# Establishment and Function of Chromatin Architecture at Eukaryotic Chromosome Replication Origins

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**The Origin Recognition Complex (ORC) is essential for the initiation of eukaryotic chromosome replication as it loads the replicative helicase, the minichromosome maintenance (MCM) complex, at replication origins[1].**

**These origins display a stereotypic chromatin structure. They are nucleosome depleted at ORC binding sites and flanked by regularly spaced nucleosome arrays[2,3]. Although discovered over a decade ago, we still do not know how this chromatin struture is established and if it matters for replication.**

**Here we show that ORC has an essential role in generating the origin chromatin structure. By genome-wide biochemical reconstitution we screened ~ 400 individual origins and 17 purified chromatin factors from budding yeast and found that ORC establishes both nucleosome depletion over origins as well as flanking nucleosome arrays by orchestrating the four chromatin remodelers INO80, ISW1a, ISW2 and Chd1. The importance of ORC's chromatin organizing activity was underscored by ORC mutations that maintained classical MCM loader but lost array generation activity. These mutations were lethal *in vivo*.**

**Our results demonstrate that ORC, besides its canonical role as MCM loader, has a second essential function as master regulator of chromatin structure at replication origins, which is critical for chromosome replication.**

## Main:

In budding yeast, origins contain specific DNA domains, the autonomous replicating sequences (ARS), which bear the ARS-consensus sequence (ACS), a conserved 11 base pair motif essential for ORC binding[4]. Budding yeast uses ~ 400 confirmed core origins equally distributed across its 16 chromosomes while replicating its ~12 mega base pair-sized genome[2,5–7]. In multi-cellular eukaryotes, due to a lack of conserved ORC binding sites, it has proven difficult to map origins of replication, but it is estimated that timely replication of human genomes involves ~ 30.000 origins[8]. Eukaryotic genomes are packaged into nucleosomes, the basic units of chromatin[9]. Interestingly, yeast origins are symmetrically flanked by arrays of regularly-spaced nucleosomes, while the ACS element lies in an A-T-rich nucleosome-free region (NFR)[2,3] (Fig. 1a).

It was suggested that ORC has a role in the establishment of these arrays as mutants defective in DNA binding showed disruption of this chromatin structure at origins[2,3]. However, the molecular basis by which ORC achieves this remains unknown.

To tackle these questions and to obtain a detailed mechanistic understanding of chromatin structure establishment at origins, we chose an *in vitro* reconstitution approach. While reconstitutions using single origins were undertaken before[2,10–12], although not for our specific question, we wished to go beyond a single origin model and leverage the advantage of comparing many origins in parallel. This way, confounding effects due to individual variation average out and consistent trends among many origins become apparent. This global approach has been highly successful, both for *in vivo* mapping as well as *in vitro* reconstitution approaches, for example, regarding yeast promoter architectures[13–16]. However, it was not applied to the reconstitution of replication origins so far. Here we set out to biochemically reconstitute origin chromatin with purified components on a global scale.

**ORC works in conjunction with chromatin remodelers**

We first asked if the origin flanking DNA sequences have sufficient intrinsic information to position nucleosomes, especially arrays.

To uncover general principles beyond individual origins, we generated a plasmid library containing most budding yeast origins as the DNA templates (~ 400 individual origins) and used purified histones and salt gradient dialysis for chromatin assembly (SGD-chromatin) onto those plasmids. Nucleosome positions were determined by limited digestion with micrococcal nuclease (MNase) coupled to high-throughput sequencing (MNase-seq)[14] (Fig. 1b, c). A composite plot of SGD chromatin at all origins aligned to their ACS element (Fig. 1d) did not show intrinsic formation of phased arrays as *in vivo* (Fig. 1a), but an NFR-like region over the ACSs. The latter was expected as origins are A-T-rich sequences, which are known to exclude nucleosomes[2,5,17].

We next tested if ORC can set up phased arrays, as previously suggested[2,3,10]. Purified ORC (Fig. 1e) on its own added to SGD chromatin could not form phased arrays at origins (Fig. 1f), likely because nucleosomes could not be mobilized under these physiological temperature and ionic strength conditions. Therefore, we hypothesized that other chromatin factors, like histone chaperones and ATP-dependent chromatin remodelers, that help to transfer/mobilize histone octamers, had to be involved. We performed an unbiased biochemical screen. We purified most histone chaperones and chromatin remodelers from yeast (9 histone chaperones (Asf1, CAF-1, HIR, FACT, Nap1, Nhp6, Spt6, Rtt106 and Vps75; Extended Data Fig. 1a) as well as 8 chromatin remodelers (INO80, ISW1a, ISW2, Chd1, Fun30, RSC, SWI/SNF and SWR1; Extended Data Fig. 1b) as well as and tested them in our assay. As with ORC, none of the 17 tested chromatin factors could form regular arrays at origins on their own (Extended Data Figs. 2, 3).

Thus, we next asked if ORC might function together with one of these chromatin factors and assayed all 17 together with ORC. Remarkably, of all the tested chromatin factors, only the chromatin remodelers INO80, ISW1a, ISW2 and Chd1 could generate *in vivo*-like nucleosome arrays in the presence of ORC (Fig. 1g, h and Extended Data Figs. 4, 5), similar to those at gene promoters, where sequence-specific "barriers" operate together with chromatin remodelers in array phasing[14,15].

Together, from our *in vitro* screen we learned that ORC in conjunction with positioning remodelers INO80, ISW1a, ISW2 or Chd1 has an essential role in arranging the chromatin landscape at origins. Our data further suggest that these four chromatin remodelers might operate redundantly *in vivo*.

**Chromatin remodellers operate redundantly**

To study this, we made use of a quadruple mutant[18] where the gene encoding the non-catalytic Arp8 subunit of INO80 was deleted on top of a *isw1Δ isw2Δ chd1Δ* triple remodeler mutant[19] (*arp8Δ isw1Δ isw2Δ chd1Δ* (QKO), Extended Data Fig. 6a). Eliminating the catalytic subunit Ino80 would not be viable in this triple mutant background. We also tested the respective single deletion mutants and determined chromatin profiles at origins *in vivo*. In support of our hypothesis, the single remodeller deletion mutants had little effect (Extended Data Fig. 7), whereas the QKO mutant showed a significant reduction of nucleosomal arrays at origins compared to wild type (Fig. 2a).

We next asked if the chromatin defects in the QKO mutant might influence DNA synthesis. We followed bulk S phase progression after pheromone arrest/release by flow cytometry. We observed a severe replication phenotype of the QKO mutant compared to wild type where most

cells arrested the cell cycle with G1 DNA content and did not even enter S phase (Fig. 2b). These *in vivo* experiments suggest that accurate nucleosome positioning around origins might be critical for chromosome replication. However, given that a QKO mutant shows significant global defects in chromatin composition[18], it is impossible, at this point, to exclude indirect effects on DNA synthesis by e. g. transcriptional dysregulation.

**Orc1 bears ORC´s chromatin function**

We learned from our *in vitro* experiments that INO80, ISW1a, ISW2 and Chd1 cannot operate without ORC at origins. For INO80, this is in contrast to promoter regions, where INO80 is able to properly position +1 nucleosomes and generate rudimentary arrays on its own[14,15]. We reasoned that, instead of eliminating chromatin remodelers, finding regions in ORC responsible for its function in chromatin organization would locally disrupt chromatin architecture at origins and possible defects in replication would be a direct consequence.

The Orc1 subunit is known to associate with chromatin. It bears a bromo-adjacent homology domain (BAH) that can interact with nucleosomes[20,21] (Fig. 2c) and an intrinsically disordered region (IDR) of unknown function[22]. IDRs are inherently flexible and we speculated that this feature might be important to interact with INO80, ISW1a, ISW2 or Chd1. Finally, Orc1 has a AAA$^+$-ATPase domain (Fig. 2c), where ATP binding is essential for DNA association and function as the helicase loader[23]. The role of ATP hydrolysis, however, is less clear. We therefore hypothesized that ATP hydrolysis by Orc1 might be important for ORC´s function to organize chromatin at origins.

We generated mutants lacking the BAH (*orc1*-BAH[24]) or the IDR domains (*orc1*-IDR) as well as a Walker B ATP-hydrolysis point mutant in the AAA$^+$-ATPase domain (*orc1*-Walker B[25]).

Whereas *orc1*-BAH did not show a growth phenotype under optimal conditions, elimination of the IDR showed a growth defect (Extended Data Fig. 6b). Upon induction of replication stress using hydroxyurea (HU), both mutations resulted in slow growth, where *orc1*-IDR was more affected than *orc1*-BAH (Extended Data Fig. 6c). Elimination of both (*orc1*-BAH-IDR) resulted in lethality (Fig. 2d). In contrast, an *orc1*-Walker B mutation on its own is lethal consistent with previous results[25] (Fig. 2d). We separately studied the viable and lethal mutations in detail. We first asked if the viable BAH- or IDR-mutations influenced origin-adjacent nucleosome positioning as well as DNA synthesis. Consistent with the growth assay, *orc1*-BAH did not show defects in nucleosome positioning at origins (Fig. 2e) and had very minor replication defects (Fig. 2f). In contrast, *orc1*-IDR mutants showed a mild but robust reduction of nucleosome occupancy (Fig. 2e), which was accompanied with a replication phenotype, with a delay in S phase entry combined with a significant fraction of cells arrested with G1 DNA content (Fig. 2f). This was similar to the QKO experiment (Fig. 2b), although less pronounced. Together, our *in vivo* analyses show that the IDR domain of Orc1 is important for nucleosome positioning at origins and for chromosome replication, whereas the BAH domain seems less involved. However, both domains together are required for viability, as was the ability of Orc1 to hydrolyze ATP.

**ORC´s chromatin function is essential**

We next aimed to determine the molecular basis of the lethal phenotypes of the *orc1*-BAH-IDR and the *orc1*-Walker B mutants. We hypothesized that they are caused by the disruption of chromatin structure at origins. Alternatively, Orc1 mutant proteins might influence ORC complex formation, ORC-DNA binding or loading of the motor of the replicative helicase, the MCM complex. To test these possibilities, we went back to *in vitro* systems and firstly expressed and

purified ORC complexes. Neither mutation impaired ORC complex formation (Fig. 3a). Thus, we next investigated ORC-DNA binding and MCM loading[12,26,27] (Fig. 3b). ORC-DNA binding and MCM loading were similar for ORC wild type (WT) and mutants (Fig. 3c), with the exception of the Orc1-Walker B mutant, which showed reduced MCM loading (lane 4). This might be due to defects in reiterate MCM loading, as previously suggested[28]. We next tested the effects of these mutant ORC complexes in combination with INO80 on the reconstitution of regular arrays at origins. Consistent with our *in vivo* results, Orc1-BAH mutant complexes had little effect, whereas Orc1-IDR mutants displayed a significant reduction in array generation (Fig. 3d). The observed defects were more pronounced compared to the *in vivo* scenario (Fig. 2e), likely because of a partial functional redundancy with other factors. Interestingly and in line with our hypothesis, nucleosome architecture was completely disrupted using an Orc1-Walker B mutant complex (Fig. 3d), comparable to reconstitutions without ORC or without remodelers (Fig. 1f and Extended Data Figs. 2, 4). Similar results as for reconstitutions with INO80 were obtained with ISW1a, ISW2 and Chd1 (Extended Data Fig. 8), although to a lesser extent, suggesting that INO80 might be the major chromatin remodeler at origins.

We next analyzed the second lethal scenario, the *orc1*-BAH-IDR mutation (Fig. 2d). Complex formation (Fig. 3e), DNA binding and MCM loading (Fig. 3f) were not affected. Consistent with our hypothesis, array reconstruction with the Orc1-BAH-IDR mutant complex and INO80 was equally disrupted as with the Orc1-Walker B mutation (Fig. 3g). Again, other remodelers showed similar results (Extended Data Fig. 9). Our *in vitro* results confirmed our hypothesis that cell death of both *orc1*-BAH-IDR and *orc1*-Walker B mutants is caused by disrupted chromatin structure at origins of replication rather than problems in MCM loading.

## Discussion

In summary, we propose that ORC has a second essential function beside its canonical role as the MCM loader. ORC is a master regulator of origin-adjacent chromatin architecture and this is an essential function for chromosome replication (Fig. 3h). ORC cannot do this alone – it must collaborate with chromatin remodelers, like INO80, ISW1a, ISW2 or Chd1.

The Orc1 subunit is critical for ORC´s chromatin function. The BAH and IDR domains operate redundantly, whereas ATP hydrolysis by the AAA$^+$-ATPase domain is essential. All these three domains are highly conserved, therefore it is very likely that ORC´s chromatin function broadly applies across species[21,29–32]. We speculate that the BAH domain binds origin-flanking nucleosomes, which might be important for the accurate positioning of ORC at the ACS, consistent with previous results[21]. We further speculate that the flexibility of the IDR might be important for facilitating ORC/remodeler interactions.

An involvement of ATP hydrolysis by the Orc1 AAA$^+$-ATPase domain is surprising, given that general regulatory factors at gene promoters act as "phasers" without enzymatic activity[14,15]. It is possible that ORC uses ATP hydrolysis to actively manipulate chromatin structure. Supporting this idea, recent work showed that ORC can evict H2A-H2B dimers[33].

Orc1 was identified in a global screen for nucleosome depletion factors, together with general regulatory factors, like Reb1 or Abf1, that are known to participate in nucleosome organization[34]. It was proposed that such factors deplete nucleosomes due to special properties of their DNA binding domain and thereby effective binding competition with the histone octamer, similar to pioneer factors[35]. Our results add a new aspect to this discussion. The Orc1-BAH-IDR mutant complex was not impaired in DNA binding (Fig. 3f). It could still increase nucleosome depletion over the ACS compared to SGD chromatin, although less so than the WT ORC complex

(Fig. 3g vs. Fig. 1h), but could not direct the generation of nucleosomal arrays anymore. This strongly argues that the nucleosome organizing activity may mainly rely on the DNA binding domain with regard to nucleosome depletion, but requires additional properties with regard to array generation. These likely include interactions with remodelers and, in the case of ORC, an ATPase activity.

Our result that chromatin architecture at origins is essential for cell viability may seem unexpected at first sight, given that chromatin per se is a major barrier to replication[11,12,36]. However, even though nucleosomes are repressive for transcription[37], proper nucleosome positioning at gene starts and in gene bodies is instrumental for transcription start site selection and suppression of cryptic transcription[38–41]. Analogously, we propose that well-positioned nucleosomes flanking replication origins might cause activated helicases to pause, to allow for complete and accurate assembly of the replication machinery in order to start bidirectional replication in a synchronous manner.

