^{\mathrm{N489W}}$. Y-axis is genomic origin firing peak heights in $\log_{10}$. Each dot denotes an annotated ACS. Box plots elements: the minimum height, first (lower) quartile, median, third (upper) quartile, and maximum height. Diamond denotes outliers that exhibited aberrantly large values.

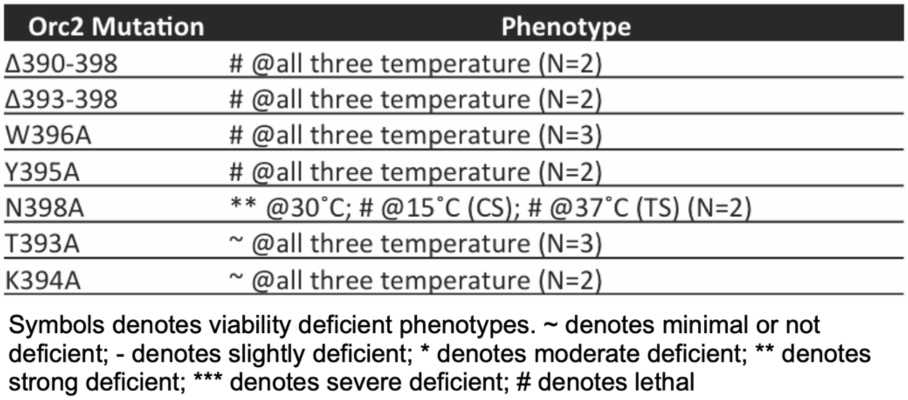
Supplementary Tables



Orc4 Mutation	Phenotype
Klα-helix	# @30˚C (N=4)
Δα-helix	# @all three temperature (N=2)
F485I, Y486Q	*** @30˚C; # @15˚C (CS); # @37˚C (TS) (N=3)
N489W	*** @30˚C; # @15˚C (CS); # @37˚C (TS) (N=3)
N489A	*** @30˚C; # @15˚C (CS); # @37˚C (TS) (N=3)
R478A	** @30˚C; *** @15˚C (CS); # @37˚C (TS) (N=3)
F485I	* @30˚C; ** @15˚C (CS); * @37˚C (N=3)
F485A, Y486A	* @30˚C; ** @15˚C (CS); * @37˚C (N=3)
Y490L	* @30˚C; ** @15˚C (CS); * @37˚C (N=2)
F492S	* @30˚C; ** @15˚C (CS); * @37˚C (N=2)
R478K	- @all three temperature (N=3)
Y486Q	- @all three temperature (N=3)
F485A	~ @30˚C; - @15˚C; ~ @37˚C (N=3)
Y486A	~ @30˚C; - @15˚C; ~ @37˚C (N=3)
Y490A	~ @30˚C; - @15˚C; ~ @37˚C (N=3)
F492A	~ @30˚C; - @15˚C; ~ @37˚C (N=3)
Q493R	~ @all three temperature (N=2)
D479R	~ @all three temperature (N=2)
V226A	~ @all three temperature (N=2)
V226R	~ @all three temperature (N=2)
R227A	~ @all three temperature (N=2)
Q493A	~ @all three temperature (N=3)
K402A	~ @all three temperature (N=3)
K402E	~ @all three temperature (N=3)
T482D	~ @all three temperature (N=5)
T482R	~ @all three temperature (N=3)
T482A	~ @all three temperature (N=3)
T482I	~ @all three temperature (N=3)
F485Y, Y486F	~ @all three temperature (N=3)
Y490R	~ @all three temperature (N=3)
F492L	~ @all three temperature (N=3)
R227D	~ @all three temperature (N=3)

Symbols denotes viability deficient phenotypes. ~ denotes minimal or not deficient; - denotes slightly deficient; * denotes moderate deficient; ** denotes strong deficient; *** denotes severe deficient; # denotes lethal

