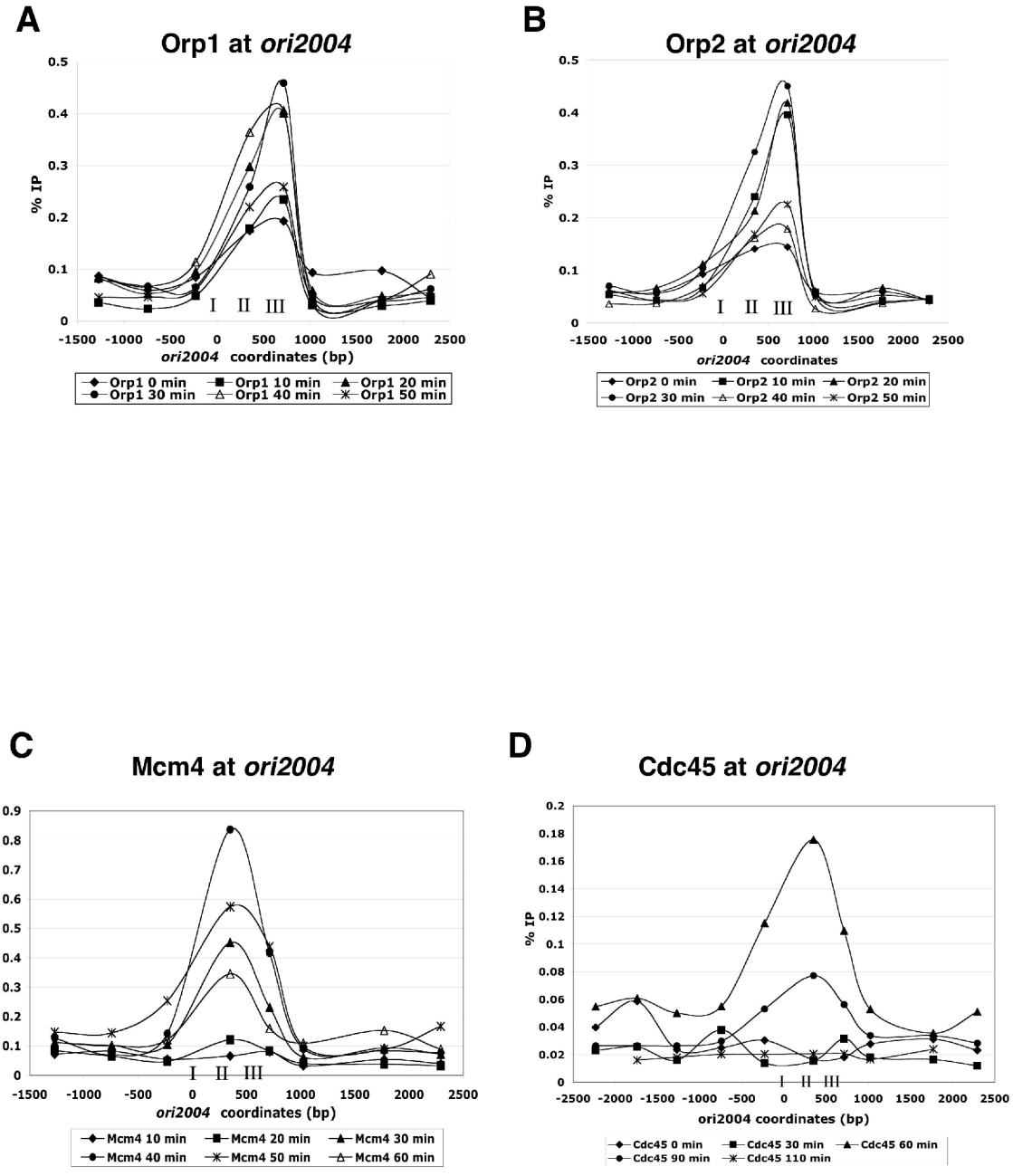
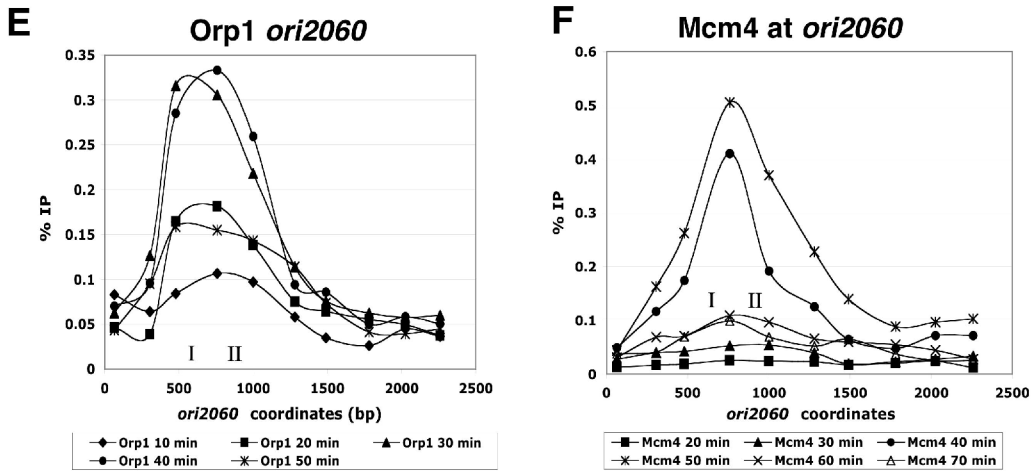
Supplementary Table 1. *Schizosaccharomyces pombe* strains used in this study

