Supporting Information

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SI Materials and Methods

Yeast Strains. Strains used in this study were derived from CK213 and SAY538 (Table S1). The *sir2*Δ*::NatMX*, *sir4*Δ*::URA3, sum1*Δ*::NatMX, SIR2*–HA, *SIR4*–Flag, and myc–*SUM1* alleles were previously described (1). The *ORC1*–V5, *ORC4*–V5, and *ORC5*–Flag alleles were constructed by integrating the epitope tag plus a selectable marker at the end of the ORF. Tagging cassettes were generated from p3FLAG–KanMX (2) or pFA6a–6xGLY–V5–hphMX4 (3). Antibiotic supplements were used at 200 μg/mL for geneticin and 300 μg/mL for hygromycin B. The *orc1–*Δ*bah, orc1–E124K*, and *orc1–P185L* mutations were obtained by first cloning the complete *ORC1*–V5 ORF plus flanking DNA into a plasmid, followed by PCR-mediated mutagenesis to delete the first 217 amino acids or to mutate the indicated amino acid. Mutations were confirmed by sequencing, and the entire *orc1–*Δ*bah–V5, orc1–E124K–V5*, and *orc1–P185L–V5* cassettes were integrated at the endogenous *ORC1* locus.

Alleles were moved into various genetic backgrounds through genetic crosses. Mating was carried out by mixing the two parental strains on malt extract (ME) medium (2% malt extract, 2% agar) and incubating at 30 °C for 2–3 d. Cells were then streaked on medium to select for diploids and subsequently transferred to ME plates for sporulation. After 3–4 d, the sporulated culture was suspended in 500 μL water, incubated at 56 °C for 15 min, and plated on medium to select for meiotic progeny of interest. Genotypes were confirmed by PCR.

Growth Rates. Yeast strains were grown in YPD overnight and diluted to an optical density (OD_{600}) of ≈0.25 in YPD. Growth was monitored by measuring the absorbance at 600 nm. Data from individual growth curves were used to calculate the doubling time using the Website (http://www.doubling-time.com/compute.php).

Flow Cytometery. Flow cytometery was conducted as previously described (4). Briefly, yeast were grown in YPD to an OD_{600} of ≈0.6 and fixed in 70% ethanol for 3 h. To digest RNA, cells were incubated in 2 mg/mL RNase A (Sigma, R-5503) at 4 °C for 36 h. To digest protein, cells were incubated in 5 mg/mL pepsin (Sigma, P-7000) at 37 °C for 15 min. Cells were then washed and resuspended in 1 mM Sytox Green (Molecular Probes, S-7020), sonicated 5 s at setting 1 on a Branson Sonifier 450, and analyzed by flow cytometry.

Budding Index. Yeast were grown to an OD_{600} of ≈0.8 and fixed in 1% formaldehyde for 10 min. Cells were collected, resuspended in PBS at an optical density of 0.5, and sonicated twice for 5 s at setting 2 on a Branson Sonifier 450. Samples were placed on a hemocytometer and viewed on a phase contrast microscope to count the numbers of unbudded and budded cells.

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4. Haase SB (2004) Cell cycle analysis of budding yeast using SYTOX Green. *Curr Protoc Cytom* Chapter 7:Unit 7.23.
5. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599.
6. Hou Z, Bernstein DA, Fox CA, Keck JL (2005) Structural basis of the Sir1-origin recognition complex interaction in transcriptional silencing. *Proc Natl Acad Sci USA* 102:8489–8494.
7. Zhang Z, Hayashi MK, Merkel O, Stillman B, Xu R-M (2002) Structure and function of the BAH-containing domain of Orc1p in epigenetic silencing. *EMBO J* 21:4600–4611.
8. Buchberger JR, et al. (2008) Sir3-nucleosome interactions in spreading of silent chromatin in *Saccharomyces cerevisiae*. *Mol Cell Biol* 28:6903–6918.
9. Sampath V, et al. (2009) Mutational analysis of the Sir3 BAH domain reveals multiple points of interaction with nucleosomes. *Mol Cell Biol* 29:2532–2545.