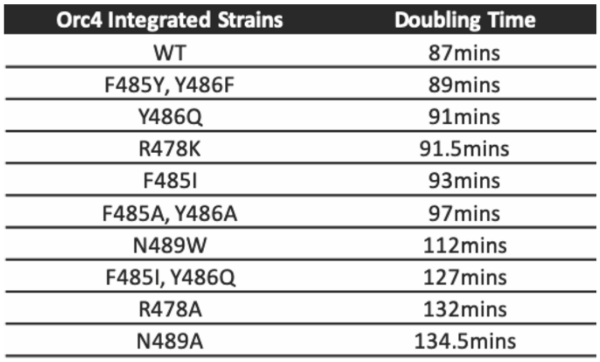
Supplementary Table 1 | Summary of plasmid shuffle assay *Orc4* mutant phenotypes. *Orc4* mutant viability phenotypes summarized from plasmid shuffle assay (Supplementary Fig. 1). Indicated symbols denotes different viability phenotypes.



Orc2 Mutation	Phenotype
Δ390-398	# @all three temperature (N=2)
Δ393-398	# @all three temperature (N=2)
W396A	# @all three temperature (N=3)
Y395A	# @all three temperature (N=2)
N398A	** @30˚C; # @15˚C (CS); # @37˚C (TS) (N=2)
T393A	~ @all three temperature (N=3)
K394A	~ @all three temperature (N=2)

Symbols denotes viability deficient phenotypes. ~ denotes minimal or not deficient; - denotes slightly deficient; * denotes moderate deficient; ** denotes strong deficient; *** denotes severe deficient; # denotes lethal

Supplementary Table 2 | Summary of plasmid shuffle assay *Orc2* mutant phenotypes. *Orc2* mutant viability phenotypes summarized from plasmid shuffle assay (Supplementary Fig. 2). Indicated symbols denotes different viability phenotypes.



Orc4 Integrated Strains	Doubling Time
WT	87mins
F485Y, Y486F	89mins
Y486Q	91mins
R478K	91.5mins
F485I	93mins
F485A, Y486A	97mins
N489W	112mins
F485I, Y486Q	127mins
R478A	132mins
N489A	134.5mins

Supplementary Table 3 | Summary of doubling time of NTAP-Orc4 integrated strains. Doubling time were calculated based on growth curves (see Fig. 1f) log phase cell concentration.

$$k = \frac{\Delta \log(Cell\ Conc.)}{\Delta Time} = \frac{\log(2)}{Doubling\ Time} \qquad (4)$$

$$Doubling\ Time = {}^{\log(2)}/_{k} \qquad (5)$$

k dictates the slope of linear regression line of growth curves log phase.

Supplementary Methods

Strain	Genotype	Source
YB51	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS415/ORC4*	This lab
YS856	*MATa ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100*	This study
YB410	*MATα orc2::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS316/ORC2*	This lab
YS3285	*MATa mrc1Δ::KanMX6 bar1Δ::TRP1 URA3::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100*	This study
YB 1519	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/ORC4*	This study
YB 1515	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415*	This study
YB1639	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4Δα-helix*	This study
YB 1514	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4Klα-helix*	This study
YB 1520	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4Y486Q*	This study
YB 1521	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4Y486A*	This study
YB 1522	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4F485I*	This study
YB 1523	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4F485A*	This study
YB 1524	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4N489A*	This study
YB 1525	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4N489W*	This study
YB 1526	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4F492A*	This study
YB 1527	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4Y490A*	This study
YB 1528	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4R478A*	This study
YB 1555	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4Q493A*	This study
YB 1556	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4K402A*	This study
YB 1557	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4K402E*	This study
YB 1558	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4T482A*	This study
YB 1559	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4T482R*	This study

YB 1560	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4T482I*	This study
YB 1571	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4T482D*	This study
YB 1562	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4R478K*	This study
YB 1563	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4F485A, Y486A*	This study
YB 1564	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4F485Y, Y486F*	This study
YB1635	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4F485I, Y486Q*	This study
YKC01	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4F492L*	This study
YKC02	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4Y490L*	This study
YKC03	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4Y490R*	This study
YKC04	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4F492S*	This study
YB1637	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4R227D*	This study
YEH003	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4V226A*	This study
YEH004	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4V226R*	This study
YEH005	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4R227A*	This study
YEH002	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4Q493R*	This study
YEH001	*MATα orc4::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4 + pRS415/orc4D479R*	This study
YS2251	*MATa bar1Δ::TRP1 ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100*	
YB 1549	*MATa bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100*	This study
YB1588	*MATa orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4*	This study
YB 1623	*MATa his3::TAP-ORC4 orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4*	This study
YB 1624	*MATa his3::TAP-orc4F485I orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4*	This study