Strain	Genotype	Source
PN143	*h- cdc25-22*	Our stock
PN1840	*h- cdc25-22 Orp1-HA*	Grallert and Nurse, 1996
YRC16	*h- cdc25-22 leu1-32 ura4-D18 ade6-M210 orp4::3HA[ura4+]*	Chuang and Kelly, 1999
EOY68	*h+ cdc25-22 ori2004ΔI*	Takahashi et al., 2003
EOY72	*h+ cdc25-22 ori2004ΔIII*	Takahashi et al., 2003
EOY90	*h- cdc25-22 ori2004ΔIΔIII*	Takahashi et al., 2003
AK300	*h- [Msmt-0] cdc25-22 leu1-32 his7-366 leu1::pFS181*(*leu1 adh1:hENT1*) *pJL218* (*his7 adh1:tk*)	Our stock
PN10488	*h+ cdc25-22 cdc45-YFP::ura4+ ura4-D18*	This study
PN10489	*h- cdc25-22 ori2004ΔI* Orp1-HA	This study
PN10490	*h+ cdc25-22 ori2004ΔIII* Orp1-HA	This study
PN10491	*h- cdc25-22 ori2004ΔIΔIII* Orp1-HA	This study
PN10492	*h+ cdc25-22 ori2004ΔI cdc45-YFP::ura4+*	This study
PN10493	*h+ cdc25-22 ori2004ΔIII cdc45YFP::ura4+ ura4-D18*	This study
PN10494	*h- cdc25-22 ori2004ΔIΔIII cdc45YFP::ura4+*	This study
PN10495	*h- cdc25-22 his7-366 leu1::pFS181*(*leu1 adh1:hENT1*) *pJL218* (*his7 adh1:tk*) *kanMX6:nmt1:3HA-cdc45*	This study
PN10496	*h- cdc25-22 his7-366 leu1::pFS181*(*leu1 adh1:hENT1*) *pJL218* (*his7 adh1:tk*) *kanMX6:nmt1:3HA-hsk1*	This study
PN10497	*h+ cdc25-22 leu1::pFS181*(*leu1 adh1:hENT1*) *pJL218* (*his7 adh1:tk*) *kanMX6:nmt1-3HA-dfp1*	This study

PN10498	*h- cdc25-22 his7-366 leu1::pFS181*(*leu1 adh1:hENT1*) *pJL218* (*his7 adh1:tk*) *kanMX6:nmt1-3HA-cdt1*	This study

