Supplementary Data

Experimental Procedures

Replication origin arrays

The replication origin array used in this study was designed in our laboratory. The array contained 623 loci that were spotted on poly-L-lysine coated glass slides. Spots represent 424 ORC/MCM binding sites (pro-ARSs) that were identified in the genome-wide study by Wyrick et al. (2001). In addition, there were 195 origin flanking regions, which include 36 negative controls that were used for normalization, as well as 4 additional controls (described below). The origin flanking regions are sites located at 5 kb intervals between two adjacent ORC binding sites that are at least 40 kb apart. The 36 negative controls used for normalization are sites that showed no ORC/MCM binding and are located >20 kb away from an ORC/MCM binding site (see Sup. Table I). The respective DNA fragments were all amplified from yeast genomic DNA (ResGen Invitrogen) and verified by agarose gel electrophoresis, followed by ethidium bromide staining. The primers for amplification are described in Wyrick et al. (2001) and were synthesized by Integrated DNA Technologies. Sup. Table I contains the details of the replication origin array features. The array also contains 4 *Arabidopsis* genes, Arab_F7H2.16, Arab_T16B5.5, Arab_F6D8.11 and Arab_T1N6.24 (gift from J. Berman) that were each spotted in triplicate. The arrays were post-processed as described in DeRisi et al. (1997).

Yeast strains and culture

All strains are derivatives of BF264-*15DU MAT**a** ade1 his2 leu2-3, 112 trp1-1*^{a}*ura3 Δns* (Richardson *et al*, 1989) and the genotypes are DCY1671, *MAT**a** bar1Δ ura3::HIS3:GFP:TUB1(URA3)*; DCY1793, *MAT**a** bar1Δ ura3::HIS3:GFP:TUB1(URA3) rad53-1*; DCY2563, *MAT**a** bar1Δ ura3::HIS3:GFP:TUB1(URA3) leu2 mec1-1 sml1Δ*. *RAD52* was deleted using the LEU2 marker in the above strains by PCR-mediated gene disruption (YB126, DCY1671 *rad52::leu2*; YB127, DCY1793 *rad52::leu2*; YB133, DCY2563 *rad52::leu2*). *SML1* was deleted using the *LEU2* marker as above (YB194, DCY1671 *sml1::leu2*; YB195, DCY1793 *sml1::leu2*). *MCM10* was Myc-tagged using nine Myc epitopes (*TRP1)* (YB231, DCY1671 *MCM10-9MYC*; YB232, DCY2563 *MCM10-9MYC*; YB233, DCY1793 *MCM10-9MYC*). *ARS315* was replaced by the *TRP1* marker (YB204, DCY1671 *ARS315::trp1*; YB205, DCY1793 *ARS315::trp1*; YB206, DCY2563 *ARS315::trp1*) and *RAD52* was deleted in these strains as described above (YB207, YB204 *rad52::leu2*; YB208, YB205 *rad52::leu2*; YB209, YB206 *rad52::leu2*). An extra copy of *ARS315* (inserted into pRS306) was integrated into the *URA3* locus in wild-type, *rad53-1* and *mec1-1* strains derived from the same background but lacking the *GFP-TUB1* construct (YB234, DCY1180 *pRS306+ARS315::ura3*; YB235, DCY1768 *pRS306+ARS315::ura3*; YB236, DCY1808 *pRS306+ARS315::ura3*). *RAD52* was deleted in these strains using the LEU2 marker (YB237, YB234 *rad52::leu2*; YB238, YB235 *rad52::leu2*; YB239, YB236 *rad52::leu2*) and *MCM10* was Myc-tagged in these strains as described earlier (YB248, YB234 9Myc-*MCM10*; YB249, YB235 9Myc-*MCM10*; YB250, YB236 9Myc- *MCM10*). Cells were grown at 30°C unless indicated otherwise. For each experiment, the initial culture was prepared using cells from stocks. Cells were blocked in G1 with α factor (200 ng/ml) and in S phase with HU as indicated.