**Fig. 1**

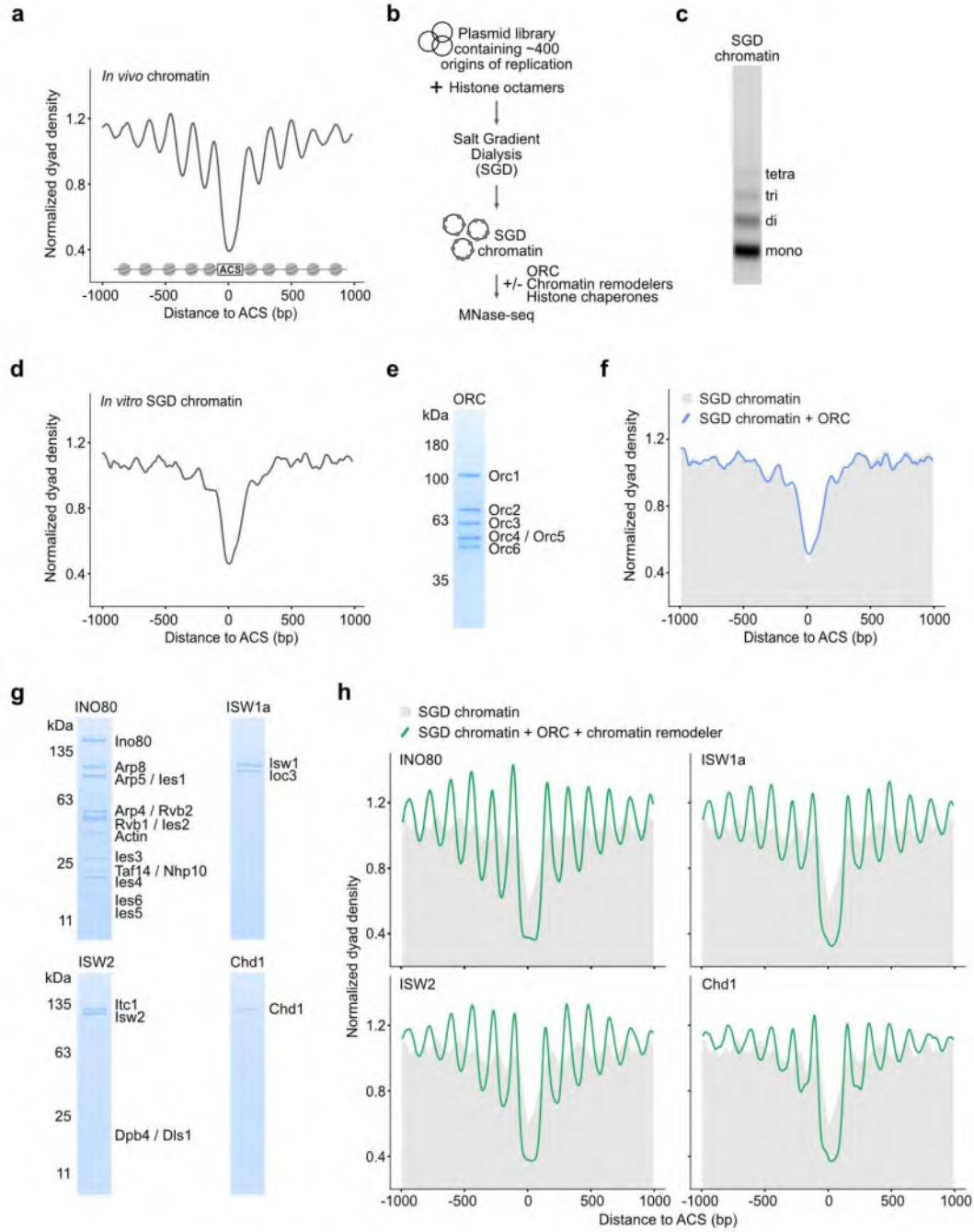

a
In vivo chromatin
Normalized dyad density
1.2
0.8
0.4
ACS
-1000
-500
0
500
1000
Distance to ACS (bp)
b
Plasmid library
containing ~400
origins of replication
+ Histone octamers
Salt Gradient
Dialysis
(SGD)
SGD
chromatin
ORC
+/- Chromatin remodelers
Histone chaperones
MNase-seq
c
SGD
chromatin
tetra
tri
di
mono
d
In vitro SGD chromatin
Normalized dyad density
Distance to ACS (bp)
e
ORC
kDa
180
100
63
35
Orc1
Orc2
Orc3
Orc4 / Orc5
Orc6
f
SGD chromatin
SGD chromatin + ORC
Normalized dyad density
Distance to ACS (bp)
g
INO80
kDa
135
63
25
11
Ino80
Arp8
Arp5 / Ies1
Arp4 / Rvb2
Rvb1 / Ies2
Actin
Ies3
Taf14 / Nhp10
Ies4
Ies6
Ies5
ISW1a
Isw1
Ioc3
ISW2
Itc1
Isw2
Dpb4 / Dls1
Chd1
Chd1
h
SGD chromatin
SGD chromatin + ORC + chromatin remodeler
INO80
ISW1a
ISW2
Chd1
Normalized dyad density
Distance to ACS (bp)

**Fig. 2**

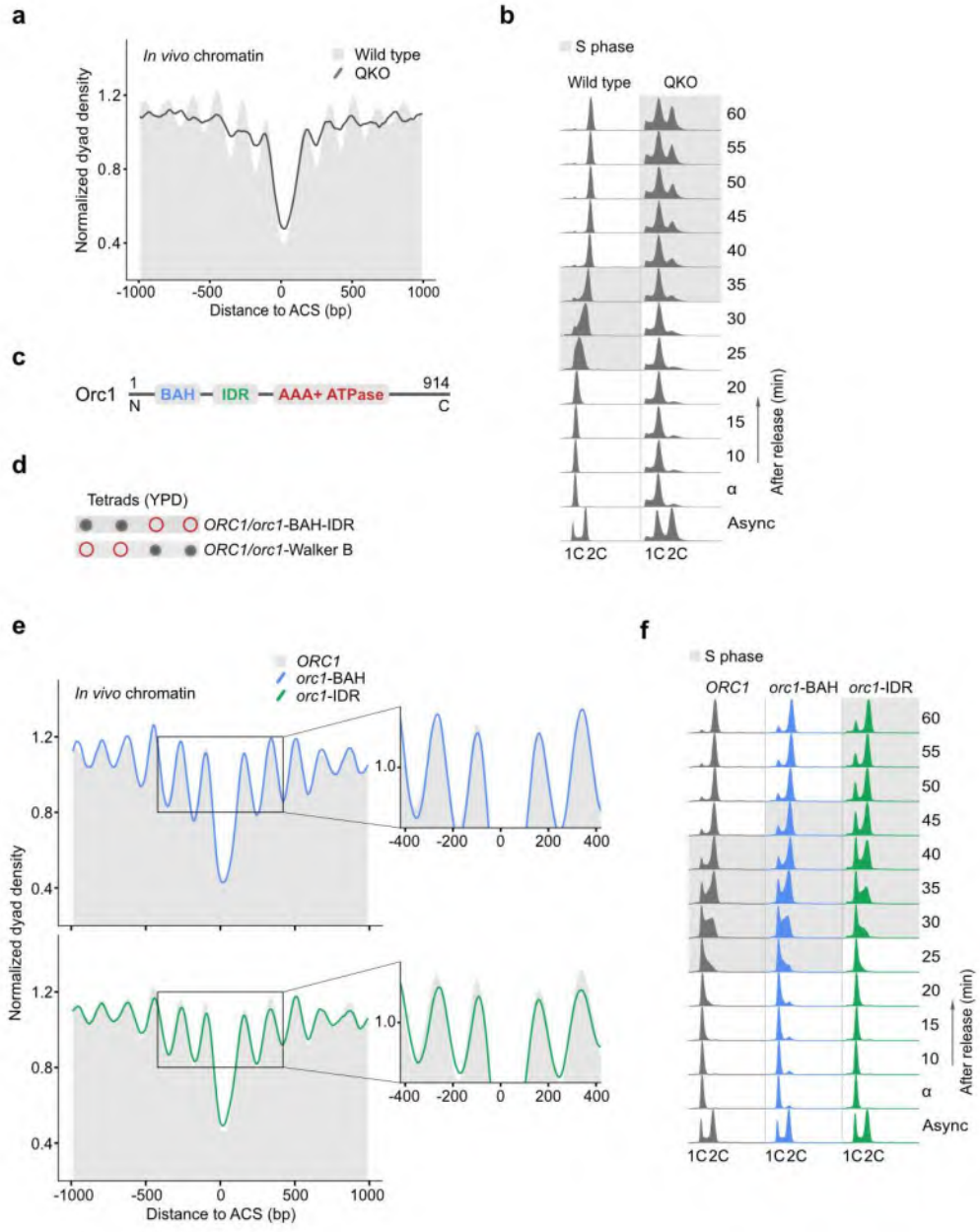
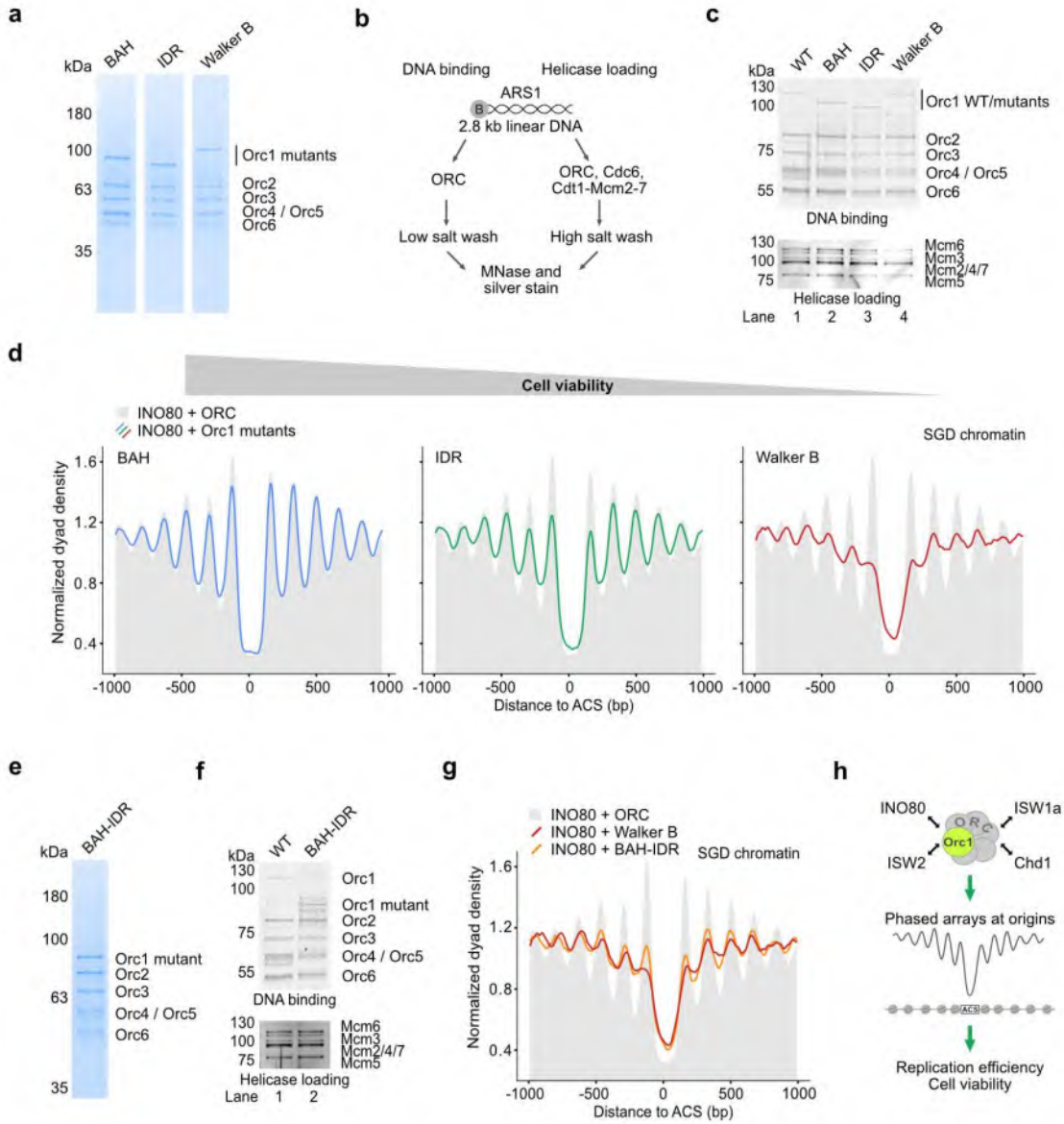

a
In vivo chromatin
Wild type
QKO
Normalized dyad density
1.2
0.8
0.4
-1000
-500
0
500
1000
Distance to ACS (bp)
b
S phase
Wild type
QKO
60
55
50
45
40
35
30
25
20
15
10
α
Async
After release (min)
1C 2C
1C 2C
c
Orc1
1
N
BAH
IDR
AAA+ ATPase
914
C
d
Tetrads (YPD)
ORC1/orc1-BAH-IDR
ORC1/orc1-Walker B
e
ORC1
orc1-BAH
orc1-IDR
In vivo chromatin
Normalized dyad density
1.2
0.8
0.4
1.0
-400
-200
0
200
400
-1000
-500
0
500
1000
Distance to ACS (bp)
f
S phase
ORC1
orc1-BAH
orc1-IDR
60
55
50
45
40
35
30
25
20
15
10
α
Async
After release (min)
1C 2C
1C 2C
1C 2C

**Fig. 3**


a
kDa
BAH
IDR
Walker B
180
100
63
35
Orc1 mutants
Orc2
Orc3
Orc4 / Orc5
Orc6
b
DNA binding
Helicase loading
ARS1
B
2.8 kb linear DNA
ORC
ORC, Cdc6,
Cdt1-Mcm2-7
Low salt wash
High salt wash
MNase and
silver stain
c
kDa
WT
BAH
IDR
Walker B
130
100
75
55
Orc1 WT/mutants
Orc2
Orc3
Orc4 / Orc5
Orc6
DNA binding
130
100
75
Mcm6
Mcm3
Mcm2/4/7
Mcm5
Helicase loading
Lane 1 2 3 4
d
Cell viability
INO80 + ORC
INO80 + Orc1 mutants
SGD chromatin
BAH
IDR
Walker B
Normalized dyad density
1.6
1.2
0.8
0.4
-1000
-500
0
500
1000
Distance to ACS (bp)
e
kDa
BAH-IDR
180
100
63
35
Orc1 mutant
Orc2
Orc3
Orc4 / Orc5
Orc6
f
kDa
WT
BAH-IDR
130
100
75
55
Orc1
Orc1 mutant
Orc2
Orc3
Orc4 / Orc5
Orc6
DNA binding
130
100
75
Mcm6
Mcm3
Mcm2/4/7
Mcm5
Helicase loading
Lane 1 2
g
INO80 + ORC
INO80 + Walker B
INO80 + BAH-IDR
SGD chromatin
Normalized dyad density
1.6
1.2
0.8
0.4
-1000
-500
0
500
1000
Distance to ACS (bp)
h
INO80
ISW1a
ISW2
Chd1
Orc1
Phased arrays at origins
ACS
Replication efficiency
Cell viability

## Legends to Figures:

**Fig. 1: ORC is a master regulator of chromatin structure at origins of replication.**

**a)** Composite plot of MNase-seq data of ~ 300 replication origins from wild type cells *in vivo* aligned to the ORC binding sites ACS (ARS-Consensus Sequence). Grey circles: Nucleosomes.

**b)** Outline of the chromatin reconstitution assay.

**c)** Bulk nucleosome spacing of *in vitro* assembled SGD chromatin was determined by partial MNase digestion. After digestion, proteins were removed and the nucleosomal DNA fragments were resolved through 1% agarose and stained with ethidium bromide.

**d)** Composite plot of MNase-seq data of SGD chromatin aligned as in **a)**.

**e)** SDS-PAGE analysis of purified ORC.

**f)** Composite plot of MNase -seq data aligned as in **a)** of SGD chromatin incubated with ORC.

**g)** SDS-PAGE analysis of purified INO80, ISW1a, ISW2 and Chd1.

**h)** Composite plots of MNase-seq data aligned as in **a)** of SGD chromatin incubated with ORC plus either INO80 or ISW1a or ISW2 or Chd1.

**Fig. 2: Orc1 is important for ORC´s chromatin function *in vivo*.**

**a)** Composite plots of MNase-seq *in vivo* data aligned as in Fig. 1A of wild type cells versus of a quadruple mutant (QKO), deleted for *ARP8*, *ISW1*, *ISW2* and *CHD1*.

**b)** Wild type and QKO cells were synchronized in G1 phase with alpha-factor. After release, S phase progression was monitored using flow cytometry. 1C and 2C indicate non-replicated or replicated DNA, respectively.

**c)** Domain organization of Orc1 subunit of ORC, highlighting the bromo-adjacent homology domain (BAH), the intrinsically disordered region (IDR) and the $AAA^+$-ATPase domain.

**d)** Representative results of tetrad dissections from *ORC1/orc1*-BAH-IDR *and ORC1/orc1*-Walker B heterozygotes. Red circles indicate positions of spores bearing *orc1* mutations.

**e)** Composite plots of MNase-seq *in vivo* data aligned as in Fig. 1a of *ORC1* wild type compared to *orc1* mutants as indicated. Similar results were obtained with three independent replicates.

**f)** Flow cytometry analyses as in Fig. 2b with the same strains as in **e)**.

**Fig. 3: Lethal phenotypes of Orc1 mutations are associated with disrupted chromatin architecture at origins**

**a)** SDS-PAGE analysis of ORCs with Orc1 mutants as indicated.

**b)** Outline of the assay of the ORC-DNA binding assay and the MCM loading assay. Salt-stable association of Mcm2-7 subunits is indicative of productive MCM loading[26,27].

**c)** Silver-stained gels of the assays described in **b)** with ORC wild type (WT) and ORCs with Orc1 mutants.

**d)** Composite plots of MNase-seq data aligned as in Fig. 1a of SGD chromatin incubated with INO80 and ORC wild type (grey background) or ORCs with mutant Orc1 as indicated. Wedge on top indicates cell viability of mutants bearing the respective *orc1* mutations.

**e)** SDS-PAGE analysis of purified ORC with the Orc1-BAH-IDR mutant.

**f)** Silver-stained gels of the assays described in **b)** with ORC wild type or ORC with the Orc1-BAH-IDR mutant.

**g)** Composite plot of MNase-seq data aligned as in **a)** of SGD chromatin incubated with indicated ORC variants.

**h)** Model of ORC function in chromatin organization at replication origins. See text for details.

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# Methods

## Strains

Yeast strains were generated using standard genetic techniques. A list of strains used in this study can be found in Extended Data Table 1.

## Origin plasmid library

The yeast origin plasmid library was generated using the *S. cerevisiae* genomic library (pGP546) from Open Biosystems[42]. A list of confirmed ARS available in OriDB[43] was used to select plasmids containing an origin of replication located at least 1000 bp away from the border between plasmid backbone and yeast genomic insert. In this way, a smaller *E.coli* clone collection containing 357 ARS was generated and stored as glycerol stocks in 96-well plates. The library was expanded as previously described[44].