YB 1625	*MATa his3::TAP-orc4N489A orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4*	This study
YB 1630	*MATa his3::TAP-orc4KLaH orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4*	This study
YB 1631	*MATa his3::TAP-orc4F485Y+Y486F orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4*	This study
YB 1632	*MATa his3::TAP-orc4R478K orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4*	This study
YB 1633	*MATa his3::TAP-orc4ΔaH orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4*	This study
YB1647	*MATa his3::TAP-orc4N489W orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4*	This study
YB1648	*MATa his3::TAP-orc4R478A orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4*	This study
YB1652	*MATa his3::TAP-orc4Y486Q orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4*	This study
YB1653	*MATa his3::TAP-orc4F485A+Y486A orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4*	This study
YB1654	*MATa his3::TAP-orc4F485I+Y486Q orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS416/ORC4*	This study
YB1649	*MATa his3::TAP-ORC4 orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100*	This study
YB1650	*MATa his3::TAP-orc4F485I orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100*	This study
YB1655	*MATa his3::TAP-orc4N489A orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100*	This study
YB1656	*MATa his3::TAP-orc4F485Y+Y486F orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100*	This study
YB1657	*MATa his3::TAP-orc4R478K orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100*	This study

YB1658	*MATa his3::TAP-orc4N489W orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100*	This study
YB1659	*MATa his3::TAP-orc4R478A orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100*	This study
YB1660	*MATa his3::TAP-orc4Y486Q orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100*	This study
YB1661	*MATa his3::TAP-orc4F485A+Y486A orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100*	This study
YB1662	*MATa his3::TAP-orc4F485I+Y486Q orc4::TRP1 bar1Δ::TRP1 LEU2::BrdU-Inc ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3,112 can1-100*	This study
YB 1620	*MATα orc2::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS316/ORC2 + pRS415*	This study
YB 1619	*MATα orc2::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS316/ORC2 + pRS415/ORC2*	This study
YB 1621	*MATα orc2::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS316/ORC2 + pRS415/orc2T393A*	This study
YB 1611	*MATα orc2::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS316/ORC2 + pRS415/orc2W396A*	This study
YKC05	*MATα orc2::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS316/ORC2 + pRS415/orc2K394A*	This study
YKC06	*MATα orc2::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS316/ORC2 + pRS415/orc2Y395A*	This study
YKC07	*MATα orc2::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS316/ORC2 + pRS415/orc2N398A*	This study
YKC08	*MATα orc2::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS316/ORC2 + pRS415/orc2Δ393-398*	This study
YKC09	*MATα orc2::TRP1 ade2-1 ura3-1 his 3-11, 15 trp1-1 leu2-3,112 can1-100 + pRS316/ORC2 + pRS415/orc2Δ390-398*	This study

Supplementary Methods Table 1 | Genotype and resources of yeast strains used in this study

Primer Names	Primer Sequence	Note
YEH001	GCGACCGCAGCATTTcaaGCTAGCAATTATCAATTTCAGGG	Used for Orc4 mutagenesis
YEH002	CCCTGAAATTGATAATTGCTAGCttgAAATGCTGCGGTCGC	Used for Orc4 mutagenesis
YEH003	CGACCGCAGCATTTgccGCTAGCAATTATCAATTTCAG	Used for Orc4 mutagenesis
YEH004	CTGAAATTGATAATTGCTAGCggcAAATGCTGCGGTCGC	Used for Orc4 mutagenesis
YEH005	CGACCGCAGCAattTACGCTAGCAATTATCAATTTCAGGG	Used for Orc4 mutagenesis
YEH006	CCCTGAAATTGATAATTGCTAGCGTAaatTGCTGCGGTCG	Used for Orc4 mutagenesis
YEH007	CGACCGCAGCAgctTACGCTAGCAATTATCAATTTCAGGG	Used for Orc4 mutagenesis
YEH008	CCCTGAAATTGATAATTGCTAGCGTAagcTGCTGCGGTCG	Used for Orc4 mutagenesis
YEH009	CGACCGCAGCATTTTACGCTAGCgctTATCAATTTCAGGG	Used for Orc4 mutagenesis
YEH010	CCTGAAATTGATAagcGCTAGCGTAAAATGCTGCGGTCGC	Used for Orc4 mutagenesis
YEH011	GCATTTTACGCTAGCtggTATCAATTTCAGGGCACCATGATCCCG	Used for Orc4 mutagenesis