FACS analysis and fluorescence microscopy

Fluorescence microscopy and flow cytometry was carried out as described (Clarke *et al* 2001) for each copy number change experiment. G1-arrested cells were released into HU and aliquots were taken every 5 min for FACS analysis and fluorescence microscopy. Cells were scored using a Zeiss Axioplan II microscope with a DIC Plan Fluar 63x/1.4 objective and Endow GFP filter set Chroma 41017. Spindle microtubules were detected by expression of a protein fusion between GFP and Tub1, the major alpha tubulin. The number of budded cells and short (1-3 μm) or long (>3 μm) spindles were counted for ~300 cells without fixation. Cell cycle progression was verified for all arrest-release experiments using flow cytometry (data not shown).

DNA isolation and labeling of DNA with fluorescent nucleotides

DNA was isolated using CsCl gradients as described (Bielinsky & Gerbi, 1999). The isolated DNA was partially digested with DpnII (NEB) to yield fragments between 0.5 – 5 kb. The digested DNA was then labeled with either fluorescent Cy5- or Cy3-conjugated dUTP (Amersham Biosciences) using the Bioprime DNA Labeling System (Invitrogen) as described in Pollack et al. (2001) except for the following changes. For each labeling reaction, 2 μg of DNA was used as template and the reaction was carried out for 2 hr at 37°C. Following the labeling, the sample was purified using the QIAQuick PCR purification kit (Qiagen). The entire labeling reaction was used for each hybridization experiment. For copy number change experiments, DNA isolated from α factor-arrested cells was labeled with Cy3 and DNA isolated from HU-arrested cells was labeled with Cy5. For the "alpha vs. alpha" control experiment, DNA isolated from α factor-arrested cells was labeled with Cy5 and Cy3 in two separate reactions.

Array hybridization

The purified and labeled DNA samples were pooled (Cy5-HU and Cy3-α or Cy5-α and Cy3-α) and 32 μg of yeast tRNA (Invitrogen) was added to block non-specific hybridizations. The probes were concentrated using a Microcon-30 filter (Millipore). The volumes of recovered DNA probes were adjusted with dH_2O, and 20X SSC and 0.1% SDS was added to obtain a final concentration of 3X SSC and 0.1% SDS in the hybridization buffer. The hybridization reaction was boiled for 2 min, and was incubated at 60°C for 5 min before application to the microarray. Hybridizations were carried out at 60°C for 16 hr. The slides were hybridized and washed as described (Pollack *et al*, 2001; DeRisi *et al*, 1997). Copy number change experiments were performed in triplicate.

Data analysis

The slides were scanned using a Scan Array 5000 scanner (GSI Lumonics) and TIFF images in each channel were obtained. Images were quantified using GenePix Pro 6.0 (Axon Instruments Inc.). Spots with background-subtracted Cy5 and Cy3 median values (obtained from GenePix Pro 6.0) with at least 150 pixel units were considered in the subsequent data analysis. Cy5/Cy3 ratios were normalized using either the 36 negative control regions from *S. cerevisiae* or using the 4 *Arabidopsis* genes, similar to using house-keeping genes for normalizing expression arrays. The alpha vs. alpha control experiment was used to estimate background variation. P-values were calculated using the Student's t-test by comparing data from HU/alpha and alpha/alpha hybridizations or, in the case of the CEOs, comparing HU/alpha hybridizations among wild-type and mutant cells. A fold change greater than one standard deviation above background (significance level 1) with a $p\text{-value} < 0.05$ was considered significant. The average fold change for each spot was calculated and plotted against the middle of its chromosomal position (kb). Theoretically, ratios should not have a numerical value < 1, however experimental values < 1 have also been observed by others (Pollack *et al*, 2001). Origins activated in wild-type cells (above significance level 1 and a p-value (HU/alpha wild-type vs. mutant) < 0.05) but not in *mec1-1* or *rad53-1* mutants (below significance level 2 – defined as one half of a standard deviation above background and a $p\text{-value} < 0.05$) were classified as CEOs. Primary data will be made available upon request.