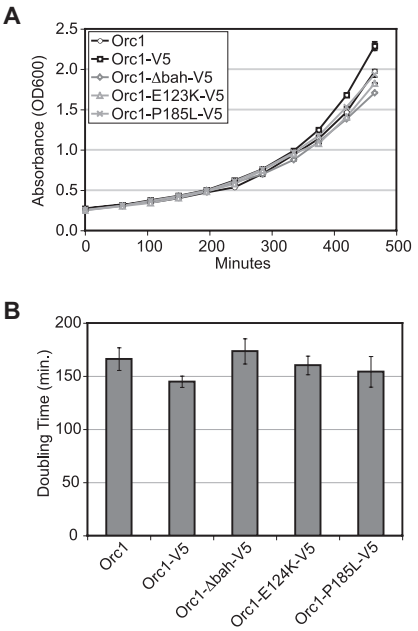
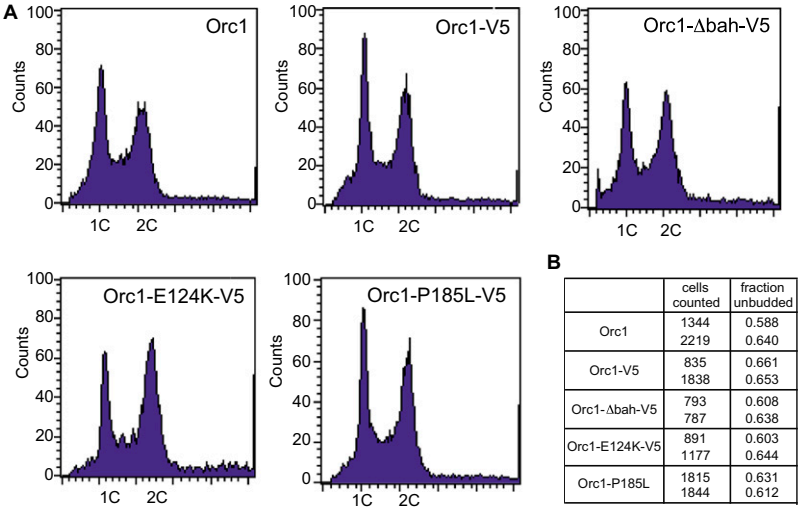


Fig. S1. Strains expressing mutant KlOrc1 had wild-type growth rates. (*A*) Strains expressing KlOrc1 (CK213), KlOrc1–V5 (LRY2561), KlOrc1–Δbah–V5 (LRY2562), KlOrc1–E124K–V5 (LRY2656), and KlOrc1–P185L–V5 (LRY2657) were grown in YPD overnight, diluted to an optical density of ≈0.25 in YPD, and monitored for 8 h. (*B*) The doubling time of each strain was calculated using data generated in experiments such as the one shown in *A*. Doubling times represent the average of at least two independent experiments.



B

	cells counted	fraction unbudded
Orc1	1344 2219	0.588 0.640
Orc1-V5	835 1838	0.661 0.653
Orc1-Δbah-V5	793 787	0.608 0.638
Orc1-E124K-V5	891 1177	0.603 0.644
Orc1-P185L	1815 1844	0.631 0.612

Fig. S2. Strains expressing mutant KlOrc1 proceeded through the cell cycle normally. (*A*) The same strains used in Fig. S1 were grown in YPD to an optical density (OD_{600}) of ≈0.6, fixed in ethanol, stained with Sytox Green, and analyzed by flow cytometry. (*B*) Two independent cultures of each strain were grown to an OD_{600} of ≈0.8, fixed in formaldehyde, and the numbers of budded and unbudded cells were counted using a phase contrast microscope.