YEH012	CGGGATCATGGTGCCCTGAAATTGATAccaGCTAGCGTAAAATGC	Used for Orc4 mutagenesis
YEH013	ACCGCAGCATTTTACGCTAGCAATTATCAAgctCAGGGCACC	Used for Orc4 mutagenesis
YEH014	GGTGCCCTGagcTTGATAATTGCTAGCGTAAAATGCTGCGGT	Used for Orc4 mutagenesis
YEH015	ACCGCAGCATTTTACGCTAGCAATgctCAATTTCAGGGC	Used for Orc4 mutagenesis
YEH016	GCCCTGAAATTGagcATTGCTAGCGTAAAATGCTGCGGT	Used for Orc4 mutagenesis
YEH017	CCGAGAAATCAGCCGTTGGTTTGgcaGATAATGCGAC	Used for Orc4 mutagenesis
YEH018	GTCGCATTATCtgcCAAACCAACGGCTGATTTCTCGG	Used for Orc4 mutagenesis
YEH024	CCGTTGGTTTGAGAGATAATGAAGACTCTGCTATACAATCAGCAAACTACAATTCATCCGGCACCATGATCCCGTTTGAC	Used for Orc4 mutagenesis
YEH025	GTCAAACGGGATCATGGTGCCGGATGAATTGTAGTTTGCTGATTGTATAGCAGAGTCTTCATTATCTCTCAAACCAACGG	Used for Orc4 mutagenesis
YEH026	GCCGTTGGTTTGAGAGATAATGGCACCATGATCCCGTTTGAC	Used for Orc4 mutagenesis
YEH027	GTCAAACGGGATCATGGTGCCATTATCTCTCAAACCAACGGC	Used for Orc4 mutagenesis
YEH032	GCATTTTACGCTAGCAATTATCAATTTgccGGCACCATGATCCC	Used for Orc4

		mutagenesis
YEH033	GGGATCATGGTGCCggcAAATTGATAATTGCTAGCGTAAAATGC	Used for Orc4 mutagenesis
YEH034	GCATTTTACGCTAGCAATTATCAATTTagaGGCACCATGATCCCG	Used for Orc4 mutagenesis
YEH035	CGGGATCATGGTGCCtctAAATTGATAATTGCTAGCGTAAAATGC	Used for Orc4 mutagenesis
YEH036	GGTTGCCTTAAGGGCGgccGACGGATCTTTTAATTTTAATTTAGC	Used for Orc4 mutagenesis
YEH037	GCTAAATTAAAATTAAAAGATCCGTCggcCGCCCTTAAGGCAACC	Used for Orc4 mutagenesis
YEH038	GGTTGCCTTAAGGGCGgagGACGGATCTTTTAATTTTAATTTAGC	Used for Orc4 mutagenesis
YEH039	GCTAAATTAAAATTAAAAGATCCGTCctcCGCCCTTAAGGCAACC	Used for Orc4 mutagenesis
YEH040	CGTTGGTTTGAGAGATAATGCGgcgGCAGCATTTTACGCTAGC	Used for Orc4 mutagenesis
YEH041	GCTAGCGTAAAATGCTGCcgcCGCATTATCTCTCAAACCAACG	Used for Orc4 mutagenesis
YEH042	CGTTGGTTTGAGAGATAATGCGcggGCAGCATTTTACGCTAGC	Used for Orc4 mutagenesis
YEH043	GCTAGCGTAAAATGCTGCccgCGCATTATCTCTCAAACCAACG	Used for Orc4 mutagenesis