## SGD chromatin assembly

Chromatin was assembled by salt gradient dialysis (SGD) as previously described[14,15]. Briefly, 10 µg of origin plasmid library DNA were combined with *Drosophila* embryo histone octamers in 100 µL SGD buffer (10 mM Tris-HCl pH 7.6, 2 M NaCl, 1 mM EDTA, 20 µg BSA, 0.05 % IGEPAL CA630) to a saturated assembly degree[44,45]. Samples were placed in Slide-A-Lyzer devices (Thermo-Fisher) in 300 mL high salt buffer (10 mM Tris-HCl pH 7.6, 2 M NaCl, 1 mM EDTA, 0.05 % IGEPAL CA630, 14.3 mM β-mercaptoethanol). This was dialyzed against 3 L low salt buffer (10 mM Tris-HCl pH 7.6, 50 mM NaCl, 1 mM EDTA, 0.05 % IGEPAL CA630, 1.4 mM β-mercaptoethanol), using a peristaltic pump at 7.5 rpm, 30 ˚C for 16 h. Chromatin was then dialyzed for 1 h with 1 L low salt buffer at 30 ˚C and stored at 4 ˚C.

## Protein expression and purification

The embryonic *D. melanogaster* histone octamers, INO80, ISW1a, RSC, Fun30, Asf1, CAF-1, FACT, Nap1, and Nhp6 were expressed and purified as previously described[12,45–53].

*ORC expression and purification*

Codon optimized wild-type ORC was purified from ySD-ORC as described before with modifications[54]. Cells were grown in YP media + 2 % raffinose at 30 ˚C with shaking until reaching a density of ~ 2-4 x $10^7$ cells/mL. Cells were arrested with 100 ng/mL α-factor (GenScript) for 2 ½ h and protein expression was induced by the addition of 2 % galactose for 2 ½ h at 30 ˚C. Cells were collected by centrifugation, washed in lysis buffer without protease inhibitors (25 mM HEPES-KOH pH 7.5, 300 mM KCl, 0.05 % NP-40, 2 mM β-mercaptoethanol, 10 % glycerol), resuspended in an equal volume of 2X lysis buffer plus protease inhibitors (cOmplete (Roche), 0.2 mM PMSF (Roth), 1 µM pepstatin A (Genaxxon), 1 µg/mL aprotinin (Genaxxon), and 2 µM leupeptin (Genaxxon)) and frozen in liquid nitrogen in a dropwise manner. The frozen cells were crushed using a Freezer Mill (SPEX SamplePrep 6875 Freezer/Mill) (6 cycles for 2 min, crushing rate 15). To purify ORC, the frozen powder was thawed and resuspended in an equal volume of

lysis buffer plus protease inhibitors. Insoluble material was cleared by ultracentrifugation (235,000 g, 1 h, 4 ˚C), the supernatant was then collected and supplemented with 2 mM $CaCl_2$ before binding to 1 mL pre-washed calmodulin affinity resin (Agilent) in batch for 1 h at 4 ˚C. This was then transferred into a disposable column (Bio-Rad) and washed with lysis buffer containing 2 mM $CaCl_2$ (100 CV). ORC was eluted in 6 CV of lysis buffer containing 1 mM EDTA and 2 mM EGTA; pooled, concentrated (Amicon Ultra centrifugal filter units, MWCO 30 kDa, Millipore) and further purified using a Superdex 200 Increase 10/300 GL column (Cytiva) in elution buffer (25 mM HEPES-KOH pH 7.5, 200 mM KOAc, 0.05 % NP-40, 2 mM β-mercaptoethanol, 10 % glycerol). Fractions containing ORC were pooled, concentrated and stored in aliquots at - 80 ˚C.

*Orc1 mutants' expression and purification*

Codon optimized versions of ORC containing a mutated Orc1 (BAH, IDR, Walker B, and BAH-IDR) were chromosomally integrated and the expression of all subunits was under the control of a GAL1/10 promoter. The endogenous Orc1 allele was tagged with a 3xFLAG to remove the complexes containing WT Orc1 subunits. The purification of the mutants was done as for SD-ORC but with the following additional purification step: pooled fractions from the calmodulin affinity resin step were applied to 0.5 mL pre-washed anti-FLAG M2 affinity gel (Sigma) and the eluted fractions were then concentrated before proceeding to the size exclusion purification step as described above.

*ISW2 expression and purification*

Cells were grown in 6 L YPD to saturation, collected and resuspended in an equal volume of 2X lysis buffer with protease inhibitors (lysis buffer (1X): 25 mM HEPES-KOH pH 7.5, 300 mM KCl, 2 mM $MgCl_2$, 0.02 % NP-40, 1 mM DTT, 100 mM EDTA, 20 % glycerol). Cells were frozen in liquid nitrogen in a dropwise manner, thawed and crushed using a Freezer Mill. The frozen cell powder was thawed, resuspended in an equal volume of lysis buffer with protease inhibitors and the insoluble material was removed by ultracentrifugation (235,000 g, 1 h, 4 ˚C). The supernatant containing Isw2-TAP was incubated with 1 mL of pre-washed IgG Sepharose 6 Fast Flow resin (VWR) in batch for 2 h at 4 ˚C. This was applied to a disposable column, washed with 100 CV wash buffer (same as lysis buffer but containing 100 mM KCl) and the resin was incubated in 1 mL wash buffer containing 0.1 mg/mL TEV protease for 16 h while rotating at 4 ˚C. ISW2 was eluted in 8 CV lysis buffer and the peak fractions were pooled, mixed with 3 volumes of wash buffer and supplemented with 2 mM $CaCl_2$. This was then incubated with 1 mL pre-washed calmodulin affinity resin for 1 h at 4 ˚C, transferred to a disposable column, washed with 75 CV of wash buffer containing 2 mM $CaCl_2$, and eluted in 10 CV of elution buffer (wash buffer containing 2 mM EDTA and 3 mM EGTA). The fractions were analyzed by SDS-PAGE, pooled and concentrated using a Vivaspin 20 (100 kDa MWCO Polyethersulfone, Merck).

*Chd1 expression and purification*

ΔNC-Chd1 was expressed and purified as described previously with modifications[55]. Insoluble material was cleared by centrifugation (235,000 g, 1 h, 4 ˚C) and the supernatant was incubated with 1 mL pre-washed Ni-NTA agarose in batch for 1 h at 4 ˚C. This was transferred to a disposable column, washed with 25 CV of lysis buffer, 50 CV of lysis buffer containing 25 mM

imidazole and eluted 10 CV with lysis buffer containing 400 mM imidazole. Peak fractions were pooled and dialyzed against 2 L of 25 mM Tris-HCl pH 7.6, 250 mM NaCl, 1 mM DTT and 10% glycerol. Chd1 was further purified in a Mono Q 5/50 GL column and eluted with a 20 CV gradient from 50 mM to 1 M NaCl (50 mM Tris-HCl pH 7.6, 1 mM DTT, 10 % glycerol). Peak fractions were analyzed by SDS-PAGE, pooled and applied to a Superdex 200 Increase 10/300 GL column using a buffer containing 50 mM Tris-HCl pH 7.6, 250 mM NaCl, 1 mM DTT and 10 % glycerol. Fractions containing Chd1 were pooled and stored at -80 ˚C.

*SWI/SNF expression and purification*

SWI/SNF was purified as described previously with modifications[56]. 6 L of cells were grown in YPD to saturation. Cells were collected and crushed in a Freezer Mill as described above. The cell powder was thawed and resuspend in an equal volume of lysis buffer (40 mM HEPES-KOH pH 7.5, 350 mM KCl, 0.1 % NP-40, 10 % glycerol) plus protease inhibitors, centrifuged at 235,000 g for 1 h at 4 ˚C and the supernatant was incubated with pre-washed anti-FLAG M2 affinity gel in batch for 1 h at 4 ˚C. This was transferred into a disposable column, washed with 100 CV of lysis buffer and 50 CV of wash buffer (10 mM Tris-HCl pH 7.2, 150 mM NaCl, 0.5 mM EDTA, 1 mM DTT, 0.1 % NP-40, 10 % glycerol). SWI/SNF was eluted in 1 CV of wash buffer with 0.5 mg/mL 3xFLAG peptide (Sigma), followed by 2 CV of wash buffer with 0.25 mg/mL 3xFLAG peptide. Fractions were analyzed by SDS-PAGE, pooled and dialyzed against 2 L of wash salt containing 40 % glycerol.

*SWR1 expression and purification*

The yeast powder was thawed and resuspended in 2 volumes of lysis buffer (25 mM HEPES-KOH pH 7.6, 500 mM KCl, 1 mM EDTA pH 8.0, 4 mM $MgCl_2$, 0.05 % NP-40, 10% glycerol) plus protease inhibitors. The lysate was centrifuged at 136,000 g for 2 h at 4 ˚C and the supernatant was incubated with 1.5 mL of pre-washed anti-FLAG M2 affinity gel in batch for 1 ½ h at 4 ˚C. This was transferred into a disposable column, washed with 100 CV of lysis buffer and 25 CV of wash buffer (25 mM HEPES-KOH pH 7.6, 150 mM KCl, 1 mM EDTA pH 8.0, 4 mM $MgCl_2$, 0.05 % NP-40, 10% glycerol). SWR1 was eluted in 1 CV of wash buffer with 0.5 mg/mL 3xFLAG peptide (Sigma), followed by 2 CV of wash buffer with 0.25 mg/mL 3xFLAG peptide. Fractions were analyzed by SDS-PAGE, pooled and dialyzed against 2 L of 25 mM Tris-HCl pH 7.6, 100 mM NaCl, 1 mM EDTA pH 8.0, 1 mM DTT and 40% glycerol.

*HIR expression and purification*

The frozen cell powder was slowly thawed and resuspended in 1 volume of lysis buffer (30 mM HEPES-KOH pH 7.5, 300 mM KCl, 0.5 mM EDTA, 0.1 % NP-40, 10% glycerol) containing protease inhibitors and centrifuged at 235,000 g, 1 h, 4 ˚C. The supernatant was then incubated with pre-washed anti-FLAG M2 affinity gel in batch for 1 h at 4 ˚C. This was transferred into a disposable column, washed with 100 CV of lysis buffer and 50 CV of wash buffer (30 mM HEPES-KOH pH 7.5, 100 mM KCl, 0.1 % NP-40, 10% glycerol). HIR was eluted in 1 CV of wash buffer with 0.5 mg/mL 3xFLAG peptide (Sigma), followed by 2 CV of wash buffer with 0.25 mg/mL 3xFLAG peptide. Fractions were analyzed by SDS-PAGE, pooled and dialyzed against 2 L of 30

mM HEPES-KOH pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.1% NP-40, 1 mM DTT and 40 % glycerol.

*Spt6 expression and purification*

6 L of cells were grown in YPD, collected and lysed with a Freezer Mill as described above. The frozen powder was slowly thawed and resuspended in an equal volume of lysis buffer (20 mM Tris-HCl pH 7.2, 0.5 M KOAc, 0.1 % NP-40, 1 mM EDTA and 10 % glycerol) containing protease inhibitors. The insoluble material was cleared by centrifugation (235,000 g, 1 h, 4 ˚C) and the supernatant was incubated with 1.5 mL pre-washed anti-FLAG M2 affinity gel in batch for 1 h at 4 ˚C. This was transferred into a disposable column, washed with 100 CV of lysis buffer and 25 CV of wash buffer (10 mM HEPES-KOH pH 7.5, 150 mM NaCl, 0.1 % NP-40, 1 mM EDTA, 10 % glycerol). Spt6 was eluted in 1 CV of wash buffer with 0.5 mg/mL 3xFLAG peptide (Sigma), followed by 2 CV of wash buffer with 0.25 mg/mL 3xFLAG peptide. Eluted fractions were analyzed by SDS-PAGE, pooled, and further purified with a Mono Q 5/50 GL column using a 20 CV gradient from 100 mM to 1 M NaCl (10 mM HEPES-KOH pH 7.5, 0.1 % NP-40, 1 mM EDTA, 1 mM DTT and 10 % glycerol).

*Rtt106 expression and purification*

Cells were grown in 2 L of YP + 2 % raffinose to 5 x $10^7$ cells/mL at 30 ˚C and protein expression was induced by addition of 2 % galactose for 3 h. Cells were collected and crushed in a Freezer Mill. The thawed powder was resuspended in 1 volume of lysis buffer (30 mM HEPES-KOH pH 7.5, 300 mM NaCl, 0.5 mM EDTA, 0.1 % NP-40, 10% glycerol) containing protease inhibitors and centrifuged (235,000 g, 1 h, 4 ˚C). The supernatant was then incubated with 1.5 mL pre-washed anti-FLAG M2 affinity gel in batch for 1 h at 4 ˚C. This was transferred into a disposable column, washed with 100 CV of lysis buffer and 50 CV of wash buffer (30 mM HEPES-KOH pH 7.5, 100 mM NaCl, 0.1 % NP-40, 10% glycerol). Rtt106 was eluted in 1 CV of wash buffer with 0.5 mg/mL 3xFLAG peptide (Sigma), followed by 2 CV of wash buffer with 0.25 mg/mL 3xFLAG peptide. Fractions were analyzed by SDS-PAGE, pooled and dialyzed against 2 L of 30 mM HEPES-KOH pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.1% NP-40, 1 mM DTT and 40 % glycerol.

*Vps75 expression and purification*

2 L of BL21 (DE3) CodonPlus-RIL cells with a Vps75-$His_6$ expression plasmid were grown at 37 ˚C to $OD_{600}$ = 0.8. Protein expression was induced with 0.8 mM IPTG incubated for 16 h at 20 ˚C. Cells were collected by centrifugation, washed, resuspended in 2 mL of lysis buffer (20 mM HEPES-KOH pH 7.6, 100 mM NaCl and protease inhibitors) per g of cells and supplemented with 16 U DNAse I, 30 µg/mL RNase A and 0.5 mM DTT. The cells were sonicated (10 min (1 s on, 2 s off, 38 % amplitude)) and centrifuged (235,000 g, 1 h, 4 ˚C). The supernatant was incubated with 1.5 mL pre-washed Ni-NTA resin for 1 h while rotating at 4 ˚C. This was then applied to a disposable column and washed with 25 CV of lysis buffer without protease inhibitors, followed by 50 CV of wash buffer (20 mM HEPES-KOH pH 7.6, 100 mM NaCl and 25 mM imidazole). Vps75 was eluted with 10 x 1 mL elution buffer (20 mM HEPES-KOH pH 7.6, 100 mM NaCl and 400 mM imidazole). Fractions were analyzed by SDS-PAGE, pooled and dialyzed against 2 L of

20 mM HEPES-KOH pH 7.6 and 100 mM NaCl. Vps75 was further purified in a Mono Q 5/50 GL column with a 20 CV gradient from 100 mM to 1 M NaCl (HEPES-KOH pH 7.6 and 2 mM DTT). Peak fractions were analyzed by SDS-PAGE, pooled and applied to a Superdex 200 Increase 10/300 GL column equilibrated with 20 mM HEPES-KOH pH 7.6, 500 mM NaCl and 1 mM EDTA.

### *In vitro* remodeling assay and MNase-seq

The assay was done as previously described with modifications[15,44]. The following concentrations of chromatin factors were used: 30 nM ORC, Orc1 mutants (BAH, IDR, Walker B, BAH-IDR); 20 nM INO80, Chd1; 10 nM ISW1a, ISW2, Fun30, RSC, SWI/SNF, SWR1, Asf1, CAF1, FACT, HIR, Nap1, Nhp6, Spt6, Rtt106, Vps75. The reactions were started by the addition of the SGD chromatin, incubated for 2 h at 30 ˚C and stopped by addition of 0.2 U apyrase (NEB), incubated at 30 ˚C for 20 min. To generate mostly mononucleosomal DNA, the reactions were incubated with 100 U MNase (Sigma-Aldrich) and 1.5 mM $CaCl_2$ for 5 min at 30 ˚C. The digest was stopped by the addition of 10 mM EDTA and 0.5 % SDS. This was followed by a proteinase K treatment for 1 h at 37 ˚C and ethanol precipitation. Samples were run in 1.5 % agarose gels for 1.5 h at 110 V constant in 1X TAE (40 mM Tris, 20 mM acetic acid, 1 mM EDTA), mononucleosomal DNA was excised and purified.

The sequencing libraries were prepared as previously described[15,44] by using 10-50 ng mononucleosomal DNA. The samples were diluted to 10 nM, pooled according to the sequencing reads (~ 5 million reads/sample), and quantified via BioAnalyzer (Agilent). The pool was sequenced either on an Illumina HiSeq 1500 in 50 bp single-end mode or on an Illumina NextSeq 1000 in 60 bp paired-end mode (Laboratory for Functional Genome Analysis, LAFUGA, LMU Munich).

### Nuclei isolation and MNase-seq

A 500 mL YPDA culture was incubated at 30 ˚C for 16-20 h until reaching $OD_{600}$ = 0.6-1.0 (measured with a GENESYS 20 Visible Spectrophotometer (Thermo Fisher)). The cells were collected by centrifugation and the nuclei isolation was performed as previously described with modifications[44,57]. Spheroblasts were generated by incubation with zymolyase (MP Biomedicals zymolyase 100T) in 1 M sorbitol, washed and resuspended in a Ficoll solution (18 % Ficoll Type 400 (Sigma-Aldrich), 20 mM $KH_2PO_4$, 1 mM $MgCl_2$, 0.25 mM EGTA pH 8.0, 0.25 mM EDTA pH 8.0). This was then aliquoted and centrifuged for 30 min at 22,550 g 4 ˚C. The nuclei pellets were frozen in a dry ice/ethanol bath and stored at - 80˚C.