Supp. Fig. 1

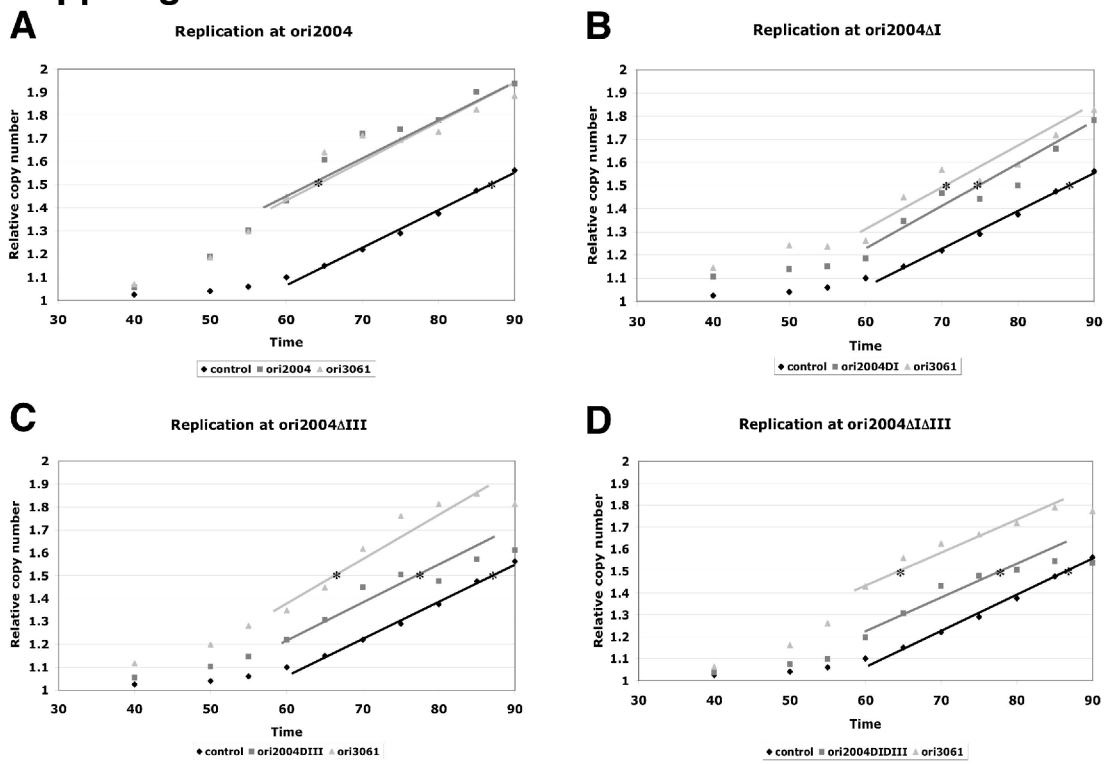

A
Orp1 at ori2004
% IP
ori2004 coordinates (bp)
I II III
Orp1 0 min
Orp1 10 min
Orp1 20 min
Orp1 30 min
Orp1 40 min
Orp1 50 min
B
Orp2 at ori2004
% IP
ori2004 coordinates
I II III
Orp2 0 min
Orp2 10 min
Orp2 20 min
Orp2 30 min
Orp2 40 min
Orp2 50 min
C
Mcm4 at ori2004
% IP
ori2004 coordinates (bp)
I II III
Mcm4 10 min
Mcm4 20 min
Mcm4 30 min
Mcm4 40 min
Mcm4 50 min
Mcm4 60 min
D
Cdc45 at ori2004
% IP
ori2004 coordinates (bp)
I II III
Cdc45 0 min
Cdc45 30 min
Cdc45 60 min
Cdc45 90 min
Cdc45 110 min



Supplementary Figure 1

Chromatin IP analysis of ORC, MCM, and Cdc45 binding at *ori2004*, *ori2060*, and *ars727*. A) Detailed scanning of Orp1 binding in a 4 kb region centered on *ori2004*. Primer sets are spaced approximately 500bp apart. The coordinates for *ori2004* and *ori2060* shown in Fig. 1A are used for all figures showing ChIP at these origins. Although Orp1 is bound at the origin throughout the cell cycle, its levels at the origin reach a maximum around 20 minutes before the start of S-phase. B) Orp2 binding at *ori2004* shows similar timing and periodicity at *ori2004* as Orp1. C) Mcm4 binding at *ori2004* peaks over region II and was displaced from and adjacent to the peak of Orp1 binding (compare Supp. Figs. 1A and 1C). Mcm4 binding is restricted to a short period during the cell cycle. D) Detailed scan of Cdc45 binding at *ori2004*. Cdc45 association occurs only during a short period in the cell cycle. E-F) Orp1 and Mcm4 binding in a 2.5 kb region centered on *ori2060*; primers are spaced approximately 250 bp apart. Both Orp1 and Mcm4 bind periodically to *ori2060*. Orp1 binding peaks around 40 minutes after *cdc25-22* release, while Mcm4 binding is maximal at 50 minutes post-release.