YEH180	CAGCAgctgccGCTAGCAATTATCAATTTCAG	Used for Orc4 mutagenesis
YEH181	CTGAAATTGATAATTGCTAGCggcagcTGCTG	Used for Orc4 mutagenesis
YEH182	CGACCGCAGCAattcaaGCTAGCAATTATCAATTTCAG	Used for Orc4 mutagenesis
YEH183	CTGAAATTGATAATTGCTAGCttgaatTGCTGCGGTCG	Used for Orc4 mutagenesis
YEH184	CGACCGCAGCAtactttGCTAGCAATTATCAATTTCAG	Used for Orc4 mutagenesis
YEH185	CTGAAATTGATAATTGCTAGCaaagtaTGCTGCGGTCG	Used for Orc4 mutagenesis
YEH186	CCGAGAAATCAGCCGTTGGTTTGaaaGATAATGCGAC	Used for Orc4 mutagenesis
YEH187	GTCGCATTATCtttCAAACCAACGGCTGATTTCTCGG	Used for Orc4 mutagenesis
YEH188	CGTTGGTTTGAGAGATAATGCGattGCAGCATTTTACGCTAGC	Used for Orc4 mutagenesis
YEH189	GCTAGCGTAAAATGCTGCaatCGCATTATCTCTCAAACCAACG	Used for Orc4 mutagenesis
YEH190	CGTTGGTTTGAGAGATAATGCGgatGCAGCATTTTACGCTAGC	Used for Orc4 mutagenesis
YEH191	GCTAGCGTAAAATGCTGCatcCGCATTATCTCTCAAACCAACG	Used for Orc4

		mutagenesis
YEH235	ACATTTGCTGGGCCTgccAGGCAAACTTTATTATACAATC	Used for Orc4 mutagenesis
YEH236	GATTGTATAATAAAGTTTGCCTggcAGGCCCAGCAAATGT	Used for Orc4 mutagenesis
YEH237	ACATTTGCTGGGCCTcgcAGGCAAACTTTATTATACAATC	Used for Orc4 mutagenesis
YEH238	GATTGTATAATAAAGTTTGCCTgcgAGGCCCAGCAAATGT	Used for Orc4 mutagenesis
YEH239	ACATTTGCTGGGCCTGTGgccCAAACTTTATTATACAATC	Used for Orc4 mutagenesis
YEH240	GATTGTATAATAAAGTTTGggcCACAGGCCCAGCAAATGT	Used for Orc4 mutagenesis
YEH241	ACATTTGCTGGGCCTGTGgacCAAACTTTATTATACAATC	Used for Orc4 mutagenesis
YEH242	GATTGTATAATAAAGTTTGgtcCACAGGCCCAGCAAATGT	Used for Orc4 mutagenesis
YEH304	CCGAGAAATCAGCCGTTGGTTTGAGAagaAATGCGAC	Used for Orc4 mutagenesis
YEH305	GTCGCATTtctTCTCAAACCAACGGCTGATTTCTCGG	Used for Orc4 mutagenesis
YEH352	CCGCAGCATTTTACGCTAGCAATttgCAATTTCAGGGCACC	Used for Orc4 mutagenesis

YEH353	GGTGCCCTGAAATTGcaaATTGCTAGCGTAAAATGCTGCGG	Used for Orc4 mutagenesis
YEH354	CCGCAGCATTTTACGCTAGCAATagaCAATTTCAGGGCACC	Used for Orc4 mutagenesis
YEH355	GGTGCCCTGAAATTGtctATTGCTAGCGTAAAATGCTGCGG	Used for Orc4 mutagenesis
YEH356	CGCTAGCAATTATCAActaCAGGGCACCATGATCCCG	Used for Orc4 mutagenesis
YEH357	CGGGATCATGGTGCCCTGtagTTGATAATTGCTAGCG	Used for Orc4 mutagenesis
YEH358	CGCTAGCAATTATCAAtcaCAGGGCACCATGATCCCG	Used for Orc4 mutagenesis
YEH359	CGGGATCATGGTGCCCTGtgaTTGATAATTGCTAGCG	Used for Orc4 mutagenesis
YEH192	GACAAGAAGCGAAgccAAGTACTGGGGCAATCATGTG	Used for Orc2 mutagenesis
YEH193	CACATGATTGCCCCAGTACTTggcTTCGCTTCTTGTC	Used for Orc2 mutagenesis
YEH194	GACAAGAAGCGAAACTgctTACTGGGGCAATCATGTG	Used for Orc2 mutagenesis
YEH195	CACATGATTGCCCCAGTAagcAGTTTCGCTTCTTGTC	Used for Orc2 mutagenesis
YEH196	GACAAGAAGCGAAACTAAGgctTGGGGCAATCATGTG	Used for Orc2