The nuclei was digested with MNase (Sigma-Aldrich) in MNase buffer (150 mM Tris-HCl pH 7.5, 500 mM NaCl, 14 mM $CaCl_2$, 2 mM EDTA, 2 mM EGTA and 50 mM β-mercaptoethanol) to get an 80 % mono- / 20 % di- nucleosome distribution. The reaction was stopped by addition of 10 mM EDTA and 0.5 % SDS. The DNA was purified by Proteinase K digestion, phenol-chloroform extraction, ethanol precipitation, RNAse A digestion and isopropanol precipitation. The samples were run in a 1.5 % agarose gel, the mononucleosomal DNA was cut out and purified using the PureLink Quick Gel Extraction kit (Invitrogen).

Sequencing libraries were prepared with the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina (NEB). The amplified DNA was run in a 1.5 % agarose gel, cut out, purified and the concentration was measured with the Qubit dsDNA HS Assay Kit (Fisher Scientific). Samples

were then diluted to 10 nM, pooled according to sequencing reads (~ 5 million reads/sample) and quantified via BioAnalyzer (Agilent). The pool was sequenced on an Illumina NextSeq 1000 in 60 bp paired-end mode (Laboratory for Functional Genome Analysis, LAFUGA, LMU Munich).

## ORC binding and MCM loading assays

The ORC binding and loading assays were done as described before with modifications[26,27]. A linear 2.8 kb fragment of yeast DNA containing an ARS1 sequence was bound at one end to magnetic beads via a biotin-streptavidin linkage. For testing the ORC binding to DNA, a 40 µL reaction was carried out by incubating together 500 ng DNA beads, 30 nM ORC and 5 mM ATP in loading buffer (25 mM HEPES-KOH pH 7.6, 10 mM MgOAc, 90 mM KOAc, 1 mM DTT, 0.1 % NP-40 and 5 % glycerol). The reaction was incubated for 90 min at 30 ˚C, 1250 rpm. The beads were washed twice with 400 µL low salt buffer (45 mM HEPES-KOH pH 7.6, 5 mM MgOAc, 300 mM KOAc, 0.02 % NP-40 and 10 % glycerol) and incubated with 860 gel units MNase (NEB) in low salt buffer containing 5 mM $CaCl_2$ for 5 min at 37 ˚C, 1250 rpm. The supernatant was analyzed in a SDS-PAGE and silver stained.

For the loading assay the DNA beads were incubated with ORC as described for the ORC binding assay, but with the addition of 80 nM Cdc6 and 50 nM Cdt1•Mcm2-7. The beads were washed twice with 400 µL low salt buffer, twice in 400 µL high salt buffer (45 mM HEPES-KOH pH 7.6, 5 mM MgOAc, 500 mM NaCl, 0.02 % NP-40 and 10 % glycerol) and one 400 µL wash in low salt buffer. The beads were incubated with MNase as above and the supernatant was analyzed in an SDS-PAGE and silver stained.

## Flow cytometry

Samples were processed as previously described[58]. Briefly, yeast cells were harvested by centrifugation and permeabilized with 70 % ethanol on ice for at least 1 h, followed by treatment with RNase A and Proteinase K. Finally, cells were stained with 0.5 µM SYTOX green and measured on a MACSquant analyzer (Miltenyi Biotec).

## Data processing

The data processing of the MNase-seq data was done as described before with modifications[44]. Sequencing reads were mapped to the *S. cerevisiae* SacCer3 (R64-1-1 build) using Bowtie2 (version 2.3.5.1)[59] and reads aligning to multiple sites were removed. This was then imported into R Studio by using GenomicAlignments[60]. For the single-end data, reads were shifted by 73 bp and extended to 50-60 bp, while for the paired-end data, reads with a fragment length between 139 and 191 bp were selected and extended to 60 bp. The coverage was calculated and aligned to a list of ACS sites[61]. The signal was normalized per ARS in a 2000 bp window centered at the ACS motif.

## Data and code availability statements

Data available on request from the authors.

## Methods References


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**Author contributions:** E.C. was setting up the assay, purified most proteins, did all the *in vitro* work and prepared the Figures; K-U.R. performed flow cytometry analyses; J.F. generated all the *in vivo orc1* mutations; L.K. purified and characterized Fun30; P.B. made the expression strain and purified Spt6; C.F.K. designed the story and wrote the paper. P.K., B.P. and C.F.K. secured funding, analyzed the data and contributing intellectually to the paper and all authors were involved in editing.

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# Extended Data for

# Establishment and Function of Chromatin Architecture at Eukaryotic Chromosome Replication Origins

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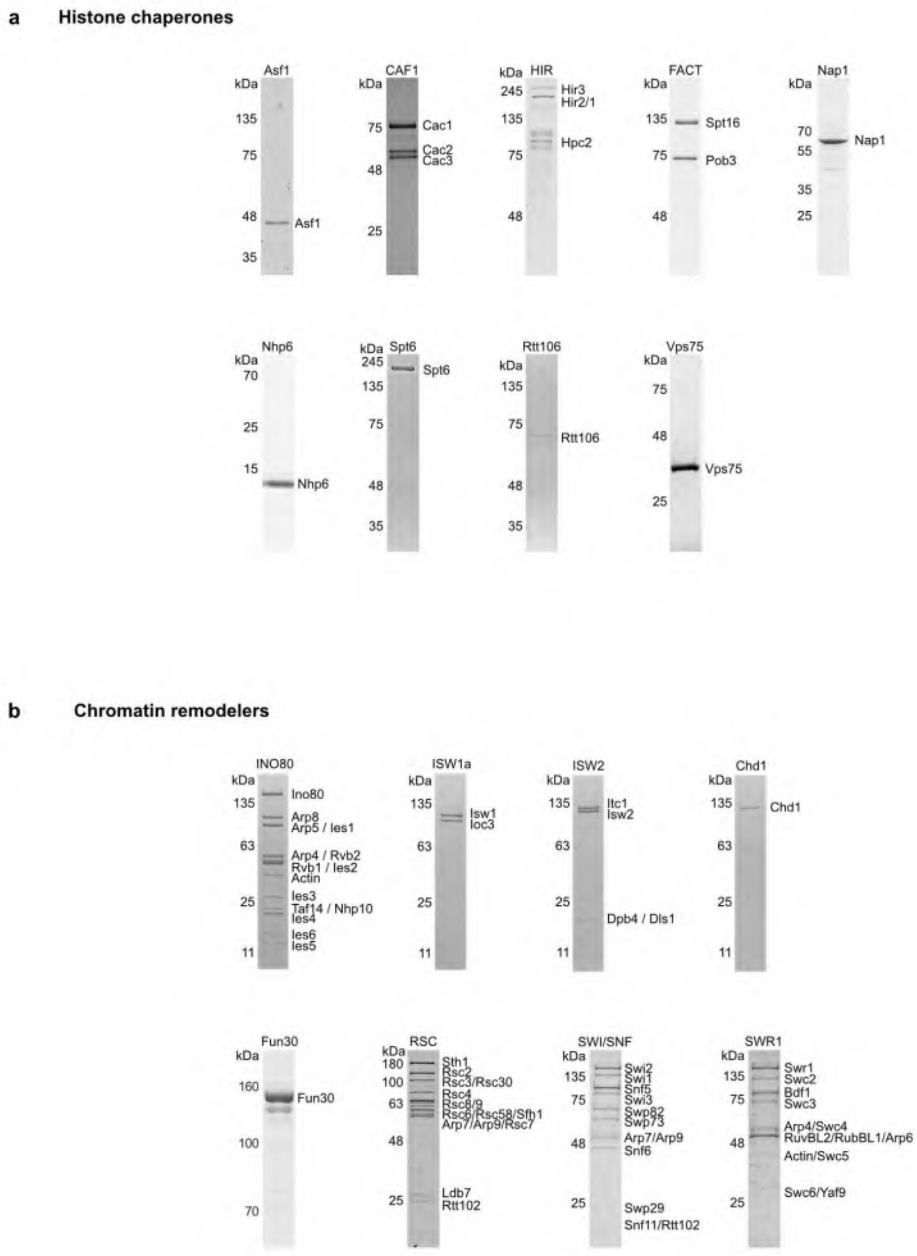
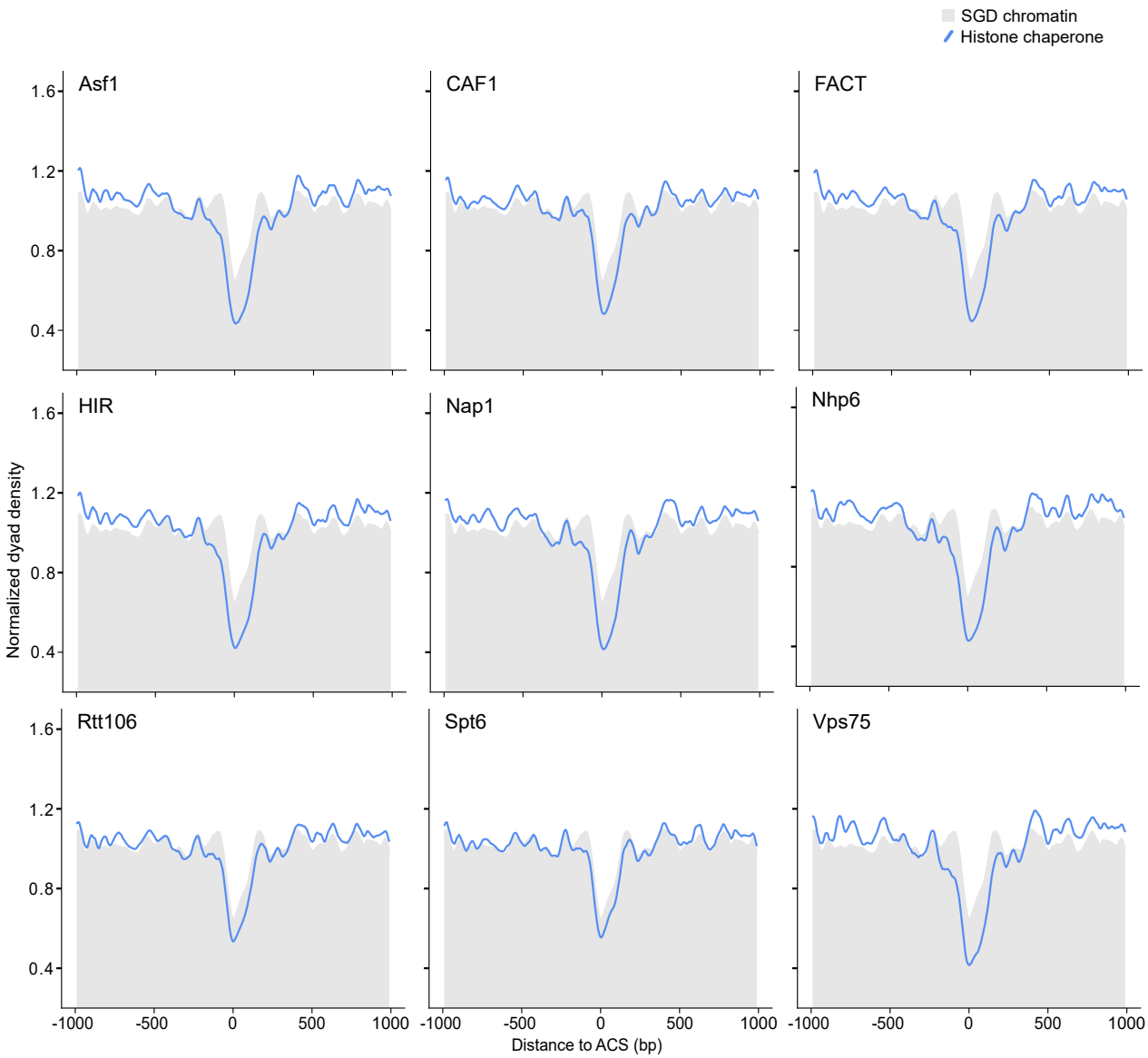
## Extended Data Figures



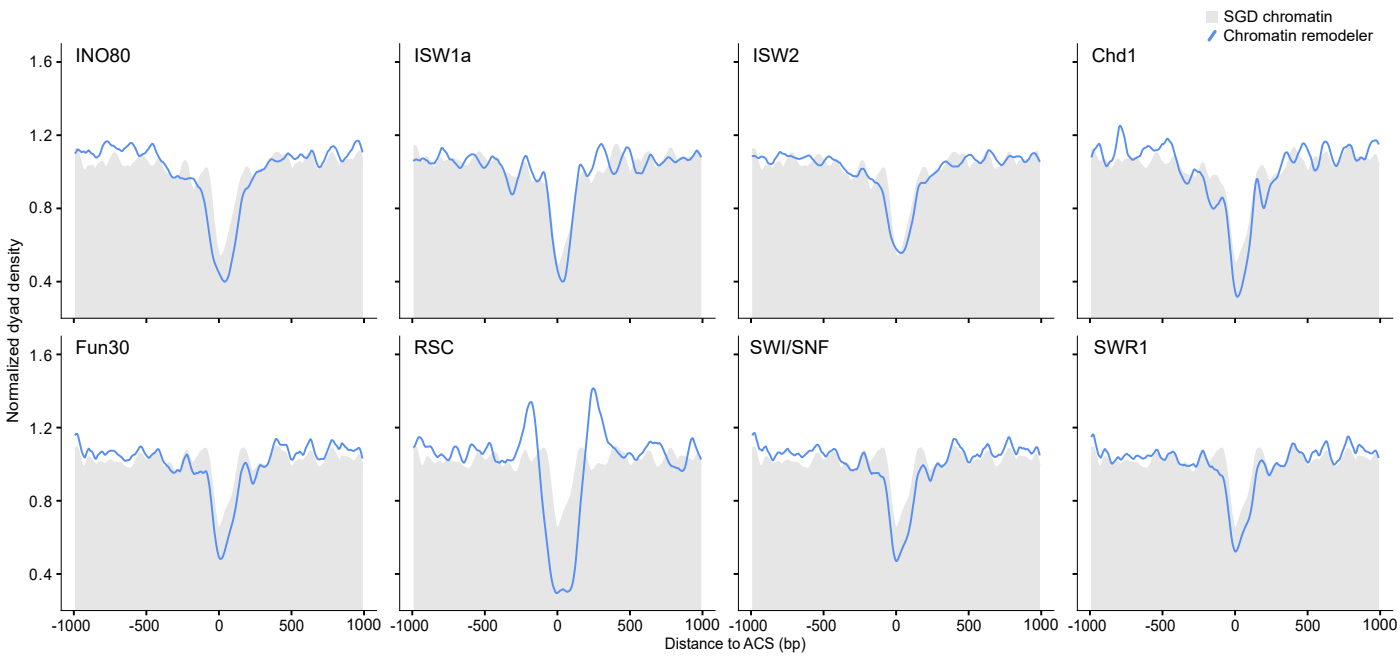

**Extended Data Fig. 1. Purified chromatin factors used in the *in vitro* screen**

SDS-PAGE analyses of purified histone chaperones **a)** and chromatin remodelers **b)**.