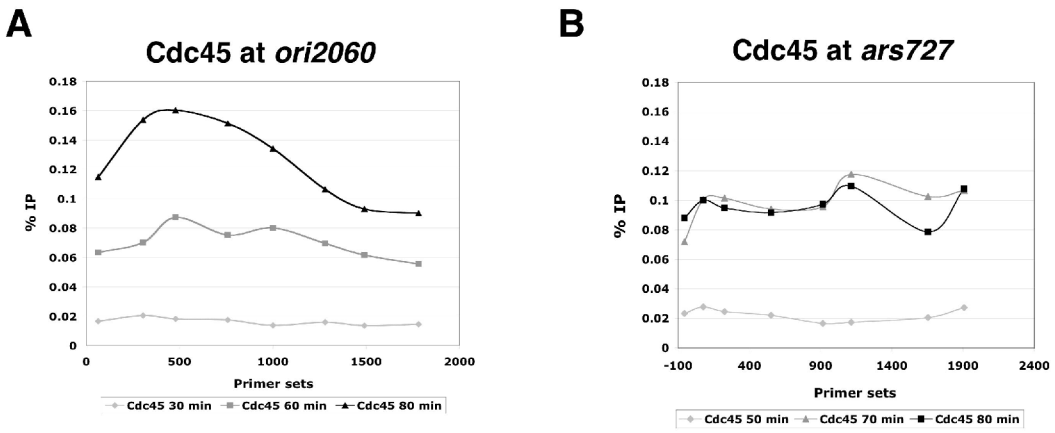
Supp. Fig. 2



Supplementary Figure 2

Timing of replication of *ori2004* deletions. Quantitative PCR was used to determine the replication of two early firing and efficient origins, *ori2004*, *ori3061*, and a late-replicating region of the genome during a synchronous time course after release from *cdc25-22* arrest. Data were normalized to the control region (diamonds) and then adjusted to generate copy number by taking into account the pattern of replication of the control region, as previously determined by Heichinger et. al (2006). The graphs represent adjusted data from representative experiments, and the replication curves for *ori2004* (squares) and *ori3061* (triangles) are shown. The point of 50% replication is marked by asterisks for each origin. *ori2004* and *ori3061* reach 50% replication at the same time, between 65 and 70 minutes; this is similar to the timing of 70 minutes reported in Heichinger et al (2006). While wild type *ori2004* (A) and *ori2004ΔI* (B) reach 50% replication around the same time as *ori3061*, *ori2004ΔIII* (C) and *ori2004ΔIΔIII* (D) show delays of 10 and 15 minutes, respectively. These results suggest that reduction in ORC binding leads to a delay in the timing of replication at an origin.