		mutagenesis
YEH197	CACATGATTGCCCCAagcCTTAGTTTCGCTTCTTGTC	Used for Orc2 mutagenesis
YEH198	GACAAGAAGCGAAACTAAGTACgctGGCAATCATGTG	Used for Orc2 mutagenesis
YEH199	CACATGATTGCCagcGTACTTAGTTTCGCTTCTTGTC	Used for Orc2 mutagenesis
YEH200	ACTAAGTACTGGGGCgctCATGTGATTTTGCAGA	Used for Orc2 mutagenesis
YEH201	TCTGCAAAATCACATGagcGCCCCAGTACTTAGT	Used for Orc2 mutagenesis
YEH326	CTGAGTTGACAAGAAGCGAACATGTGATTTTGCAGATCCA	Used for Orc2 mutagenesis
YEH327	TGGATCTGCAAAATCACATGTTCGCTTCTTGTCAACTCAG	Used for Orc2 mutagenesis
YEH328	TGGTCCCCGCTGAGTTGACACATGTGATTTTGCAGATCCA	Used for Orc2 mutagenesis
YEH329	TGGATCTGCAAAATCACATGTGTCAACTCAGCGGGGACCA	Used for Orc2 mutagenesis
YEH019	ATGACTATAAGCGAAGCTCG	Used for Orc4 sequencing

YEH020	CTCAATTGGAACAGCAGTTG	Used for Orc4 sequencing /integrated NTAP-Orc4 sequencing
YEH021	AAGAGTGATTTATATGCCGC	Used for Orc4 sequencing /integrated NTAP-Orc4 sequencing
YEH022	TCAACTCCAGAATTCCCAC	Used for Orc4 sequencing /integrated NTAP-Orc4 sequencing
YEH023	TCACAGTTGTGTCCAGGAG	Used for Orc4 sequencing
YEH221	GGAAGACTTTGTAGAGCAT	Used for Orc2 sequencing
YEH222	CCTACACGTATGCGGAA	Used for Orc2 sequencing
YEH223	ATGCTCTACAAAGTCTTCC	Used for Orc2 sequencing
YEH224	TTCCGCATACGTGTAGG	Used for Orc2 sequencing
gYEH003-F	ATTTGTAATACGCTTTACTAGTTTT	Used for gRNA003 insertion to bRA89 plasmid

gYEH003-R	TAGTAAAGCGTATTACAAATGATCA	Used for gRNA003 insertion to bRA89 plasmid
gYEH005-F	GAATATACTAAAAAATGAGCGTTTT	Used for gRNA005 insertion to bRA89 plasmid
gYEH005-R	GCTCATTTTTTAGTATATTCGATCA	Used for gRNA005 insertion to bRA89 plasmid
YEH263	AGAAGAAGCATGGCAGGCCTTG	Used for CRISPR NTAP-Orc4 template construction/integrated NTAP-Orc4 sequencing
YEH264	accagcaccTAGGGCGAATTG	Used for CRISPR NTAP-Orc4 template construction
YEH265	CTAggtgctggtATGACTATAAG	Used for CRISPR NTAP-Orc4 template construction