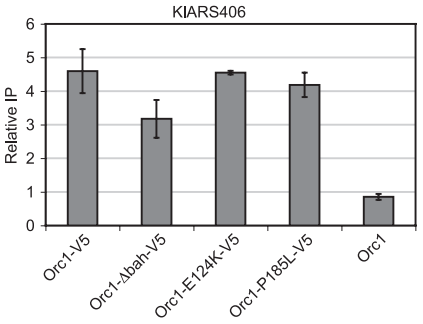


Fig. S3. Variants of KlOrc1 associated with the replication origin KlARS406. The associations of KlOrc1–V5 (LRY2561), KlOrc1–Δbah–V5 (LRY2562), KlOrc1–E124K–V5 (LRY2656), and KlOrc1–P185L–V5 (LRY2657) with *KlARS406* were analyzed as for Fig. 1. As a negative control, a mock precipitation using the V5 antibody was conducted from a strain expressing untagged KlOrc1 (CK213).

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ScOrc1    MAKTLKDLQG-WEIITTDEQGNIIDGGQKRLRRRGAKTEHYLKRS-SDGI  48
KlOrc1    MASTLAEFEVQWEIQKTDLKGNLIAETPRRRRRGDATEHEVINLVRYDGV  50
ScSir3    MAKTLKDLDG-WQVIITDDQGRVIDDNNRRRSRKRGGENVFLKRI-SDGL  48

ScOrc1    KLGRGDSVVMHNEAAGTYSVYMIQELRLNTLNNVVELWALTYLRWFHVNP  98
KlOrc1    RLYPGVTIVCKVEGADELSAYMIHEVRLNTSN-YVELWCLNYLSWYHINA  99
ScSir3    SFGKGESVIFNDNVTETYSVYLIHEIRLNTLNNVVEIWVFSYLRWFHLKP  98

ScOrc1    LAHYRQFNPDANILNRP--LNYYNKLFSETANKNELYLTAELAELQLFNF 146
KlOrc1    AERYKQLDGEFYETNKEKGDKFFETFASQSIKNELYLTAELSEIYLRDL 149
ScSir3    KLYYEQFRPDLIKEDHP--LEFYKDKFFNEVNKSELYLTAELSEIWLKDF 146

ScOrc1    IRVANVMDGSKWEVLKGN-VDPERDFTVRYICEPTGEKFVDINIEDVKAY 195
KlOrc1    QFVANIKNEKEYLDSVNEGKMDSNMFLCRSACLPSGTNLADLDIHFFEEK 199
ScSir3    IAVGQILPESQWNDSSID-KIEDRDFLVRYACEPTAEKFVPIDIFQIIRR 195

ScOrc1    IKKVEPREAQEYLKDLTLPSKKKEIKRGPQKKDKATQTAQISDAE      240
KlOrc1    IRSSNPKVSLEYLRDITLPKLPKPLNKSKVHAREKVVATKL          240
ScSir3    VKEMEPKQSDEYLKRVSVPVSGQKTNRQVMHKMGVERSSKRLAKK      240
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Fig. S4. Alignment of the BAH domains from ScOrc1, ScSir3, and KlOrc1. The first 240 amino acids from ScOrc1, ScSir3, and KlOrc1 were aligned using MEGA 4 software (5). Conserved residues are indicated with a vertical bar, and similar residues are indicated with a colon. Residues in ScOrc1, which are important for interacting with Sir1 (*) (6, 7) and residues in ScSir3, which are important for interactions with nucleosomes (^) (8, 9) are indicated. Boxed and bolded residues indicate the H domain. KlOrc1–E124 and KlOrc1–P185 are highlighted in red.