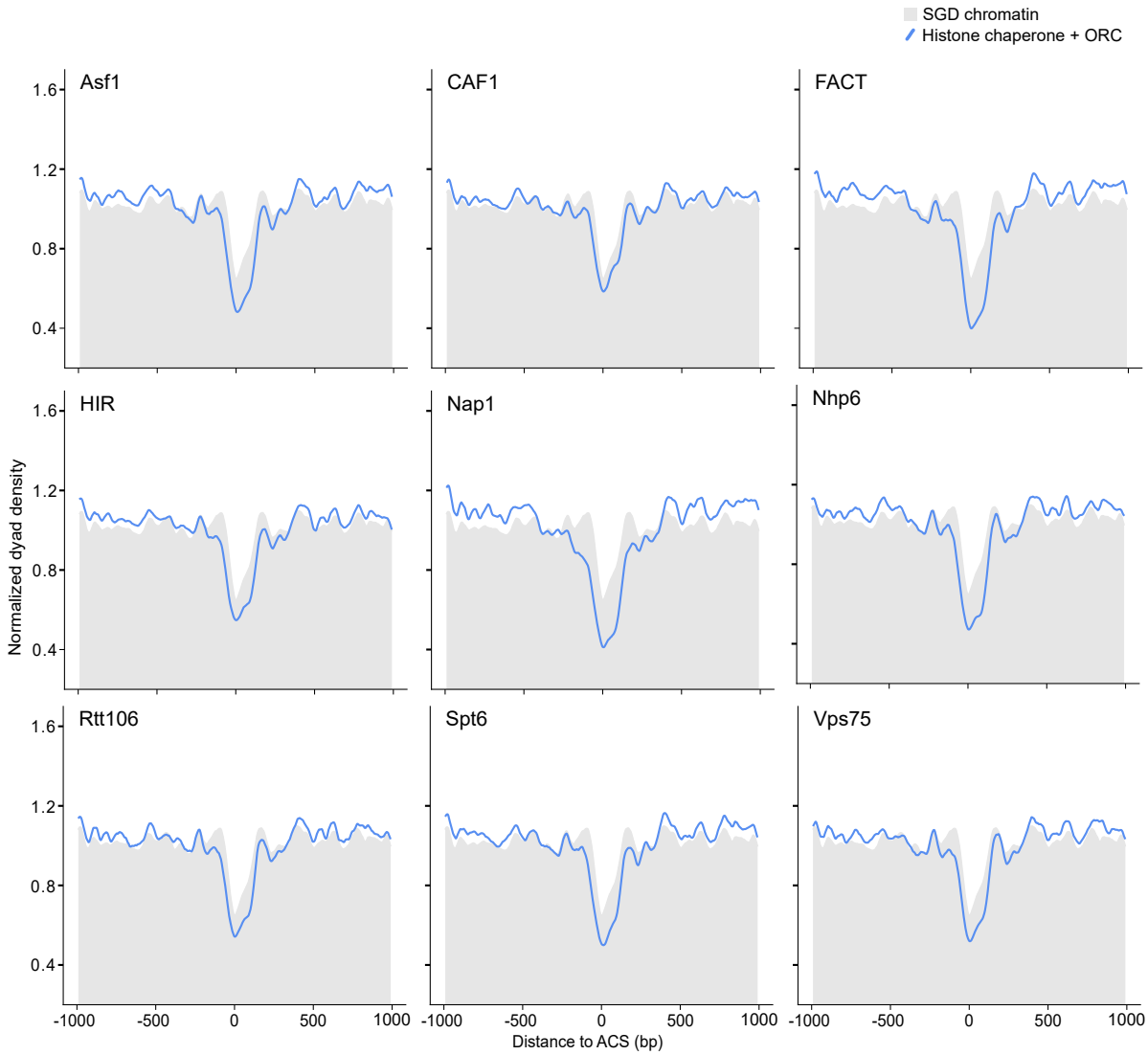

**Extended Data Fig. 2. Influence of histone chaperones on nucleosome positioning at origins**

Composite plots of MNase-seq data of SGD chromatin incubated with the indicated histone chaperones aligned as in Fig. 1a. All experiments were repeated three times with different protein preparations. SGD (Salt Gradient Dialysis).



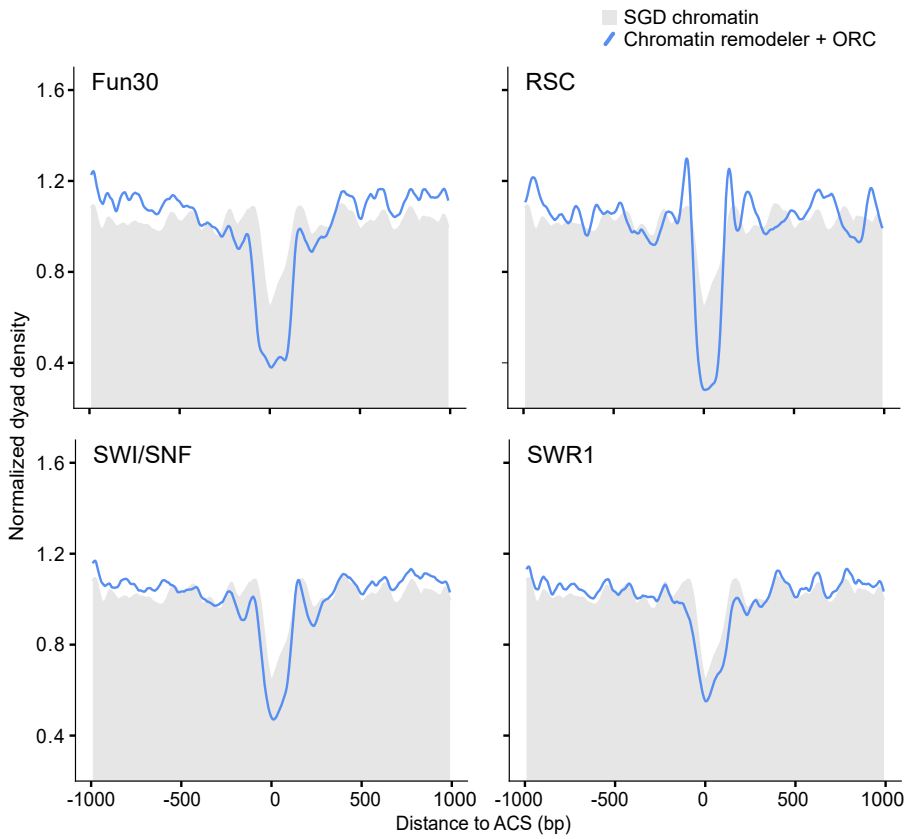

**Extended Data Fig. 3. Influence of chromatin remodelers on nucleosome positioning at origins**

Composite plots aligned as in Fig. 1a of MNase-seq data of SGD chromatin incubated with the indicated chromatin remodelers aligned as in Fig. 1a. All experiments were repeated three times with different protein preparations. SGD (Salt Gradient Dialysis).



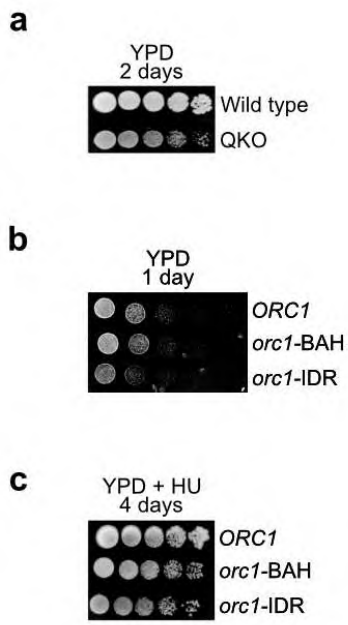

**Extended Data Fig. 4. Influence of histone chaperones in combination with ORC on nucleosome positioning at origins**

Composite plots aligned as in Fig. 1a of MNase-seq data of SGD chromatin incubated with including indicated histone chaperones plus ORC. All experiments were repeated three times with different protein preparations. SGD (Salt Gradient Dialysis).



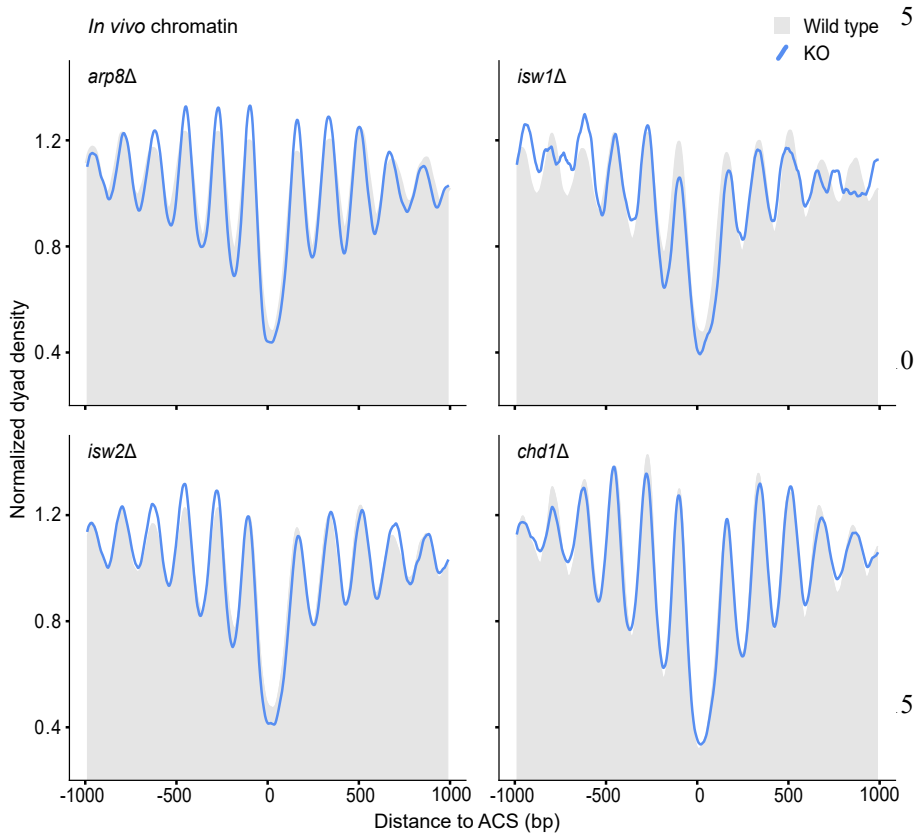

**Extended Data Fig. 5. Influence of Fun30, RSC, SWI/SNF and SWR1 plus ORC on nucleosome positioning at origins**

Composite plots aligned as in Fig. 1a of MNase-seq data of SGD chromatin including indicated chromatin remodelers plus ORC. All experiments were repeated three times with different protein preparations. SGD (Salt Gradient Dialysis).



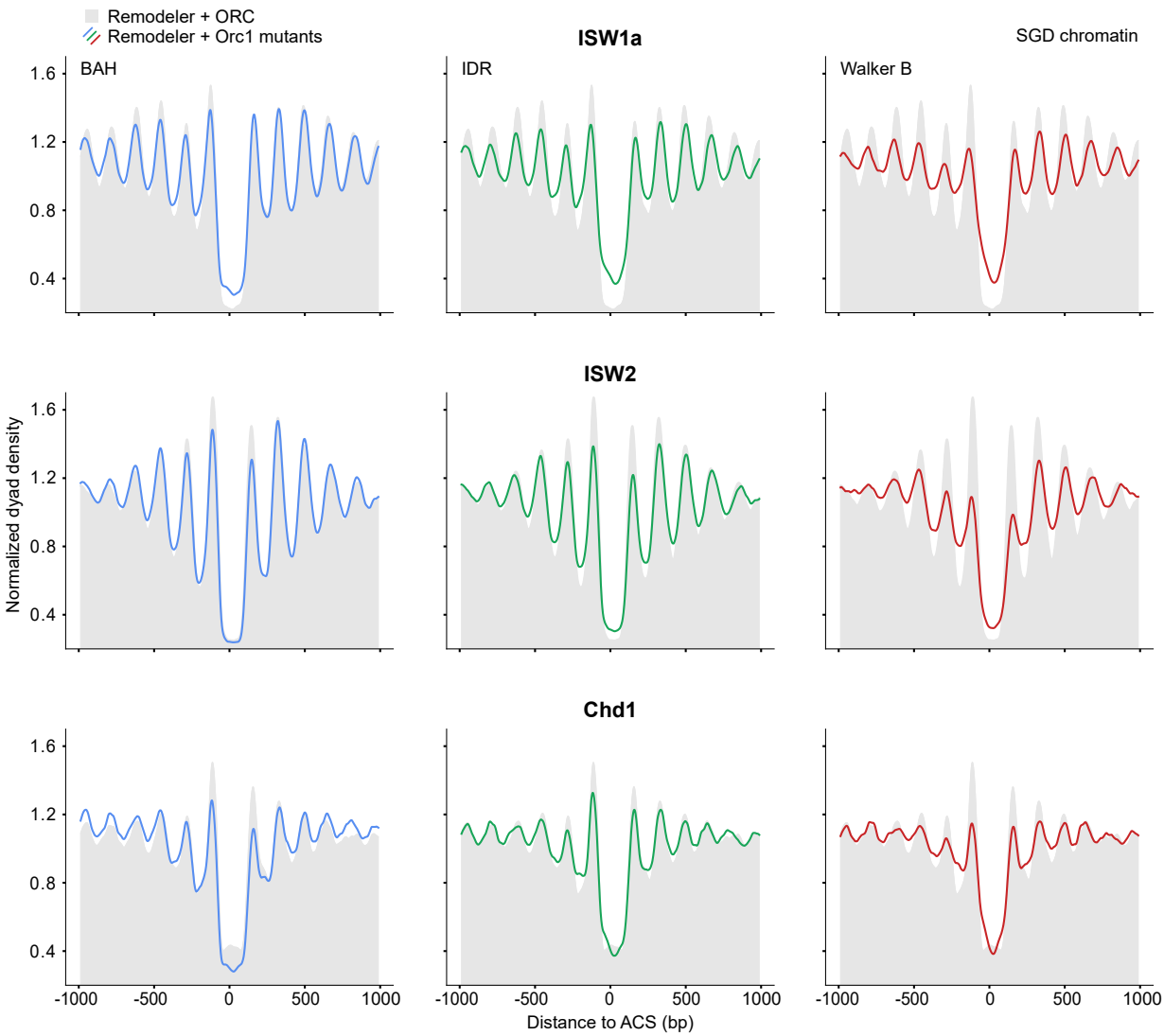

**Extended Data Fig. 6. Influence of QKO and viable *orc1* mutations on cell growth**

Spot dilution assays (10-fold serial dilutions) with the indicated yeast strains. HU: hydroxyurea (100 mM). Assays were repeated at least three times.



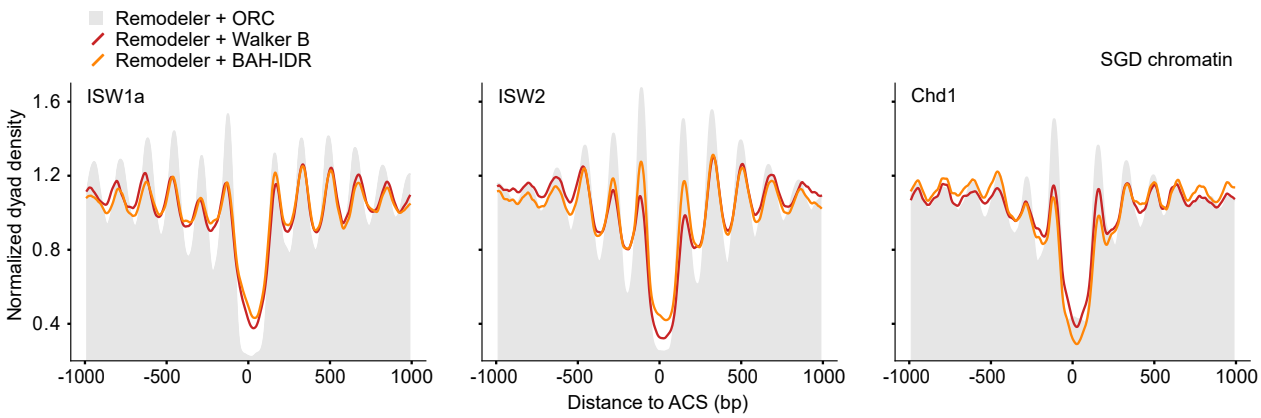

**Extended Data Fig. 7. Effect of single remodeler deletion mutations on chromatin at origins *in vivo***

Composite plots aligned to the ACS as in Fig. 1a of MNase-seq data of *in vivo* chromatin of wild type versus indicated deletion mutants. All experiments were repeated three times independently.




**Extended Data Fig. 8. Orc1 mutations influence nucleosome positioning at origins to different degrees**

Composite plots of MNase-seq data of SGD chromatin incubated with ISW1a, ISW2 and Chd1 in the presence of wild type ORC or with Orc1-BAH, Orc1-IDR and Orc1-Walker B mutant complexes, as indicated. All data were aligned as in Fig. 1a and all experiments were repeated three times with different protein preparations. SGD (Salt Gradient Dialysis).




**Extended Data Fig. 9. Orc1-Walker B and Orc1-BAH-IDR mutations have a similar defect in chromatin organization at origins**

Composite plots of MNase -seq data of SDG chromatin assembled with ISW1a, ISW2 and Chd1 in the presence of wild type ORC or the indicated Orc1-Walker B or with Orc1-BAH-IDR mutant complexes. All data were aligned as in Fig. 1a and all experiments were repeated three times with different protein preparations. SGD (Salt Gradient Dialysis).