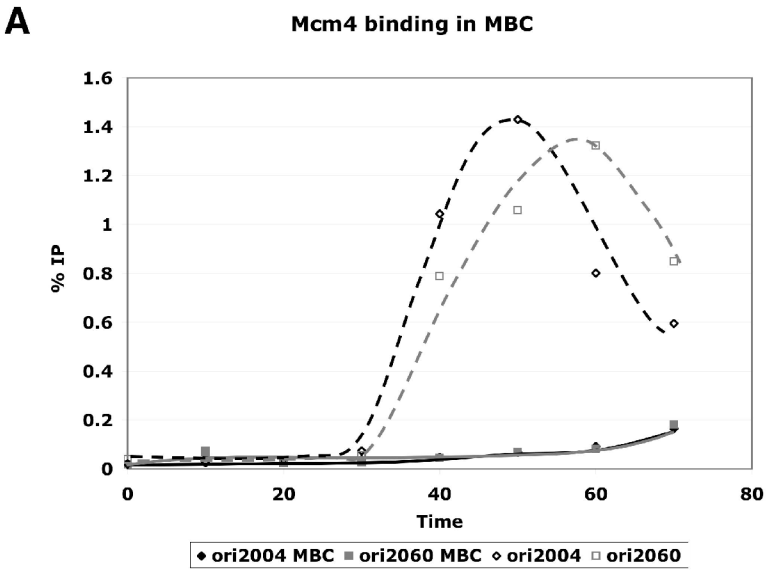
Supp. Fig. 3

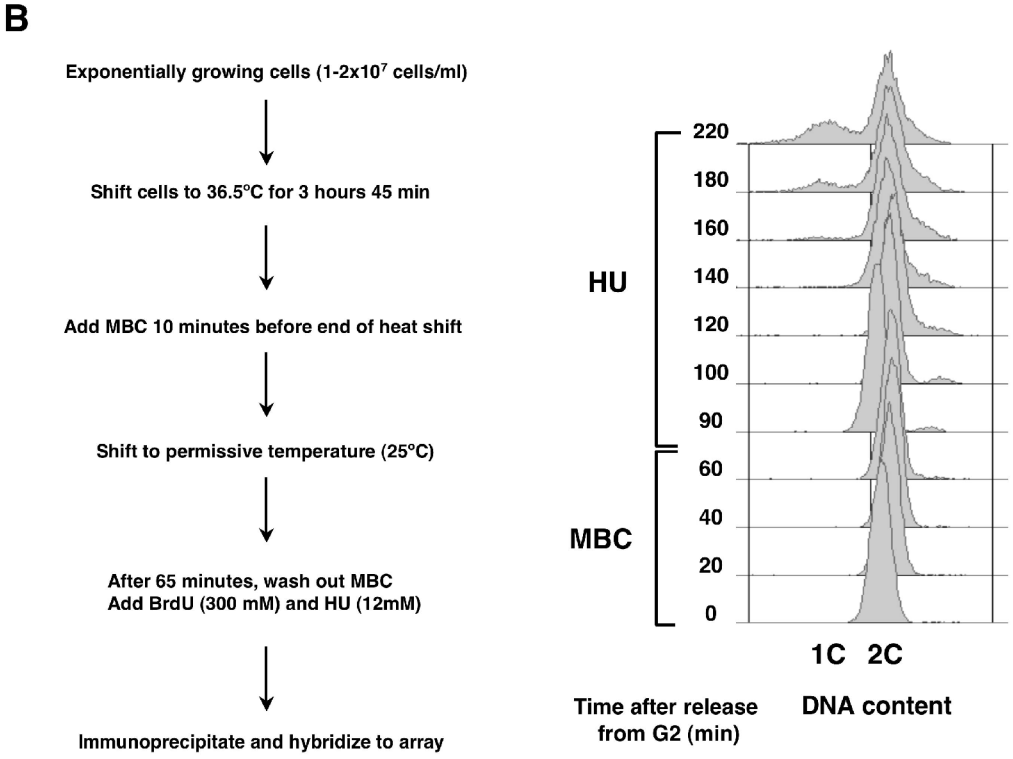


Supplementary Figure 3

Cdc45 binding to regions containing *ori2060* and *ars727*. (A, B) Quantitative real-time PCR analysis of Cdc45 binding at *ori2060* and *ars727* during S phase. Unlike Orp1 and Mcm4 binding, during S phase, Cdc45 binding occurs throughout the region and not just at the origins, suggesting that passive replication is responsible for a large part of the replication through both *ori2060* (A) and *ars727* (B).

Supp. Fig. 4


A
Mcm4 binding in MBC
% IP
Time
ori2004 MBC
ori2060 MBC
ori2004
ori2060


B
Exponentially growing cells (1-2x10^7 cells/ml)
Shift cells to 36.5°C for 3 hours 45 min
Add MBC 10 minutes before end of heat shift
Shift to permissive temperature (25°C)
After 65 minutes, wash out MBC
Add BrdU (300 mM) and HU (12mM)
Immunoprecipitate and hybridize to array
HU
MBC
220
180
160
140
120
100
90
60
40
20
0
1C 2C
Time after release from G2 (min)
DNA content

Supplementary Figure 4

Treatment by MBC, a drug that destabilizes microtubules, prolongs M phase.
A) Mcm4 binding at *ori2004* and *ori2060* during the MBC arrest. Cells containing *cdc25-22* were arrested at 36.5º for 3 hours and 45 minutes, and MBC was added 10 minutes before release into permissive temperature. Mcm4 occupancy is minimal during this time period, reaching only around 0.1% IP, while normally Mcm4 has an occupancy of around 1% (shown by the dotted lines). B) Left panel: Schematic of the experimental design for MBC treatment followed by HU arrest. Right panel: FACS profile for the experimental scheme. Cells do not undergo a complete round of replication and divide by the end of the experiment.