Alkaline gel electrophoresis of replicating DNA

Replication intermediates from wild-type and *rad53-1* cells were analyzed using alkaline agarose gel electrophoresis as described (Diffley & Santocanale, 1998). Cells were grown at 25°C unless indicated otherwise. α factor-arrested cells were released into S phase in the presence of HU as indicated. 10 ml aliquots were taken in 30-min intervals. A 10 ml aliquot was also removed from the G1-arrested cells as a negative control. Cells were lysed using lyticase, and DNA enriched for replication intermediates was isolated using a partial phenol-chloroform fractionation. For each fractionation, ~400 μl of supernatant (which contain all the RI DNA) was removed and the entire sample was loaded on the alkaline gel. Alkaline gel electrophoresis was carried out for ~16 hr at 20 V. DNA was transferred from gels to Hybond N$^+$ membranes (Amersham Biosciences) according to the manufacturer's instructions. Origin-specific probes were PCR amplified using primers described in Sup. Table I. These probes were biotinylated using the Bioprime DNA Labeling System (Invitrogen). Southern blots were hybridized and visualized using the North2South Chemiluminescent Hybridization and Detection Kit (Pierce) according to the manufacturer's instructions.

2D gel electrophoresis of replication intermediates

Replication intermediates from wild-type, *mec1-1* and *rad53-1* cells were analyzed using two-dimensional (2D) agarose gel electrophoresis as described (Friedman & Brewer, 1995; Tourrière *et al*, 2005). α factor-arrested cells were released into S phase in the presence of 0.2 M HU at 30°C. DNA was isolated and cleaved with suitable restriction enzymes as described (Wu & Gilbert, 1995; Tourrière *et al*, 2005). DNA fragments were separated by molecular weight in a 0.4 % agarose gel at ~1 V/cm for 16 – 18 hr. Separation in the second dimension was carried out in a 1.5 % agarose gel at 5 V/cm for ~12 – 15 hr at 4°C. DNA was transferred from gels to Hybond N$^+$ membranes (Amersham Biosciences) according to the manufacturer's instructions. Origin-specific probes were PCR amplified (primer sequences available upon request) and labeled with α-P32-dATP (MP) using the RediPrimeII Kit (Stratagene). Blots were hybridized using PerfectHybPlus hybridization buffer (Sigma) and detected on a PhosphorImager (Fujifilm FLA500).

Linker-mediated PCR analysis of DNA breaks

The presence of DNA breaks adjacent to replication origins was tested using a two-step linker-mediated PCR method. Genomic DNA was isolated from cells grown in the absence of HU, unless otherwise stated. A linker was ligated to 500 ng – 1 μg of double-stranded DNA (Mueller & Wold, 1989), after conducting a fill-in reaction to yield blunt DNA ends as described (Ren *et al*, 2001). PCR was carried out under standard conditions except for the following changes. 30 - 60 ng of linker-ligated DNA was amplified by a 25-cycle PCR reaction (94 °C 2'; 25 X [94 °C 30", 55 °C 30", 72 °C 2']) with one origin-specific and one linker-specific primer. This was followed by a nested 10-cycle PCR reaction (94 °C 2'; 10 X [94 °C 30", 58 °C 30", 72 °C 1:30']) using 5 μl of the first PCR reaction as template and a second origin-specific primer and the same linker-specific primer. All primers were used at 30 μM (except for the ARS606 primer, which was used at 90 μM). PCR fragments were fractionated on agarose gels. In parallel, PCR products were cloned into T-overhang vectors and sequenced to verify specificity. To ensure that all primer pairs worked at equal efficiency, breaks were introduced at specific restriction sites ~ 1 kb downstream of each origin, and tested using the same two-step PCR protocol (10 ng of ligated DNA as template). Restriction enzymes used were PstI (*ARS305*), TaqI (*ARS121*), SnaBI (*ARS1*), PstI (*ARS310*), HpaII (*ARS1305*), AccI (*ARS315*), DraI (*ARS606*) and BglII (*ARS315* in *URA3*). All enzymes were obtained from NEB. Primer sequences are available upon request.