Table S1. List of *K. lactis* strains used in this study

Strain	Genotype	Source
CK213	*MAT***a** *lysA1 leu2 trp1 uraA1*	Chen and Clark-Walker (1)
SAY538	*MATα nej1Δ::LEU2 ade1 leu2 trp1 uraA1*	Astrom (2)
LRY2235	*MAT***a** *ORC5-Flag::KanMX nej1Δ::LEU2 leu2 trp1 uraA1*	
LRY2239	*MATa SIR2-HA::NatMX SIR4-Flag::KanMX myc-SUM1 nej1::LEU2 leu2 trp1 uraA1*	
LRY2285	*MATα SIR2-HA::NatMX SIR4-Flag::KanMX myc-SUM1 nej1Δ::LEU2 leu2 trp1 uraA1*	
LRY2561	*MAT***a** *ORC1-V5::HphMX lysA1 leu2 trp1 uraA1*	
LRY2562	*MATa orc1-Δbah-V5::HphMX lysA1 leu2 trp1 uraA1*	
LRY2563	*MAT***a** *orc1-Δbah::HphMX SIR2-HA::NatMX SIR4-Flag::KanMX myc-SUM1 nej1Δ::LEU2 leu2 trp1 uraA1*	
LRY2566	*MAT***a** *orc1-V5::HphMX SIR2-HA::NatMX SIR4-Flag::KanMX myc-SUM1 nej1Δ::LEU2 leu2 trp1 uraA1*	
LRY2572	*MAT***a** *ORC1-V5::HphMX sir2Δ::NatMX SIR4-Flag::KanMX myc-SUM1 nej1Δ::LEU2 leu2 trp1 uraA1*	
LRY2573	*MAT***a** *ORC1-V5::HphMX SIR2-HA::NatMX sir4Δ::URA3 myc-SUM1 nej1Δ::LEU2 leu2 trp1 uraA1*	
LRY2574	*MAT***a** *ORC1-V5::HphMX SIR2-HA::NatMX SIR4-Flag::KanMX sum1Δ::NatMX nej1Δ::LEU2 leu2 trp1 uraA1 lysA1*	
LRY2576	*MAT***a** *ORC1-V5::HphMX sir4Δ::URA3 sum1Δ::NatMX nej1Δ::LEU2 leu2 trp1 uraA1*	
LRY2577	*MAT***a** *ORC1-V5::HphMX sir2Δ::NatMX sir4Δ::URA3 myc-SUM1 nej1Δ::LEU2 leu2 trp1 uraA1*	
LRY2578	*MAT***a** *ORC1-V5::HphMX sir2Δ::NatMX SIR4-Flag::KanMX sum1Δ::NatMX nej1Δ::LEU2 leu2 trp1 uraA1*	
LRY2581	*MATα ORC1-V5::HphMX nej1Δ::LEU2 ade1 leu2 trp1 uraA1*	
LRY2656	*MAT***a** *orc1-E124K-V5::HphMX lysA1 leu2 trp1 uraA1*	
LRY2657	*MAT***a** *orc1-P185L-V5::HphMX lysA1 leu2 trp1 uraA1*	
LRY2709	*MATα orc1-Δbah-V5::HphMX nej1Δ::LEU2 ade1 leu2 trp1 uraA1*	
LRY2711	*MAT***a** *ORC4-V5::HphMX lysA1 leu2 trp1 uraA1*	

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2. Barsoum E, Sjöstrand JOO, Aström SU (2010) Ume6 is required for the MATa/MATα cellular identity and transcriptional silencing in *Kluyveromyces lactis*. *Genetics* 184:999–1011.