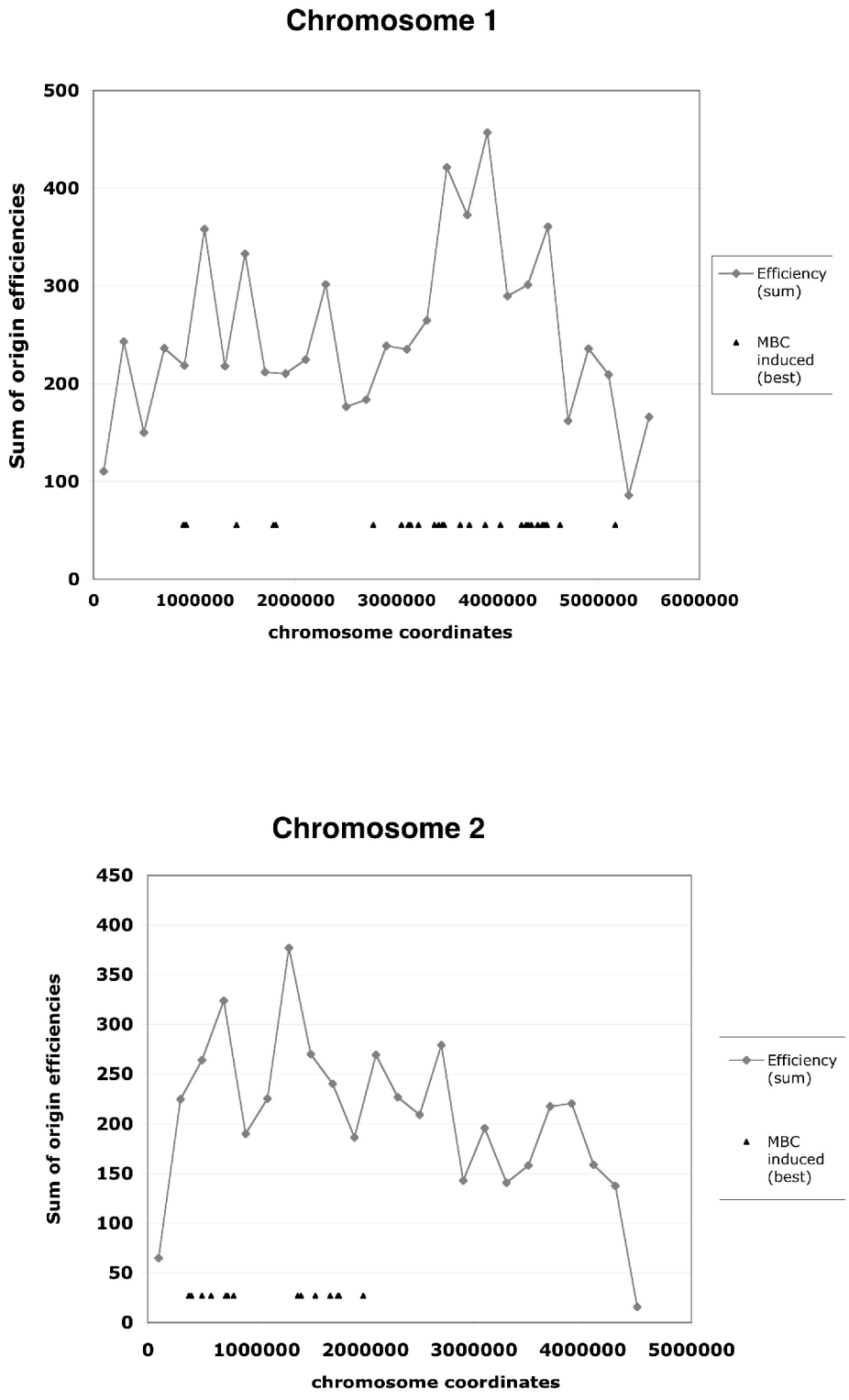
Supp. Fig. 5

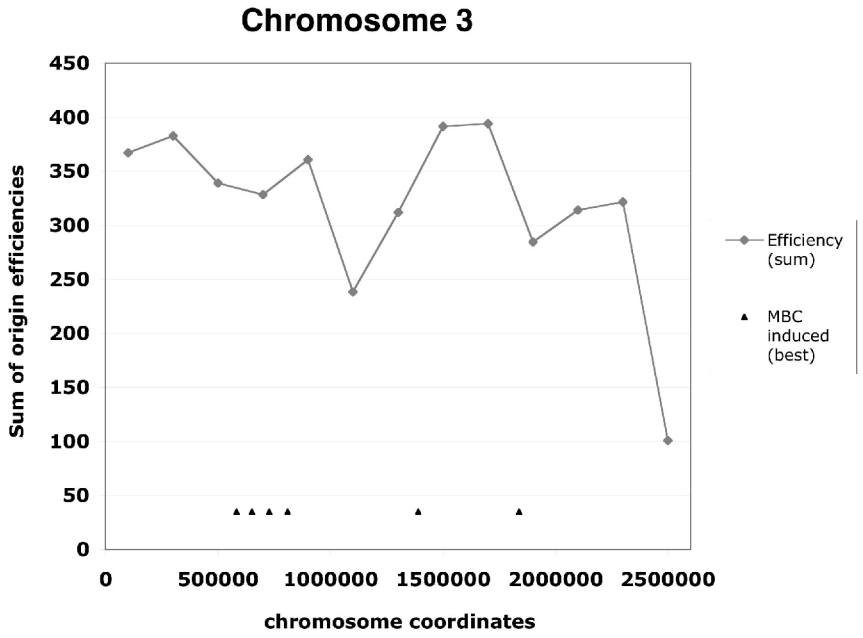
ORI Number	Efficiency Mitosis	Reduced in MBC
3061	76	+
1128	73	+
2026	65	+
3006	65	+
3033	64	+
3021	62	-
2024	62	+
1132	61	+
1138	60	-
1035	60	+
1129	59	-
1109	59	+
2042	58	+
1028	57	+
3049	57	+
1110	56	-
3044	56	+
1126	56	+
1043	55	+
3046	55	+
1120	54	-
3077	54	+
2040	54	+
3032	53	+
3056	53	+
1103	53	+
3050	53	+
3045	52	+
3025	52	+
3057	52	+
3035	52	+
3009	52	+
3065	52	+
1024	51	+
2050	51	+
1019	51	+
1123	50	+
1116	50	+
1104	50	+
2015	50	-
3007	49	+
3018	49	+
3037	49	+
3059	49	-
1034	49	+
3060	49	+
3062	49	+
3083	49	+
3024	48	+
3047	48	+

Supplementary Figure 5

Early-firing efficient origins are reduced in efficiency after MBC treatment. List showing the top 50 most efficient origins according to Heichinger et al. (2006) and the effect of prolonging mitosis. Origins that are reduced in efficiency after MBC treatment in three biological repeat experiments are marked with +.