YEH266	TTTTTCAACGCTAACTATAATTT	Used for CRISPR NTAP-Orc4 template construction/integrated NTAP-Orc4 sequencing
YEH267	CTCGAGGCCACCAAGAAGAGAAAGAGAAGAGC	Used for CRISPR NTAP-Orc4 template construction
YEH268	AGGCCTGCCATGCTTCTTCTTTTGAATATTACCGATATT	Used for CRISPR NTAP-Orc4 template construction
YEH311	TTCCTGCAGCCCGGGGGATCGAAGAGAAAGAGAAGAGCCA	Used for CRISPR NTAP-Orc4 template construction
YEH312	GCTCCACCGCGGTGGCGGCCTTTTTCAACGCTAACTATAA	Used for CRISPR NTAP-Orc4 template construction

ssYEH001	AATGAGCAGGCAAGATAAACGAAGGCAAAGATGACAGAGCAGAAAGCCCGAAGAGAAAGAGAAGAGCCAGATATTGACTG	Used for CRIPSR NTAP-Orc4 integration yeast transformation (coupled with gRNA003)
ssYEH002	ACAAGTGGCAATGAAAAAAATTATAGTTAGCGTTGAAAAATgcgAAAGCGTATTACAAATGAAACCAAGATTCAGATTGC	Used for CRIPSR NTAP-Orc4 integration yeast transformation (coupled with gRNA003)
ssYEH003	TTATACATTATATAAAGTAATGTGATTTCTTCGAAGAATATACTAAAAAGAAGAGAAAGAGAAGAGCCAGATATTGACTG	Used for CRIPSR NTAP-Orc4 integration yeast transformation (coupled with gRNA005)
ssYEH004	ACAAGTGGCAATGAAAAAAATTATAGTTAGCGTTGAAAAAATatcCAGGCAAGATAAACGAAGGCAAAGATGACAGAGCA	Used for CRIPSR NTAP-Orc4 integration yeast transformation (coupled with gRNA005)

YEH298	TTAAGAGCTTGGTGAGCGCT	Used for CRISPR NTAP-Orc4 insertion checking
YEH299	ATGCTTGGCAGAGCATGTAT	Used for CRISPR NTAP-Orc4 insertion checking
YEH173	GTGCCCCGGAGGATGAGA	Used for integrated NTAP-Orc4 sequencing
YEH279	TCACCATCTTCAACAGTCAA	Used for integrated NTAP-Orc4 sequencing
YEH281	CGCGAATTGTGATATACCTA	Used for integrated NTAP-Orc4 sequencing
YEH282	ATATGAGTGATAATGGTTCC	Used for integrated NTAP-Orc4 sequencing
YEH294	GAAGAGAAAGAGAAGAGCCA	Used for integrated NTAP-Orc4 sequencing
ARS416 L1 AR	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTCAAATGATTTAGCATTATCTTTAC	Used for MPOS assay ARS416 sequencing library barcoding

ARS317 L1 AR	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCT TTTTATGGAAGATTAAGCTCA	Used for MPOS assay ARS317 sequencing library barcoding
ARS416 L1 AF01	ACTCTTTCCCTACACGACGCTCTTCCGATCT ACAT AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF02	ACTCTTTCCCTACACGACGCTCTTCCGATCT AGCC AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF03	ACTCTTTCCCTACACGACGCTCTTCCGATCT ATGT AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF04	ACTCTTTCCCTACACGACGCTCTTCCGATCT CATA AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF05	ACTCTTTCCCTACACGACGCTCTTCCGATCT CGAT AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding

ARS416 L1 AF06	ACTCTTTCCCTACACGACGCTCTTCCGATCT CTCT AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF07	ACTCTTTCCCTACACGACGCTCTTCCGATCT GAGA AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF08	ACTCTTTCCCTACACGACGCTCTTCCGATCT GCTA AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF09	ACTCTTTCCCTACACGACGCTCTTCCGATCT GTAT AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF10	ACTCTTTCCCTACACGACGCTCTTCCGATCT TACA AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF11	ACTCTTTCCCTACACGACGCTCTTCCGATCT TCGG AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding

ARS416 L1 AF12	ACTCTTTCCCTACACGACGCTCTTCCGATCT TGTA AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF13	ACTCTTTCCCTACACGACGCTCTTCCGATCT AAAA AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF14	ACTCTTTCCCTACACGACGCTCTTCCGATCT ACCA AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF15	ACTCTTTCCCTACACGACGCTCTTCCGATCT AGGA AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF16	ACTCTTTCCCTACACGACGCTCTTCCGATCT ATTA AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF17	ACTCTTTCCCTACACGACGCTCTTCCGATCT CCAG AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding

ARS416 L1 AF18	ACTCTTTCCCTACACGACGCTCTTCCGATCT CGCA AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF19	ACTCTTTCCCTACACGACGCTCTTCCGATCT CTGG AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF20	ACTCTTTCCCTACACGACGCTCTTCCGATCT GATT AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF21	ACTCTTTCCCTACACGACGCTCTTCCGATCT GGAA AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF22	ACTCTTTCCCTACACGACGCTCTTCCGATCT GTCA AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS416 L1 AF23	ACTCTTTCCCTACACGACGCTCTTCCGATCT TAGC AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding

ARS416 L1 AF24	ACTCTTTCCCTACACGACGCTCTTCCGATCT TCTC AGACAAATGGTGTAAAAGAC	Used for MPOS assay ARS416 sequencing library barcoding
ARS317 L1 AF01	ACTCTTTCCCTACACGACGCTCTTCCGATCT ACAT CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF02	ACTCTTTCCCTACACGACGCTCTTCCGATCT AGCC CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF03	ACTCTTTCCCTACACGACGCTCTTCCGATCT ATGT CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF04	ACTCTTTCCCTACACGACGCTCTTCCGATCT CATA CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF05	ACTCTTTCCCTACACGACGCTCTTCCGATCT CGAT CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding

ARS317 L1 AF06	ACTCTTTCCCTACACGACGCTCTTCCGATCT CTCT CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF07	ACTCTTTCCCTACACGACGCTCTTCCGATCT GAGA CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF08	ACTCTTTCCCTACACGACGCTCTTCCGATCT GCTA CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF09	ACTCTTTCCCTACACGACGCTCTTCCGATCT GTAT CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF10	ACTCTTTCCCTACACGACGCTCTTCCGATCT TACA CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF11	ACTCTTTCCCTACACGACGCTCTTCCGATCT TCGG CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding

ARS317 L1 AF12	ACTCTTTCCCTACACGACGCTCTTCCGATCT TGTA CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF13	ACTCTTTCCCTACACGACGCTCTTCCGATCT AAAA CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF14	ACTCTTTCCCTACACGACGCTCTTCCGATCT ACCA CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF15	ACTCTTTCCCTACACGACGCTCTTCCGATCT AGGA CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF16	ACTCTTTCCCTACACGACGCTCTTCCGATCT ATTA CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF17	ACTCTTTCCCTACACGACGCTCTTCCGATCT CCAG CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding

ARS317 L1 AF18	ACTCTTTCCCTACACGACGCTCTTCCGATCT CGCA CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF19	ACTCTTTCCCTACACGACGCTCTTCCGATCT CTGG CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF20	ACTCTTTCCCTACACGACGCTCTTCCGATCT GATT CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF21	ACTCTTTCCCTACACGACGCTCTTCCGATCT GGAA CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF22	ACTCTTTCCCTACACGACGCTCTTCCGATCT GTCA CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
ARS317 L1 AF23	ACTCTTTCCCTACACGACGCTCTTCCGATCT TAGC CAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding

ARS317 L1 AF24	ACTCTTTCCCTACACGACGCTCTTCCGATCT TCTCCAGTGTTTTCAATTTTTTATTAAAC	Used for MPOS assay ARS317 sequencing library barcoding
YEH229	GCCGCAAAAAAGGGAATAAG	Used for MPOS assay sequencing library contrusction
YEH230	CGTGTTCAAACGATACCTGG	Used for MPOS assay sequencing library contrusction
YEH231	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACG	Used for MPOS assay sequencing library contrusction
YEH232	AAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCT	Used for MPOS assay sequencing library contrusction

Supplementary Methods Table 2 | Primers and oligos sequences used in this study