Table S2. List of oligonucleotides used for this study

RRP7	GCAACAACAGATACTGTGGG
	CCTACTACTAATGTGAAACCATC
ACT1	CGTCGCTTTGGACTTCGAACAA
	GTGGTACCACCGGACATGAC
KARS12	
1	CAAACACTCGGCTTGGCTAG
	GAAAGACTGTAGATTAGTAGACC
2	CCA GCA ATG CGA ACA TAA CAC
	GGA TTT CAC CAT GGT TCT TGA AG
3	CTATTCTCTGCCAAGCTATCC
	GGTTCTGTCTACAGATTTCCAC
4	GGGCCTCTATTCAAATTACCTATG
	GTTGGTGTTCAAGTAACGACTAC
5	GGAAGAGCTTCAGGGTC
	GCGATCGAAATACGTAGATTGC
6	CACAGCATTAAGCACCACTTC
	GATCGACCTTCGATCCGTC
7	GAGCATGCGGTTCTCTTCC
	GTTGCGATTTGGGCAGCG
HML	
1	GTTCATCGTTGTCATAACTTTCTCG
	GATGATGGGGTGCCCGAAAG
2	CGTATTATACATGACAGCCAAAGG
	GTTGGGTGAATAAACCATCTCAC
3	CCTGTACAATTGCTATGCCTACAG
	CATGCCGCTTCTGTCTTTAGC
4	CCACTTTAAAGGTTGGAGCATTC
	GGGTTTAGCAATGAGTGTATGGG
5	GGGTCCGTTTCTTGGGATTAATG
	CCGCTGCCGTTCTAAGTCTG
6	GATACTCTGAGACTTTCTCTTGG
	CAGTGACTGGTCATTAGCCGAG
7	GTACTGTCTAAGGGAAGCTGG
	GGACAGGATACCGTGTAAATACC
8	GCTGTTGAATATTGGATTGGGCTG
	GTACTTGCTTTGTTCCATTAACGTG
9	GGCTGAAGTTCGATGAACTAGG
	GGTGCTCGGCATACAAACTTC
10	CAAGCCCCTACTACCAAACATG
	GTCACTCTGACTTCGTGGCC
11	GAAGGGATTTAGTGGTATCTCAAC
	CCAACATAAGGTATCTCTTCTTATG
12	GCATATATTTCAGTACGGTGAGC
	CTTTCAAAAAGAATAGTGCTTACTGG
HMR	
1	CTACTACCACTGCCTCTGAAAAC
	CTGGAATCGTTGTTAGAGCCTG
2	CTGGTGCGTCATCAACTAGC
	GGGATAAATACGGATAGGGTATC
3	CCAGTAAAGAACACACACCGC
	GAGCGAAGACCCTTTTCTGAG
4	GCGACAACAAGTGGAAGAGTTG
	CTCTAGACATGCATGTCCGAC
5	CAGGAGATGCAGACCATAGC
	CGAGCTAAAATAGCTCGGGTC
6	CGCCTTCTTCACAAAACTAC
	GTCGAATGACATGGGAGAC
7	CCACATAGATACGTCCCTGTTTTAG
	GACTCCGGTCAATGCTGAGG
8	CCATCAAATGGTGTGAATTGAATCTC
	CCTGAATATTAACGATTGCTCACC

Table S2. Cont.

9	GACCAACCATGTCTTCCTTTCC
	GGTTGGTCGATGGATTTTCGTG
10	GGAACGAATGGTCACCGGAC
	GATGAACCTGCACCTACAG
11	CAAGGCAGTATCTCTCCGAAC
	GGCATTCTACCGACCTCCG
12	CCCGTATCTGCCATACCAAAC
	GTCTTCGTGGCTGCTAATAGGC
13	GGGCGTATCGCATCACGTAG
	GGTCGCTCTTACGTGAGCTG
14	GGCCAGGTTGAAATACGCAAG
	CAGCAACAAGAACTGTAGACATC
TEL–BR	
1	CCCGCTATATTTGGTCCATCATC
	AACAAAGAGAATGCAGGGAGAGT
2	CATCCCCAGCATAAATTCATCA
	ACAGGAAAGAAAGGAGTAGAGGTG
3	TTGCAACGATTCGAACATGCTGT
	ATCACGTGACTGGAAGTCGAGT
4	CTTCTGGGGTATTAATGCTGCTG
	AGCTCTAGTGTTGTTGTTGGCTC
5	GTGAACGAATCCGATGTCTGTG
	TGGAGAGTTCTATTACTTCCGCC
6	GAGTAAACACCGTTGTGGTAGGA
	CAAACACCAGAAATTGAAACTGCC
7	ATATACGGTACCGGTCCAAGGA
	TCGAGACCCCAGAGTTTAAGAC
Tel–EL	GTCCTTGCATAGGATACACACGTT
	AGGTGAAGAGAAGCAGGTGATG
Tel–ER	CCAAGCATTGGCCATGGCAAG
	CTCGAAACGGATTTCCTCTTTCG
Tel–FR	GGTGAAAAACAGTCAAGAATACGC
	CTCTAGACGGATTTCCTCTTCTG