**Extended Data Table 1. Strain list**

| Strain | Genotype | Source |
|---|---|---|
| yCFK3 | Frigola et al., 2013; Orc1 tagged with a CBP tag | Diffley lab |
| yCFK31 | *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::Hyg pep4::KanMX* *his3::HIS3*pRS303/Ino80-TEV-3XFLAG, Nhp10 *his3::HIS3*pRS303/Gal4,Taf14 *ura3::URA3*pRS306/Ies1, Ies2 *ura3::URA3*pRS306/Act1,Arp4 *leu2::LEU2*pRS305/Ies5, Ies6 *leu2::LEU2*pRS305/Arp5, Arp8 *trp1::TRP1*pRS304/Ies3, Ies4 *trp1::TRP1*pRS304/Rvb1, Rvb2 | [12] |
| yCFK99 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100, bar1::Hyg, pep4::KanMX*, IOC3-3xFLAG-NAT | [12] |
| yCFK86 | MATa *ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0* ISW2-TAP::HIS3MX6 | Pugh lab |
| ySB784 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100, bar1Δ::TRP1 pep4Δ::LEU2 GAL4* pGAl1-10 Fun30-3xFLAG::HIS3 | [50] |
| yCFK27 | Wittmeyer et al., 2004; Rsc2 tagged with a TAP tag at the C terminus | Kornberg lab |
| yCFK84 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100, bar1::Hyg, pep4::KanMX*, SWR1-3xFlag::NAT | This study |
| yCFK 78 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100, bar1::Hyg, pep4::KanMX*, SNF6-3xFLAG-NAT | This study |
| yCFK25 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::Hyg pep4::KanMX* *his3::HIS3*pRS303/Asf1-CBP,Gal4 | [12] |
| yCFK26 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::Hyg pep4::KanMX* *his3::HIS3*pRS303/Cac1-CBP,Gal4 | [53] |
| yCFK82 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::Hyg pep4::KanMX* HIR1-3xFLAG | This study |
| yCFK81 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100, bar1::Hyg, pep4::KanMX*, SPT6-3xFLAG::NAT | This study |
| yCFK74 | MATa *ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0* Rtt106-TAP::*HIS3* | This study |

| | | |
|---|---|---|
| yAM4 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100, bar1::Hyg, pep4::KanMX*, *his::HIS*pRS303/SDORC3,4, *trp1::TRP1*pRS304/SDORC5,6 | Diffley lab |
| yJF5 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100, bar1::Hyg, pep4::KanMX*, ORC1-3xFLAG *his::HIS*pRS303/SDORC3,4, *trp1::TRP1*pRS304/SDORC5,6 | This study |
| yJF16 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100, bar1::Hyg, pep4::KanMX*, ORC1-3xFLAG *his::HIS*pRS303/SDORC3,4, *trp1::TRP1p*RS304/SDORC5,6, *ura3::URA3*pRS306/ΔBAH-SDORC1,ORC2 | This study |
| yJF20 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100, bar1::Hyg, pep4::KanMX*, ORC1-3xFLAG *his::HIS*pRS303/SDORC3,4, *trp1::TRP1*pRS304/SDORC5,6, *ura3::URA3*pRS306/ΔIDR-SDORC1,ORC2 | This study |
| yJF27 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100, bar1::Hyg, pep4::KanMX*, ORC1-3xFLAG *his::HIS*pRS303/SDORC3,4, *trp1::TRP1*pRS304/SDORC5,6, *ura3::URA3*pRS306/D569Y-SDORC1,ORC2 | This study |
| yJF52 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100, bar1::Hyg, pep4::KanMX*, ORC1-3xFLAG *his::HIS*pRS303/SDORC3,4, *trp1::TRP1*pRS304/SDORC5,6, *ura3::URA3*pRS306/ΔBAH-ΔIDR-SDORC1,ORC2 | This study |
| W1588-4C | MATa *leu2-3, 112 trp1-1, can1-100, ura3-1, ade2-1, his3-11,15, RAD5+* | [19] |
| YTT186 | MATa *leu2-3, 112 trp1-1, can1-100, ura3-1, ade2-1, his3-11,15, RAD5+, isw1::ADE2* | [19] |
| YTT196 | MATa *leu2-3, 112 trp1-1, can1-100, ura3-1, ade2-1, his3-11,15, RAD5+, isw2::LEU2* | [19] |
| yFMP476 | *MATa leu2-3, 112 trp1-1, can1-100, ura3-1, ade2-1, his3-11,15, RAD5+, arp8Δ::NATMX6* | [18] |
| yFMP382 | MATa, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, chd1::hphNT1* | Mueller-Planitz lab |
| yFMP480 | MATa *leu2-3, 112 trp1-1, can1-100, ura3-1, ade2-1, his3-11,15, RAD5+, isw1Δ:ADE2 isw2Δ::LEU2 chd1Δ::TRP1 arp8Δ::NATMX6* | [18] |

| | | |
|---|---|---|
| yEC26 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100, orc1::URA3, leu2*::pRS305/ORC1 | This study |
| yEC30 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100, orc1::URA3*, *leu2*::pRS305/ΔBAH-ORC1 | This study |
| yEC27 | MATa *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100, orc1::URA3, leu2*::pRS305/ΔIDR-ORC1 | This study |

## Extended Data Table 2. DNA constructs

All the codon optimized sequences contain one 5′ AscI site, one 3′ XhoI site, and encode an N-terminal CBP tag and a Pgk1 terminator sequence.

| Construct information | DNA sequence |
|---|---|
| Orc1-ΔBAH (Δ48-188) | ATGGCAAAAACGTTGAAGGATTTACAGGGTTGGGAGATAATAACAAC<br>TGATGAGCAGGGAAATATAATCGATGGAGGTCAGAAGAGATTACGCC<br>GAAGAGGTGCAAAAACTGAACATTACTTAAAGAGAAGTTCTGATGGA<br>ATTGAGGATGTCAAAGCTTACATAAAGAAAGTGGAGCCAAGGGAAGC<br>CCAGGAATATTTGAAAGATTTAACACTTCCATCAAAGAAGAAAGAGA<br>TCAAAAGAGGTCCTCAAAAGAAAGATAAGGCTACTCAAACGGCACAA<br>ATTTCAGACGCAGAAACAAGAGCTACAGATATAACGGATAATGAGGA<br>CGGTAATGAAGATGAATCATCTGATTATGAAAGTCCGTCAGATATCGA<br>CGTTAGCGAGGATATGGACAGCGGTGAAATATCCGCAGATGAGCTTG<br>AGGAAGAAGAAGACGAAGAAGAAGACGAAGACGAAGAAGAGAAAG<br>AAGCTAGGCATACAAATTCACCAAGGAAAAGAGGCCGTAAGATAAAA<br>CTAGGTAAAGATGATATTGACGCTTCTGTACAACCTCCCCCCAAAAAA<br>AGAGGTCGTAAACCTAAAGATCCTAGTAAACCGCGTCAGATGCTATTG<br>ATATCTTCATGCCGTGCAAATAATACTCCTGTGATTAGGAAATTTACA<br>AAAAAGAATGTTGCTAGGGCGAAAAAGAAATATACCCCGTTTTCGAA<br>AAGATTTAAATCTATAGCTGCAATACCAGATTTAACTTCATTACCTGA<br>ATTTTACGGAAATTCTTCGGAATTGATGGCATCAAGGTTTGAAAACAA<br>ATTAAAAACAACCCAAAAGCATCAGATTGTAGAAACAATTTTTTCTAA<br>AGTCAAAAAACAGTTGAACTCTTCGTATGTCAAAGAAGAAATATTGA<br>AGTCTGCAAATTTCCAAGATTATTTACCGGCTAGGGAGAATGAATTCG<br>CCTCAATTTATTTAAGTGCATATAGTGCCATTGAGTCCGACTCCGCTAC<br>TACTATATACGTGGCTGGTACGCCTGGTGTAGGGAAAACTTTAACCGT<br>AAGGGAAGTCGTAAAGGAACTACTATCGTCTTCTGCACAACGAGAAA<br>TACCAGACTTTCTTTATGTGGAAATAAATGGATTGAAAATGGTAAAAC<br>CCACAGACTGTTACGAAACTTTATGGAACAAAGTGTCAGGAGAAAGG<br>TTAACATGGGCAGCTTCAATGGAGTCACTAGAGTTTTACTTTAAAAGA<br>GTTCCAAAAAATAAGAAGAAAACCATTGTAGTCTTGTTGGACGAACTC<br>GATGCCATGGTAACGAAATCTCAAGATATTATGTACAATTTTTTCAAT<br>TGGACTACTTACGAAAATGCCAAACTTATTGTCATTGCAGTAGCCAAT<br>ACAATGGACTTACCAGAACGTCAGCTAGGCAATAAGATTACTTCAAG<br>AATTGGGTTTACCAGAATTATGTTCACTGGGTATACGCACGAAGAGCT<br>AAAAAATATCATTGATTTAAGACTGAAGGGGTTGAACGACTCATTTTT<br>CTATGTTGATACAAAAACTGGCAATGCTATTTTGATTGATGCGGCTGG<br>AAACGACACTACAGTTAAGCAAACGTTGCCTGAAGACGTGAGGAAAG<br>TTCGCTTAAGAATGAGTGCTGATGCCATTGAAATAGCTTCGAGAAAAG<br>TAGCAAGTGTTAGTGGTGATGCAAGAAGAGCATTGAAGGTTTGTAAA<br>AGAGCAGCTGAAATTGCTGAAAAACACTATATGGCTAAGCATGGTTA<br>TGGATATGATGGAAAGACGGTTATTGAAGATGAAAATGAGGAGCAAA<br>TATACGATGATGAAGACAAGGATCTTATTGAAAGTAACAAAGCCAAA<br>GACGATAATGATGACGATGATGACAATGATGGGGTACAAACAGTTCA<br>CATCACGCACGTTATGAAAGCCTTAAACGAAACTTTAAATTCTCATGT<br>AATTACGTTTATGACGCGACTTTCATTTACAGCAAAACTGTTTATTTAT<br>GCATTATTAAACTTGATGAAAAAGAACGGATCTCAAGAGCAAGAACT<br>GGGCGATATTGTCGATGAAATCAAGTTACTTATTGAAGTAAATGGCAG<br>TAATAAGTTTGTCATGGAGATAGCCAAAACATTGTTCCAACAGGGAA<br>GTGATAATATTTCTGAACAATTGAGAATTATATCATGGGATTTCGTTCT<br>CAATCAGTTACTTGACGCGGGAATATTGTTTAAACAAACTATGAAGAA |

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|---|---|
| | CGATAGAATATGTTGTGTCAAGCTAAATATATCAGTAGAAGAAGCCA<br>AAAGAGCCATGAATGAGGATGAGACATTGAGAAATTTATAG |
| Orc1-ΔIDR (Δ236-345) | ATGGCAAAAACGTTGAAGGATTTACAGGGTTGGGAGATAATAACAAC<br>TGATGAGCAGGGAAATATAATCGATGGAGGTCAGAAGAGATTACGCC<br>GAAGAGGTGCAAAAACTGAACATTACTTAAAGAGAAGTTCTGATGGA<br>ATTAAACTAGGTCGTGGTGATAGTGTAGTCATGCACAACGAAGCCGCT<br>GGGACTTACTCCGTTTATATGATCCAGGAGTTGAGACTTAATACATTA<br>AATAATGTTGTCGAACTCTGGGCTCTCACCTATTTACGATGGTTTGAA<br>GTCAATCCTTTAGCTCATTATAGGCAGTTTAATCCTGACGCTAACATTT<br>TGAATCGTCCTTTAAATTATTACAATAAACTGTTTTCTGAAACTGCAA<br>ATAAAAATGAACTGTATCTCACTGCAGAATTAGCCGAATTGCAGCTAT<br>TTAACTTTATCAGGGTTGCCAACGTAATGGATGGAAGCAAATGGGAA<br>GTATTGAAAGGAAATGTCGATCCAGAAAGAGACTTTACAGTTCGTTAT<br>ATTTGTGAGCCGACTGGGGAGAAATTTGTGGACATTAATATTGAGGAT<br>GTCAAAGCTTACATAAAGAAAGTGGAGCCAAGGGAAGCCCAGGAATA<br>TTTGAAAGATTTAACACTTCCATCAAAGAAGAAAGAGATCAAAAGAG<br>GTCCTCAAAAGAAAGATAAGGCTACTCAAACGGCACAAATATCTTCA<br>TGCCGTGCAAATAATACTCCTGTGATTAGGAAATTTACAAAAAAGAAT<br>GTTGCTAGGGCGAAAAAGAAATATACCCCGTTTTCGAAAAGATTTAA<br>ATCTATAGCTGCAATACCAGATTTAACTTCATTACCTGAATTTTACGG<br>AAATTCTTCGGAATTGATGGCATCAAGGTTTGAAAACAAATTAAAAAC<br>AACCCAAAAGCATCAGATTGTAGAAACAATTTTTTCTAAAGTCAAAAA<br>ACAGTTGAACTCTTCGTATGTCAAAGAAGAAATATTGAAGTCTGCAAA<br>TTTCCAAGATTATTTACCGGCTAGGGAGAATGAATTCGCCTCAATTTA<br>TTTAAGTGCATATAGTGCCATTGAGTCCGACTCCGCTACTACTATATA<br>CGTGGCTGGTACGCCTGGTGTAGGGAAAACTTTAACCGTAAGGGAAG<br>TCGTAAAGGAACTACTATCGTCTTCTGCACAACGAGAAATACCAGACT<br>TTCTTTATGTGGAAATAAATGGATTGAAAATGGTAAAACCCACAGACT<br>GTTACGAAACTTTATGGAACAAAGTGTCAGGAGAAAGGTTAACATGG<br>GCAGCTTCAATGGAGTCACTAGAGTTTTACTTTAAAAGAGTTCCAAAA<br>AATAAGAAGAAAACCATTGTAGTCTTGTTGGACGAACTCGATGCCATG<br>GTAACGAAATCTCAAGATATTATGTACAATTTTTTCAATTGGACTACTT<br>ACGAAAATGCCAAACTTATTGTCATTGCAGTAGCCAATACAATGGACT<br>TACCAGAACGTCAGCTAGGCAATAAGATTACTTCAAGAATTGGGTTTA<br>CCAGAATTATGTTCACTGGGTATACGCACGAAGAGCTAAAAAATATC<br>ATTGATTTAAGACTGAAGGGGTTGAACGACTCATTTTTCTATGTTGAT<br>ACAAAAACTGGCAATGCTATTTTGATTGATGCGGCTGGAAACGACACT<br>ACAGTTAAGCAAACGTTGCCTGAAGACGTGAGGAAAGTTCGCTTAAG<br>AATGAGTGCTGATGCCATTGAAATAGCTTCGAGAAAAGTAGCAAGTG<br>TTAGTGGTGATGCAAGAAGAGCATTGAAGGTTTGTAAAAGAGCAGCT<br>GAAATTGCTGAAAAACACTATATGGCTAAGCATGGTTATGGATATGAT<br>GGAAAGACGGTTATTGAAGATGAAAATGAGGAGCAAATATACGATGA<br>TGAAGACAAGGATCTTATTGAAAGTAACAAAGCCAAAGACGATAATG<br>ATGACGATGATGACAATGATGGGGTACAAACAGTTCACATCACGCAC<br>GTTATGAAAGCCTTAAACGAAACTTTAAATTCTCATGTAATTACGTTT<br>ATGACGCGACTTTCATTTACAGCAAAACTGTTTATTTATGCATTATTAA<br>ACTTGATGAAAAAGAACGGATCTCAAGAGCAAGAACTGGGCGATATT<br>GTCGATGAAATCAAGTTACTTATTGAAGTAAATGGCAGTAATAAGTTT<br>GTCATGGAGATAGCCAAAACATTGTTCCAACAGGGAAGTGATAATAT<br>TTCTGAACAATTGAGAATTATATCATGGGATTTCGTTCTCAATCAGTTA<br>CTTGACGCGGGAATATTGTTTAAACAAACTATGAAGAACGATAGAAT<br>ATGTTGTGTCAAGCTAAATATATCAGTAGAAGAAGCCAAAAGAGCCA<br>TGAATGAGGATGAGACATTGAGAAATTTATAG |
| Orc1-Walker B (D569Y) | ATGGCAAAAACGTTGAAGGATTTACAGGGTTGGGAGATAATAACAAC<br>TGATGAGCAGGGAAATATAATCGATGGAGGTCAGAAGAGATTACGCC<br>GAAGAGGTGCAAAAACTGAACATTACTTAAAGAGAAGTTCTGATGGA |