Supp. Fig. 6

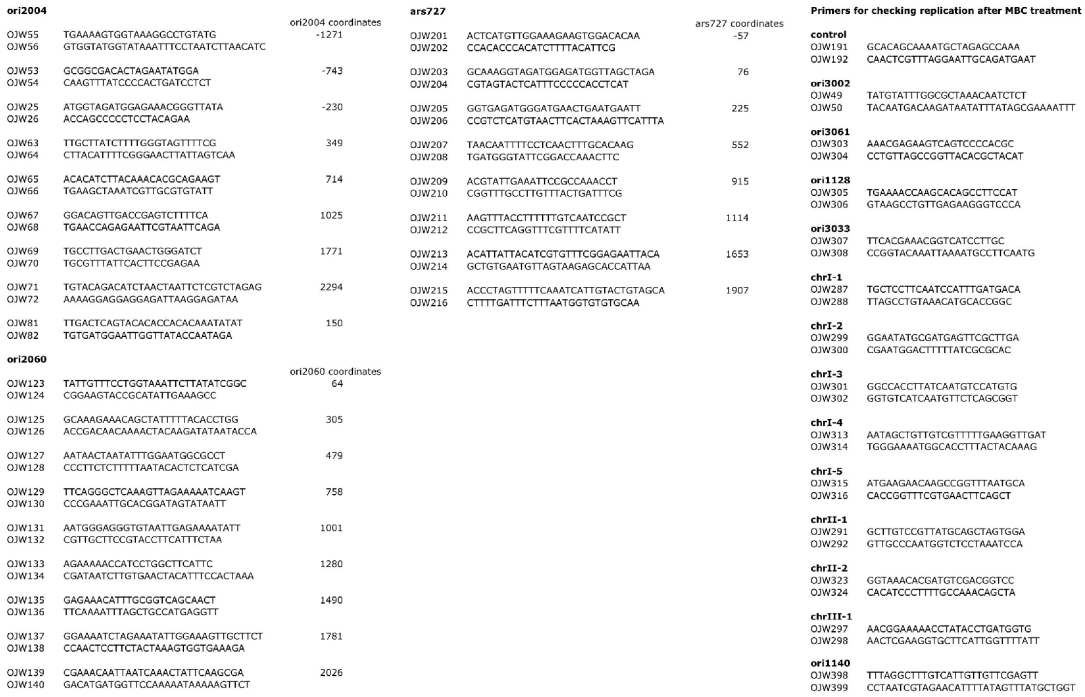

Chromosome 1
Sum of origin efficiencies
500
400
300
200
100
0
0
1000000
2000000
3000000
4000000
5000000
6000000
chromosome coordinates
Efficiency (sum)
MBC induced (best)
Chromosome 2
Sum of origin efficiencies
450
400
350
300
250
200
150
100
50
0
0
1000000
2000000
3000000
4000000
5000000
chromosome coordinates
Efficiency (sum)
MBC induced (best)



Supplementary Figure 6

Analysis of regions that show increased replication in the MBC experiment. The positions of the 50 best MBC induced regions are marked with black triangles, and the sum of the origin efficiencies for consecutive, non-overlapping 200 kb windows are indicated with a gray line. Origin efficiencies are obtained from Heichinger et al. (2006). In general, regions from which MBC-induced origins are excluded have lower efficiencies.

Supp. Fig. 7



ori2004

		ori2004 coordinates
OJW55	TGAAAAGTGGTAAAGGCCTGTATG	-1271
OJW56	GTGGTATGGTATAAATTTCCTAATCTTAACATC	
OJW53	GCGGCGACACTAGAATATGGA	-743
OJW54	CAAGTTTATCCCCACTGATCCTCT	
OJW25	ATGGTAGATGGAGAAACGGGTTATA	-230
OJW26	ACCAGCCCCCTCCTACAGAA	
OJW63	TTGCTTATCTTTTGGGTAGTTTTCG	349
OJW64	CTTACATTTTCGGGAACTTATTAGTCAA	
OJW65	ACACATCTTACAAACACGCAGAAGT	714
OJW66	TGAAGCTAAATCGTTGCGTGTATT	
OJW67	GGACAGTTGACCGAGTCTTTTCA	1025
OJW68	TGAACCAGAGAATTCGTAATTCAGA	
OJW69	TGCCTTGACTGAACTGGGATCT	1771
OJW70	TGCGTTTATTCACTTCCGAGAA	
OJW71	TGTACAGACATCTAACTAATTCTCGTCTAGAG	2294
OJW72	AAAAGGAGGAGGAGATTAAGGAGATAA	
OJW81	TTGACTCAGTACACACCACACAAATATAT	150
OJW82	TGTGATGGAATTGGTTATACCAATAGA	