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| | ATTAAACTAGGTCGTGGTGATAGTGTAGTCATGCACAACGAAGCCGCT<br>GGGACTTACTCCGTTTATATGATCCAGGAGTTGAGACTTAATACATTA<br>AATAATGTTGTCGAACTCTGGGCTCTCACCTATTTACGATGGTTTGAA<br>GTCAATCCTTTAGCTCATTATAGGCAGTTTAATCCTGACGCTAACATTT<br>TGAATCGTCCTTTAAATTATTACAATAAACTGTTTTCTGAAACTGCAA<br>ATAAAAATGAACTGTATCTCACTGCAGAATTAGCCGAATTGCAGCTAT<br>TTAACTTTATCAGGGTTGCCAACGTAATGGATGGAAGCAAATGGGAA<br>GTATTGAAAGGAAATGTCGATCCAGAAAGAGACTTTACAGTTCGTTAT<br>ATTTGTGAGCCGACTGGGGAGAAATTTGTGGACATTAATATTGAGGAT<br>GTCAAAGCTTACATAAAGAAAGTGGAGCCAAGGGAAGCCCAGGAATA<br>TTTGAAAGATTTAACACTTCCATCAAAGAAGAAAGAGATCAAAAGAG<br>GTCCTCAAAAGAAAGATAAGGCTACTCAAACGGCACAAATTTCAGAC<br>GCAGAAACAAGAGCTACAGATATAACGGATAATGAGGACGGTAATGA<br>AGATGAATCATCTGATTATGAAAGTCCGTCAGATATCGACGTTAGCGA<br>GGATATGGACAGCGGTGAAATATCCGCAGATGAGCTTGAGGAAGAAG<br>AAGACGAAGAAGAAGACGAAGACGAAGAAGAGAAAGAAGCTAGGCA<br>TACAAATTCACCAAGGAAAAGAGGCCGTAAGATAAAACTAGGTAAAG<br>ATGATATTGACGCTTCTGTACAACCTCCCCCCAAAAAAAGAGGTCGTA<br>AACCTAAAGATCCTAGTAAACCGCGTCAGATGCTATTGATATCTTCAT<br>GCCGTGCAAATAATACTCCTGTGATTAGGAAATTTACAAAAAAGAAT<br>GTTGCTAGGGCGAAAAAGAAATATACCCCGTTTTCGAAAAGATTTAA<br>ATCTATAGCTGCAATACCAGATTTAACTTCATTACCTGAATTTTACGG<br>AAATTCTTCGGAATTGATGGCATCAAGGTTTGAAAACAAATTAAAAAC<br>AACCCAAAAGCATCAGATTGTAGAAACAATTTTTTCTAAAGTCAAAAA<br>ACAGTTGAACTCTTCGTATGTCAAAGAAGAAATATTGAAGTCTGCAAA<br>TTTCCAAGATTATTTACCGGCTAGGGAGAATGAATTCGCCTCAATTTA<br>TTTAAGTGCATATAGTGCCATTGAGTCCGACTCCGCTACTACTATATA<br>CGTGGCTGGTACGCCTGGTGTAGGGAAAACTTTAACCGTAAGGGAAG<br>TCGTAAAGGAACTACTATCGTCTTCTGCACAACGAGAAATACCAGACT<br>TTCTTTATGTGGAAATAAATGGATTGAAAATGGTAAAACCCACAGACT<br>GTTACGAAACTTTATGGAACAAAGTGTCAGGAGAAAGGTTAACATGG<br>GCAGCTTCAATGGAGTCACTAGAGTTTTACTTTAAAAGAGTTCCAAAA<br>AATAAGAAGAAAACCATTGTAGTCTTGTTGGACGAACTCTATGCCATG<br>GTAACGAAATCTCAAGATATTATGTACAATTTTTTCAATTGGACTACTT<br>ACGAAAATGCCAAACTTATTGTCATTGCAGTAGCCAATACAATGGACT<br>TACCAGAACGTCAGCTAGGCAATAAGATTACTTCAAGAATTGGGTTTA<br>CCAGAATTATGTTCACTGGGTATACGCACGAAGAGCTAAAAAATATC<br>ATTGATTTAAGACTGAAGGGGTTGAACGACTCATTTTTCTATGTTGAT<br>ACAAAAACTGGCAATGCTATTTTGATTGATGCGGCTGGAAACGACACT<br>ACAGTTAAGCAAACGTTGCCTGAAGACGTGAGGAAAGTTCGCTTAAG<br>AATGAGTGCTGATGCCATTGAAATAGCTTCGAGAAAAGTAGCAAGTG<br>TTAGTGGTGATGCAAGAAGAGCATTGAAGGTTTGTAAAAGAGCAGCT<br>GAAATTGCTGAAAAACACTATATGGCTAAGCATGGTTATGGATATGAT<br>GGAAAGACGGTTATTGAAGATGAAAATGAGGAGCAAATATACGATGA<br>TGAAGACAAGGATCTTATTGAAAGTAACAAAGCCAAAGACGATAATG<br>ATGACGATGATGACAATGATGGGGTACAAACAGTTCACATCACGCAC<br>GTTATGAAAGCCTTAAACGAAACTTTAAATTCTCATGTAATTACGTTT<br>ATGACGCGACTTTCATTTACAGCAAAACTGTTTATTTATGCATTATTAA<br>ACTTGATGAAAAAGAACGGATCTCAAGAGCAAGAACTGGGCGATATT<br>GTCGATGAAATCAAGTTACTTATTGAAGTAAATGGCAGTAATAAGTTT<br>GTCATGGAGATAGCCAAAACATTGTTCCAACAGGGAAGTGATAATAT<br>TTCTGAACAATTGAGAATTATATCATGGGATTTCGTTCTCAATCAGTTA<br>CTTGACGCGGGAATATTGTTTAAACAAACTATGAAGAACGATAGAAT<br>ATGTTGTGTCAAGCTAAATATATCAGTAGAAGAAGCCAAAAGAGCCA<br>TGAATGAGGATGAGACATTGAGAAATTTATAG |

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| Orc1-ΔBAH-ΔIDR (Δ48-188, Δ236-345) | ATGGCAAAAACGTTGAAGGATTTACAGGGTTGGGAGATAATAACAAC<br>TGATGAGCAGGGAAATATAATCGATGGAGGTCAGAAGAGATTACGCC<br>GAAGAGGTGCAAAAACTGAACATTACTTAAAGAGAAGTTCTGATGGA<br>ATTGAGGATGTCAAAGCTTACATAAAGAAAGTGGAGCCAAGGGAAGC<br>CCAGGAATATTTGAAAGATTTAACACTTCCATCAAAGAAGAAAGAGA<br>TCAAAAGAGGTCCTCAAAAGAAAGATAAGGCTACTCAAACGGCACAA<br>ATATCTTCATGCCGTGCAAATAATACTCCTGTGATTAGGAAATTTACA<br>AAAAAGAATGTTGCTAGGGCGAAAAAGAAATATACCCCGTTTTCGAA<br>AAGATTTAAATCTATAGCTGCAATACCAGATTTAACTTCATTACCTGA<br>ATTTTACGGAAATTCTTCGGAATTGATGGCATCAAGGTTTGAAAACAA<br>ATTAAAAACAACCCAAAAGCATCAGATTGTAGAAACAATTTTTTCTAA<br>AGTCAAAAAACAGTTGAACTCTTCGTATGTCAAAGAAGAAATATTGA<br>AGTCTGCAAATTTCCAAGATTATTTACCGGCTAGGGAGAATGAATTCG<br>CCTCAATTTATTTAAGTGCATATAGTGCCATTGAGTCCGACTCCGCTAC<br>TACTATATACGTGGCTGGTACGCCTGGTGTAGGGAAAACTTTAACCGT<br>AAGGGAAGTCGTAAAGGAACTACTATCGTCTTCTGCACAACGAGAAA<br>TACCAGACTTTCTTTATGTGGAAATAAATGGATTGAAAATGGTAAAAC<br>CCACAGACTGTTACGAAACTTTATGGAACAAAGTGTCAGGAGAAAGG<br>TTAACATGGGCAGCTTCAATGGAGTCACTAGAGTTTTACTTTAAAAGA<br>GTTCCAAAAAATAAGAAGAAAACCATTGTAGTCTTGTTGGACGAACTC<br>GATGCCATGGTAACGAAATCTCAAGATATTATGTACAATTTTTTCAAT<br>TGGACTACTTACGAAAATGCCAAACTTATTGTCATTGCAGTAGCCAAT<br>ACAATGGACTTACCAGAACGTCAGCTAGGCAATAAGATTACTTCAAG<br>AATTGGGTTTACCAGAATTATGTTCACTGGGTATACGCACGAAGAGCT<br>AAAAAATATCATTGATTTAAGACTGAAGGGGTTGAACGACTCATTTTT<br>CTATGTTGATACAAAAACTGGCAATGCTATTTTGATTGATGCGGCTGG<br>AAACGACACTACAGTTAAGCAAACGTTGCCTGAAGACGTGAGGAAAG<br>TTCGCTTAAGAATGAGTGCTGATGCCATTGAAATAGCTTCGAGAAAAG<br>TAGCAAGTGTTAGTGGTGATGCAAGAAGAGCATTGAAGGTTTGTAAA<br>AGAGCAGCTGAAATTGCTGAAAAACACTATATGGCTAAGCATGGTTA<br>TGGATATGATGGAAAGACGGTTATTGAAGATGAAAATGAGGAGCAAA<br>TATACGATGATGAAGACAAGGATCTTATTGAAAGTAACAAAGCCAAA<br>GACGATAATGATGACGATGATGACAATGATGGGGTACAAACAGTTCA<br>CATCACGCACGTTATGAAAGCCTTAAACGAAACTTTAAATTCTCATGT<br>AATTACGTTTATGACGCGACTTTCATTTACAGCAAAACTGTTTATTTAT<br>GCATTATTAAACTTGATGAAAAAGAACGGATCTCAAGAGCAAGAACT<br>GGGCGATATTGTCGATGAAATCAAGTTACTTATTGAAGTAAATGGCAG<br>TAATAAGTTTGTCATGGAGATAGCCAAAACATTGTTCCAACAGGGAA<br>GTGATAATATTTCTGAACAATTGAGAATTATATCATGGGATTTCGTTCT<br>CAATCAGTTACTTGACGCGGGAATATTGTTTAAACAAACTATGAAGAA<br>CGATAGAATATGTTGTGTCAAGCTAAATATATCAGTAGAAGAAGCCA<br>AAAGAGCCATGAATGAGGATGAGACATTGAGAAATTTATAG |
| Codon-optimized Orc1-BAH (Δ48-188) | GGCGCGCCTATAAAACAATGAAGAGAAGATGGAAGAAGAACTTCATT<br>GCTGTTTCTGCTGCTAACAGATTCAAGAAGATTTCTTCTTCTGGTGCTT<br>TGGAAAACTTGTACTTCCAAGGTGAAATGGCTAAGACTTTGAAGGACT<br>TGCAAGGTTGGGAAATTATTACTACTGACGAACAAGGTAACATTATTG<br>ACGGTGGTCAAAAGAGATTGAGAAGAAGAGGTGCTAAGACTGAACAC<br>TACTTGAAGAGATCTTCTGACGGTATTGAAGACGTTAAGGCTTACATT<br>AAGAAGGTTGAACCAAGAGAAGCTCAAGAATACTTGAAGGACTTGAC<br>TTTGCCATCTAAGAAGAAGGAAATTAAGAGAGGTCCACAAAAGAAGG<br>ACAAGGCTACTCAAACTGCTCAAATTTCTGACGCTGAAACTAGAGCTA<br>CTGACATTACTGACAACGAAGACGGTAACGAAGACGAATCTTCTGAC<br>TACGAATCTCCATCTGACATTGACGTTTCTGAAGACATGGACTCTGGT<br>GAAATTTCTGCTGACGAATTGGAAGAAGAAGAAGACGAAGAAGAAG<br>ACGAAGACGAAGAAGAAAAGGAAGCTAGACACACTAACTCTCCAAG<br>AAAGAGAGGTAGAAAGATTAAGTTGGGTAAGGACGACATTGACGCTT |

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| | CTGTTCAACCACCACCAAAGAAGAGAGGTAGAAAGCCAAAGGACCCA<br>TCTAAGCCAAGACAAATGTTGTTGATTTCTTCTTGTAGAGCTAACAAC<br>ACTCCAGTTATTAGAAAGTTCACTAAGAAGAACGTTGCTAGAGCTAAG<br>AAGAAGTACACTCCATTCTCTAAGAGATTCAAGTCTATTGCTGCTATT<br>CCAGACTTGACTTCTTTGCCAGAATTCTACGGTAACTCTTCTGAATTGA<br>TGGCTTCTAGATTCGAAAACAAGTTGAAGACTACTCAAAAGCACCAA<br>ATTGTTGAAACTATTTTCTCTAAGGTTAAGAAGCAATTGAACTCTTCTT<br>ACGTTAAGGAAGAAATTTTGAAGTCTGCTAACTTCCAAGACTACTTGC<br>CAGCTAGAGAAAACGAATTCGCTTCTATTTACTTGTCTGCTTACTCTGC<br>TATTGAATCTGACTCTGCTACTACTATTTACGTTGCTGGTACTCCAGGT<br>GTTGGTAAGACTTTGACTGTTAGAGAAGTTGTTAAGGAATTGTTGTCT<br>TCTTCTGCTCAAAGAGAAATTCCAGACTTCTTGTACGTTGAAATTAAC<br>GGTTTGAAGATGGTTAAGCCAACTGACTGTTACGAAACTTTGTGGAAC<br>AAGGTTTCTGGTGAAAGATTGACTTGGGCTGCTTCTATGGAATCTTTG<br>GAATTCTACTTCAAGAGAGTTCCAAAGAACAAGAAGAAGACTATTGT<br>TGTTTTGTTGGACGAATTGGACGCTATGGTTACTAAGTCTCAAGACAT<br>TATGTACAACTTCTTCAACTGGACTACTTACGAAAACGCTAAGTTGAT<br>TGTTATTGCTGTTGCTAACACTATGGACTTGCCAGAAAGACAATTGGG<br>TAACAAGATTACTTCTAGAATTGGTTTCACTAGAATTATGTTCACTGGT<br>TACACTCACGAAGAATTGAAGAACATTATTGACTTGAGATTGAAGGGT<br>TTGAACGACTCTTTCTTCTACGTTGACACTAAGACTGGTAACGCTATTT<br>TGATTGACGCTGCTGGTAACGACACTACTGTTAAGCAAACTTTGCCAG<br>AAGACGTTAGAAAGGTTAGATTGAGAATGTCTGCTGACGCTATTGAA<br>ATTGCTTCTAGAAAGGTTGCTTCTGTTTCTGGTGACGCTAGAAGAGCT<br>TTGAAGGTTTGTAAGAGAGCTGCTGAAATTGCTGAAAAGCACTACATG<br>GCTAAGCACGGTTACGGTTACGACGGTAAGACTGTTATTGAAGACGA<br>AAACGAAGAACAAATTTACGACGACGAAGACAAGGACTTGATTGAAT<br>CTAACAAGGCTAAGGACGACAACGACGACGACGACGACAACGACGGT<br>GTTCAAACTGTTCACATTACTCACGTTATGAAGGCTTTGAACGAAACT<br>TTGAACTCTCACGTTATTACTTTCATGACTAGATTGTCTTTCACTGCTA<br>AGTTGTTCATTTACGCTTTGTTGAACTTGATGAAGAAGAACGGTTCTC<br>AAGAACAAGAATTGGGTGACATTGTTGACGAAATTAAGTTGTTGATTG<br>AAGTTAACGGTTCTAACAAGTTCGTTATGGAAATTGCTAAGACTTTGT<br>TCCAACAAGGTTCTGACAACATTTCTGAACAATTGAGAATTATTTCTT<br>GGGACTTCGTTTTGAACCAATTGTTGGACGCTGGTATTTTGTTCAAGC<br>AAACTATGAAGAACGACAGAATTTGTTGTGTTAAGTTGAACATTTCTG<br>TTGAAGAAGCTAAGAGAGCTATGAACGAAGACGAAACTTTGAGAAAC<br>TTGTAAATTGAATTGAATTGAAATCGATAGATCAATTTTTTTCTTTTCT<br>CTTTCCCCATCCTTTACGCTAAAATAATAGTTTATTTTATTTTTTGAAT<br>ATTTTTTATTTATATACGTATATATAGACTATTATTTATCTTTTAATGAT<br>TATTAAGATTTTTATTAAAAAAAAATTCGCTCCTCTTTTAATGCCTTTA<br>TGCCCCGGGCTGCAGGAATTCGATATCAAGCTTATCGATACCGTCGAC<br>CTCGAG |
| Codon-optimized Orc1-IDR (Δ236-345) | GGCGCGCCTATAAAACAATGAAGAGAAGATGGAAGAAGAACTTCATT<br>GCTGTTTCTGCTGCTAACAGATTCAAGAAGATTTCTTCTTCTGGTGCTT<br>TGGAAAACTTGTACTTCCAAGGTGAAATGGCTAAGACTTTGAAGGACT<br>TGCAAGGTTGGGAAATTATTACTACTGACGAACAAGGTAACATTATTG<br>ACGGTGGTCAAAAGAGATTGAGAAGAAGAGGTGCTAAGACTGAACAC<br>TACTTGAAGAGATCTTCTGACGGTATTAAGTTGGGTAGAGGTGACTCT<br>GTTGTTATGCACAACGAAGCTGCTGGTACTTACTCTGTTTACATGATTC<br>AAGAATTGAGATTGAACACTTTGAACAACGTTGTTGAATTGTGGGCTT<br>TGACTTACTTGAGATGGTTCGAAGTTAACCCATTGGCTCACTACAGAC<br>AATTCAACCCAGACGCTAACATTTTGAACAGACCATTGAACTACTACA<br>ACAAGTTGTTCTCTGAAACTGCTAACAAGAACGAATTGTACTTGACTG<br>CTGAATTGGCTGAATTGCAATTGTTCAACTTCATTAGAGTTGCTAACG<br>TTATGGACGGTTCTAAGTGGGAAGTTTTGAAGGGTAACGTTGACCCAG |

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| | AAAGAGACTTCACTGTTAGATACATTTGTGAACCAACTGGTGAAAAGT<br>TCGTTGACATTAACATTGAAGACGTTAAGGCTTACATTAAGAAGGTTG<br>AACCAAGAGAAGCTCAAGAATACTTGAAGGACTTGACTTTGCCATCTA<br>AGAAGAAGGAAATTAAGAGAGGTCCACAAAAGAAGGACAAGGCTAC<br>TCAAACTGCTCAAATTTCTTCTTGTAGAGCTAACAACACTCCAGTTATT<br>AGAAAGTTCACTAAGAAGAACGTTGCTAGAGCTAAGAAGAAGTACAC<br>TCCATTCTCTAAGAGATTCAAGTCTATTGCTGCTATTCCAGACTTGACT<br>TCTTTGCCAGAATTCTACGGTAACTCTTCTGAATTGATGGCTTCTAGAT<br>TCGAAAACAAGTTGAAGACTACTCAAAAGCACCAAATTGTTGAAACT<br>ATTTTCTCTAAGGTTAAGAAGCAATTGAACTCTTCTTACGTTAAGGAA<br>GAAATTTTGAAGTCTGCTAACTTCCAAGACTACTTGCCAGCTAGAGAA<br>AACGAATTCGCTTCTATTTACTTGTCTGCTTACTCTGCTATTGAATCTG<br>ACTCTGCTACTACTATTTACGTTGCTGGTACTCCAGGTGTTGGTAAGAC<br>TTTGACTGTTAGAGAAGTTGTTAAGGAATTGTTGTCTTCTTCTGCTCAA<br>AGAGAAATTCCAGACTTCTTGTACGTTGAAATTAACGGTTTGAAGATG<br>GTTAAGCCAACTGACTGTTACGAAACTTTGTGGAACAAGGTTTCTGGT<br>GAAAGATTGACTTGGGCTGCTTCTATGGAATCTTTGGAATTCTACTTC<br>AAGAGAGTTCCAAAGAACAAGAAGAAGACTATTGTTGTTTTGTTGGA<br>CGAATTGGACGCTATGGTTACTAAGTCTCAAGACATTATGTACAACTT<br>CTTCAACTGGACTACTTACGAAAACGCTAAGTTGATTGTTATTGCTGTT<br>GCTAACACTATGGACTTGCCAGAAAGACAATTGGGTAACAAGATTAC<br>TTCTAGAATTGGTTTCACTAGAATTATGTTCACTGGTTACACTCACGAA<br>GAATTGAAGAACATTATTGACTTGAGATTGAAGGGTTTGAACGACTCT<br>TTCTTCTACGTTGACACTAAGACTGGTAACGCTATTTTGATTGACGCTG<br>CTGGTAACGACACTACTGTTAAGCAAACTTTGCCAGAAGACGTTAGAA<br>AGGTTAGATTGAGAATGTCTGCTGACGCTATTGAAATTGCTTCTAGAA<br>AGGTTGCTTCTGTTTCTGGTGACGCTAGAAGAGCTTTGAAGGTTTGTA<br>AGAGAGCTGCTGAAATTGCTGAAAAGCACTACATGGCTAAGCACGGT<br>TACGGTTACGACGGTAAGACTGTTATTGAAGACGAAAACGAAGAACA<br>AATTTACGACGACGAAGACAAGGACTTGATTGAATCTAACAAGGCTA<br>AGGACGACAACGACGACGACGACGACAACGACGGTGTTCAAACTGTT<br>CACATTACTCACGTTATGAAGGCTTTGAACGAAACTTTGAACTCTCAC<br>GTTATTACTTTCATGACTAGATTGTCTTTCACTGCTAAGTTGTTCATTT<br>ACGCTTTGTTGAACTTGATGAAGAAGAACGGTTCTCAAGAACAAGAA<br>TTGGGTGACATTGTTGACGAAATTAAGTTGTTGATTGAAGTTAACGGT<br>TCTAACAAGTTCGTTATGGAAATTGCTAAGACTTTGTTCCAACAAGGT<br>TCTGACAACATTTCTGAACAATTGAGAATTATTTCTTGGGACTTCGTTT<br>TGAACCAATTGTTGGACGCTGGTATTTTGTTCAAGCAAACTATGAAGA<br>ACGACAGAATTTGTTGTGTTAAGTTGAACATTTCTGTTGAAGAAGCTA<br>AGAGAGCTATGAACGAAGACGAAACTTTGAGAAACTTGTAAATTGAA<br>TTGAATTGAAATCGATAGATCAATTTTTTTCTTTTCTCTTTCCCCATCCT<br>TTACGCTAAAATAATAGTTTATTTTATTTTTTGAATATTTTTTATTTATA<br>TACGTATATATAGACTATTATTTATCTTTTAATGATTATTAAGATTTTT<br>ATTAAAAAAAAAATTCGCTCCTCTTTTAATGCCTTTATGCCCCGGGCTGC<br>AGGAATTCGATATCAAGCTTATCGATACCGTCGACCTCGAG |
| Codon-optimized Orc1-Walker B (D569Y) | GCGCGCCTATAAAACAATGAAGAGAAGATGGAAGAAGAACTTCATTG<br>CTGTTTCTGCTGCTAACAGATTCAAGAAGATTTCTTCTTCTGGTGCTTT<br>GGAAAACTTGTACTTCCAAGGTGAAATGGCTAAGACTTTGAAGGACTT<br>GCAAGGTTGGGAAATTATTACTACTGACGAACAAGGTAACATTATTGA<br>CGGTGGTCAAAAGAGATTGAGAAGAAGAGGTGCTAAGACTGAACACT<br>ACTTGAAGAGATCTTCTGACGGTATTAAGTTGGGTAGAGGTGACTCTG<br>TTGTTATGCACAACGAAGCTGCTGGTACTTACTCTGTTTACATGATTCA<br>AGAATTGAGATTGAACACTTTGAACAACGTTGTTGAATTGTGGGCTTT<br>GACTTACTTGAGATGGTTCGAAGTTAACCCATTGGCTCACTACAGACA<br>ATTCAACCCAGACGCTAACATTTTGAACAGACCATTGAACTACTACAA<br>CAAGTTGTTCTCTGAAACTGCTAACAAGAACGAATTGTACTTGACTGC |

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| | TGAATTGGCTGAATTGCAATTGTTCAACTTCATTAGAGTTGCTAACGTT<br>ATGGACGGTTCTAAGTGGGAAGTTTTGAAGGGTAACGTTGACCCAGA<br>AAGAGACTTCACTGTTAGATACATTTGTGAACCAACTGGTGAAAAGTT<br>CGTTGACATTAACATTGAAGACGTTAAGGCTTACATTAAGAAGGTTGA<br>ACCAAGAGAAGCTCAAGAATACTTGAAGGACTTGACTTTGCCATCTAA<br>GAAGAAGGAAATTAAGAGAGGTCCACAAAAGAAGGACAAGGCTACT<br>CAAACTGCTCAAATTTCTGACGCTGAAACTAGAGCTACTGACATTACT<br>GACAACGAAGACGGTAACGAAGACGAATCTTCTGACTACGAATCTCC<br>ATCTGACATTGACGTTTCTGAAGACATGGACTCTGGTGAAATTTCTGC<br>TGACGAATTGGAAGAAGAAGAAGACGAAGAAGAAGACGAAGACGAA<br>GAAGAAAAGGAAGCTAGACACACTAACTCTCCAAGAAAGAGAGGTA<br>GAAAGATTAAGTTGGGTAAGGACGACATTGACGCTTCTGTTCAACCAC<br>CACCAAAGAAGAGAGGTAGAAAGCCAAAGGACCCATCTAAGCCAAG<br>ACAAATGTTGTTGATTTCTTCTTGTAGAGCTAACAACACTCCAGTTATT<br>AGAAAGTTCACTAAGAAGAACGTTGCTAGAGCTAAGAAGAAGTACAC<br>TCCATTCTCTAAGAGATTCAAGTCTATTGCTGCTATTCCAGACTTGACT<br>TCTTTGCCAGAATTCTACGGTAACTCTTCTGAATTGATGGCTTCTAGAT<br>TCGAAAACAAGTTGAAGACTACTCAAAAGCACCAAATTGTTGAAACT<br>ATTTTCTCTAAGGTTAAGAAGCAATTGAACTCTTCTTACGTTAAGGAA<br>GAAATTTTGAAGTCTGCTAACTTCCAAGACTACTTGCCAGCTAGAGAA<br>AACGAATTCGCTTCTATTTACTTGTCTGCTTACTCTGCTATTGAATCTG<br>ACTCTGCTACTACTATTTACGTTGCTGGTACTCCAGGTGTTGGTAAGAC<br>TTTGACTGTTAGAGAAGTTGTTAAGGAATTGTTGTCTTCTTCTGCTCAA<br>AGAGAAATTCCAGACTTCTTGTACGTTGAAATTAACGGTTTGAAGATG<br>GTTAAGCCAACTGACTGTTACGAAACTTTGTGGAACAAGGTTTCTGGT<br>GAAAGATTGACTTGGGCTGCTTCTATGGAATCTTTGGAATTCTACTTC<br>AAGAGAGTTCCAAAGAACAAGAAGAAGACTATTGTTGTTTTGTTGGA<br>CGAATTGTATGCTATGGTTACTAAGTCTCAAGACATTATGTACAACTT<br>CTTCAACTGGACTACTTACGAAAACGCTAAGTTGATTGTTATTGCTGTT<br>GCTAACACTATGGACTTGCCAGAAAGACAATTGGGTAACAAGATTAC<br>TTCTAGAATTGGTTTCACTAGAATTATGTTCACTGGTTACACTCACGAA<br>GAATTGAAGAACATTATTGACTTGAGATTGAAGGGTTTGAACGACTCT<br>TTCTTCTACGTTGACACTAAGACTGGTAACGCTATTTTGATTGACGCTG<br>CTGGTAACGACACTACTGTTAAGCAAACTTTGCCAGAAGACGTTAGAA<br>AGGTTAGATTGAGAATGTCTGCTGACGCTATTGAAATTGCTTCTAGAA<br>AGGTTGCTTCTGTTTCTGGTGACGCTAGAAGAGCTTTGAAGGTTTGTA<br>AGAGAGCTGCTGAAATTGCTGAAAAGCACTACATGGCTAAGCACGGT<br>TACGGTTACGACGGTAAGACTGTTATTGAAGACGAAAACGAAGAACA<br>AATTTACGACGACGAAGACAAGGACTTGATTGAATCTAACAAGGCTA<br>AGGACGACAACGACGACGACGACGACAACGACGGTGTTCAAACTGTT<br>CACATTACTCACGTTATGAAGGCTTTGAACGAAACTTTGAACTCTCAC<br>GTTATTACTTTCATGACTAGATTGTCTTTCACTGCTAAGTTGTTCATTT<br>ACGCTTTGTTGAACTTGATGAAGAAGAACGGTTCTCAAGAACAAGAA<br>TTGGGTGACATTGTTGACGAAATTAAGTTGTTGATTGAAGTTAACGGT<br>TCTAACAAGTTCGTTATGGAAATTGCTAAGACTTTGTTCCAACAAGGT<br>TCTGACAACATTTCTGAACAATTGAGAATTATTTCTTGGGACTTCGTTT<br>TGAACCAATTGTTGGACGCTGGTATTTTGTTCAAGCAAACTATGAAGA<br>ACGACAGAATTTGTTGTGTTAAGTTGAACATTTCTGTTGAAGAAGCTA<br>AGAGAGCTATGAACGAAGACGAAACTTTGAGAAACTTGTAAATTGAA<br>TTGAATTGAAATCGATAGATCAATTTTTTTCTTTTCTCTTTCCCCATCCT<br>TTACGCTAAAATAATAGTTTATTTTATTTTTTGAATATTTTTTATTTATA<br>TACGTATATATAGACTATTATTTATCTTTTAATGATTATTAAGATTTTT<br>ATTAAAAAAAAATTCGCTCCTCTTTTAATGCCTTTATGCCTCGAG |
| Codon-optimized Orc1-BAH-IDR (Δ48-188, Δ236-345) | GGCGCGCCTATAAAACAATGAAGAGAAGATGGAAGAAGAACTTCATT<br>GCTGTTTCTGCTGCTAACAGATTCAAGAAGATTTCTTCTTCTGGTGCTT<br>TGGAAAACTTGTACTTCCAAGGTGAAATGGCTAAGACTTTGAAGGACT |

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| | TGCAAGGTTGGGAAATTATTACTACTGACGAACAAGGTAACATTATTG<br>ACGGTGGTCAAAAGAGATTGAGAAGAAGAGGTGCTAAGACTGAACAC<br>TACTTGAAGAGATCTTCTGACGGTATTGAAGACGTTAAGGCTTACATT<br>AAGAAGGTTGAACCAAGAGAAGCTCAAGAATACTTGAAGGACTTGAC<br>TTTGCCATCTAAGAAGAAGGAAATTAAGAGAGGTCCACAAAAGAAGG<br>ACAAGGCTACTCAAACTGCTCAAATTTCTTCTTGTAGAGCTAACAACA<br>CTCCAGTTATTAGAAAGTTCACTAAGAAGAACGTTGCTAGAGCTAAGA<br>AGAAGTACACTCCATTCTCTAAGAGATTCAAGTCTATTGCTGCTATTC<br>CAGACTTGACTTCTTTGCCAGAATTCTACGGTAACTCTTCTGAATTGAT<br>GGCTTCTAGATTCGAAAACAAGTTGAAGACTACTCAAAAGCACCAAA<br>TTGTTGAAACTATTTTCTCTAAGGTTAAGAAGCAATTGAACTCTTCTTA<br>CGTTAAGGAAGAAATTTTGAAGTCTGCTAACTTCCAAGACTACTTGCC<br>AGCTAGAGAAAACGAATTCGCTTCTATTTACTTGTCTGCTTACTCTGCT<br>ATTGAATCTGACTCTGCTACTACTATTTACGTTGCTGGTACTCCAGGTG<br>TTGGTAAGACTTTGACTGTTAGAGAAGTTGTTAAGGAATTGTTGTCTT<br>CTTCTGCTCAAAGAGAAATTCCAGACTTCTTGTACGTTGAAATTAACG<br>GTTTGAAGATGGTTAAGCCAACTGACTGTTACGAAACTTTGTGGAACA<br>AGGTTTCTGGTGAAAGATTGACTTGGGCTGCTTCTATGGAATCTTTGG<br>AATTCTACTTCAAGAGAGTTCCAAAGAACAAGAAGAAGACTATTGTT<br>GTTTTGTTGGACGAATTGGACGCTATGGTTACTAAGTCTCAAGACATT<br>ATGTACAACTTCTTCAACTGGACTACTTACGAAAACGCTAAGTTGATT<br>GTTATTGCTGTTGCTAACACTATGGACTTGCCAGAAAGACAATTGGGT<br>AACAAGATTACTTCTAGAATTGGTTTCACTAGAATTATGTTCACTGGTT<br>ACACTCACGAAGAATTGAAGAACATTATTGACTTGAGATTGAAGGGTT<br>TGAACGACTCTTTCTTCTACGTTGACACTAAGACTGGTAACGCTATTTT<br>GATTGACGCTGCTGGTAACGACACTACTGTTAAGCAAACTTTGCCAGA<br>AGACGTTAGAAAGGTTAGATTGAGAATGTCTGCTGACGCTATTGAAAT<br>TGCTTCTAGAAAGGTTGCTTCTGTTTCTGGTGACGCTAGAAGAGCTTT<br>GAAGGTTTGTAAGAGAGCTGCTGAAATTGCTGAAAAGCACTACATGG<br>CTAAGCACGGTTACGGTTACGACGGTAAGACTGTTATTGAAGACGAA<br>AACGAAGAACAAATTTACGACGACGAAGACAAGGACTTGATTGAATC<br>TAACAAGGCTAAGGACGACAACGACGACGACGACGACAACGACGGT<br>GTTCAAACTGTTCACATTACTCACGTTATGAAGGCTTTGAACGAAACT<br>TTGAACTCTCACGTTATTACTTTCATGACTAGATTGTCTTTCACTGCTA<br>AGTTGTTCATTTACGCTTTGTTGAACTTGATGAAGAAGAACGGTTCTC<br>AAGAACAAGAATTGGGTGACATTGTTGACGAAATTAAGTTGTTGATTG<br>AAGTTAACGGTTCTAACAAGTTCGTTATGGAAATTGCTAAGACTTTGT<br>TCCAACAAGGTTCTGACAACATTTCTGAACAATTGAGAATTATTTCTT<br>GGGACTTCGTTTTGAACCAATTGTTGGACGCTGGTATTTTGTTCAAGC<br>AAACTATGAAGAACGACAGAATTTGTTGTGTTAAGTTGAACATTTCTG<br>TTGAAGAAGCTAAGAGAGCTATGAACGAAGACGAAACTTTGAGAAAC<br>TTGTAAATTGAATTGAATTGAAATCGATAGATCAATTTTTTTCTTTTCT<br>CTTTCCCCATCCTTTACGCTAAAATAATAGTTTATTTTATTTTTTGAAT<br>ATTTTTTATTTATATACGTATATATAGACTATTATTTATCTTTTAATGAT<br>TATTAAGATTTTTATTAAAAAAAAATTCGCTCCTCTTTTAATGCCTTTA<br>TGCCCCGGGCTGCAGGAATTCGATATCAAGCTTATCGATACCGTCGAC<br>CTCGAG |