ori2060

		ori2060 coordinates
OJW123	TATTGTTTCCTGGTAAATTCTTATATCGGC	64
OJW124	CGGAAGTACCGCATATTGAAAGCC	
OJW125	GCAAAGAAACAGCTATTTTTACACCTGG	305
OJW126	ACCGACAACAAAACTACAAGATATAATACCA	
OJW127	AATAACTAATATTTGGAATGGCGCCT	479
OJW128	CCCTTCTCTTTTTAATACACTCTCATCGA	
OJW129	TTCAGGGCTCAAAGTTAGAAAAATCAAGT	758
OJW130	CCCGAAATTGCACGGATAGTATAATT	
OJW131	AATGGGAGGGTGTAATTGAGAAAATATT	1001
OJW132	CGTTGCTTCCGTACCTTCATTTCTAA	
OJW133	AGAAAAACCATCCTGGCTTCATTC	1280
OJW134	CGATAATCTTGTGAACTACATTTCCACTAAA	
OJW135	GAGAAACATTTGCGGTCAGCAACT	1490
OJW136	TTCAAAATTTAGCTGCCATGAGGTT	
OJW137	GGAAAATCTAGAAATATTGGAAAGTTGCTTCT	1781
OJW138	CCAACTCCTTCTACTAAAGTGGTGAAAGA	
OJW139	CGAAACAATTAATCAAACTATTCAAGCGA	2026
OJW140	GACATGATGGTTCCAAAAATAAAAAGTTCT	

ars727

		ars727 coordinates
OJW201	ACTCATGTTGGAAAGAAGTGGACACAA	-57
OJW202	CCACACCCACATCTTTTACATTCG	
OJW203	GCAAAGGTAGATGGAGATGGTTAGCTAGA	76
OJW204	CGTAGTACTCATTTCCCCCACCTCAT	
OJW205	GGTGAGATGGGATGAACTGAATGAATT	225
OJW206	CCGTCTCATGTAACTTCACTAAAGTTCATTTA	
OJW207	TAACAATTTTCCTCAACTTTGCACAAG	552
OJW208	TGATGGGTATTCGGACCAAACTTC	
OJW209	ACGTATTGAAATTCCGCCAAACCT	915
OJW210	CGGTTTGCCTTGTTTACTGATTTCG	
OJW211	AAGTTTACCTTTTTTGTCAATCCGCT	1114
OJW212	CCGCTTCAGGTTTCGTTTTCATATT	
OJW213	ACATTATTACATCGTGTTTCGGAGAATTACA	1653
OJW214	GCTGTGAATGTTAGTAAGAGCACCATTAA	
OJW215	ACCCTAGTTTTTCAAATCATTGTACTGTAGCA	1907
OJW216	CTTTTGATTTCTTTAATGGTGTGTGCAA	

Primers for checking replication after MBC treatment

control	
OJW191	GCACAGCAAAATGCTAGAGCCAAA
OJW192	CAACTCGTTTAGGAATTGCAGATGAAT
ori3002	
OJW49	TATGTATTTGGCGCTAAACAATCTCT
OJW50	TACAATGACAAGATAATATTTATAGCGAAAATTT
ori3061	
OJW303	AAACGAGAAGTCAGTCCCCACGC
OJW304	CCTGTTAGCCGGTTACACGCTACAT
ori1128	
OJW305	TGAAAACCAAGCACAGCCTTCCAT
OJW306	GTAAGCCTGTTGAGAAGGGTCCCA
ori3033	
OJW307	TTCACGAAACGGTCATCCTTGC
OJW308	CCGGTACAAATTAAAATGCCTTCAATG
chrI-1	
OJW287	TGCTCCTTCAATCCATTTGATGACA
OJW288	TTAGCCTGTAAACATGCACCGGC
chrI-2	
OJW299	GGAATATGCGATGAGTTCGCTTGA
OJW300	CGAATGGACTTTTTATCGCGCAC
chrI-3	
OJW301	GGCCACCTTATCAATGTCCATGTG
OJW302	GGTGTCATCAATGTTCTCAGCGGT
chrI-4	
OJW313	AATAGCTGTTGTCGTTTTTGAAGGTTGAT
OJW314	TGGGAAAATGGCACCTTTACTACAAAG
chrI-5	
OJW315	ATGAAGAACAAGCCGGTTTAATGCA
OJW316	CACCGGTTTCGTGAACTTCAGCT
chrII-1	
OJW291	GCTTGTCCGTTATGCAGCTAGTGGA
OJW292	GTTGCCCAATGGTCTCCTAAATCCA
chrII-2	
OJW323	GGTAAACACGATGTCGACGGTCC
OJW324	CACATCCCTTTTGCCAAACAGCTA
chrIII-1	
OJW297	AACGGAAAAACCTATACCTGATGGTG
OJW298	AACTCGAAGGTGCTTCATTGGTTTTATT
ori1140	
OJW398	TTTAGGCTTTGTCATTGTTGTTCGAGTT
OJW399	CCTAATCGTAGAACATTTTATAGTTTATGCTGGT

Supplementary Figure 7

Primers used for real-time PCR assays. Sequences of primers used for quantitative PCR assays. The primers for *ori2004*, *ori2060*, and *ars727* are accompanied by their origin coordinates.