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Supplementary Table I. Details of replication origin array loci.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
YAL069W	102	I	335	649
YAL067C	103	I	7475	8538
iYAL063C-2	104	I	30371	31573
iYAL056W	105	I	41808	42182
iYAL039C-0	106	I	69532	70662
iYAL017W	107	I	124299	124883
iYAL002W	108	I	147533	147596
iYAR008W	109(101)	I	159794	160106
iYAR019C-1	110(ADE1)	I	175994	176855
iYARCdelta8-3	111	I	214197	215673
iYBL109W-0	201	II	6125	7151
iYBL086C	202	II	62592	63866
iYBL068W	203	II	93389	93811
iYBL067C	204	II	95877	96900
iYBL065W	205	II	100265	100329
iYBL040C	206	II	142827	143948
iYBL027W	207	II	169335	170582
iYBL001C	208	II	237425	238165
iYBR008C	209(H4)	II	254166	255327
iYBRCdelta11	210	II	258930	259101
iYBR044C	211	II	326016	326749
YBR070C	212	II	379180	379871
iYBR075W	213	II	389171	390329
iYBR082C-0	214	II	407123	408123
iYBR085W-0	215	II	416860	417958
iYBR124W	216	II	486820	487153
YBR134W	217	II	504201	504602
YBR136W	Late/Late	II	511722	512665
YBR138C	Late/Late	II	515295	513721
YBR140C	Late/Late	II	517910	518955
iYBR140C	Late/Late	II	526582	526979
iYBR142W	Late/Late	II	530592	530823
iYBR145W	Late/Late	II	534771	535214
iYBR148W	218	II	539659	539941

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
iYBR157C-1	Late/Late	II	555382	556502
iYBR160W[b]	Late/Late	II	560927	561588
YBR163W	Late/Late	II	566203	567128
iYBR166C[b]	Late/Late	II	571154	571422
YBR170C	Late/Late	II	578045	576303
iYBR172C	Late/Late	II	581476	581680
iYBR176W	Late/Late	II	584612	584761
YBR180W	219	II	589749	590936
iYBR200W	220	II	622481	623742
iYBR203W	221	II	631897	632209
iYBR241C	222	II	704010	704625
YBR250W	223	II	719235	720218
iYBR268W	224	II	741573	742139
iYBR275C	225	II	757061	757576
iYBR278W	226	II	760855	761213
iYBR284W	227	II	773588	773878
iYBR294W	228	II	791769	792803
iYBR297W	229	II	801883	802585
YCL076W	300	III	1537	2127
iYCLWomega2-0	300.5	III	4314	5392
iYCL069W	301	III	11072	11493
iYCL065W-0	302	III	14108	14947
iYCL065W-1	303/320	III	14947	15787
iYCL063W	FALSE	III	18544	18799
iYCL055W-0	304	III	28919	30175
iYCL050C	305	III	38777	39763
iYCL048W	FALSE	III	43533	43638
iYCL027W	306	III	73312	74675
YCL004W	307	III	108713	109963
YCR010C	309	III	132724	131873
iYCR026C	310	III	166050	167084
iYCR036W	313	III	193027	193143
YCR038C	314	III	197966	196350
YCR057C	FALSE	III	221958	219187

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
iYCR060W	315	III	223463	224292
YCR066W	Early/Late	III	230226	231689
YCR068W	Early/Late	III	235940	237229
iYCR076C[b]	Early/Late	III	248768	248961
YCR079W[b]	Early/Late	III	251571	252878
YCR086W	Early/Late	III	262115	262687
iYCR088W	Early/Late	III	265569	266157
iYCR090C-0	316	III	271586	272356
YCR096C	317	III	292260	291901
iYCR097W	318	III	293043	293725
iYDL247W	401	IV	7814	8683
iYDL246C-1	402	IV	10706	11657
iYDL245C-1	403	IV	14782	16204
iYDL210W	405	IV	85986	86208
iYDL189W	406	IV	123590	123866
iYDL180W	407	IV	137540	138292
iYDL170W	408	IV	157905	158065
iYDL167C	Late/Late	IV	163155	163450
YDL161W	Late/Late	IV	167715	169079
YDL159W	Late/Late	IV	172482	174029
iYDL155W[b]	Late/Late	IV	178057	178335
iYDL152W[b]	Late/Late	IV	183188	183319
iYDL149W	Late/Late	IV	187919	188155
iYDL146W[b]	Late/Late	IV	194226	194572
iYDL145C	Late/Late	IV	198177	198663
iYDL142C	Late/Late	IV	202571	203040
YDL140C	Late/Late	IV	206307	207319
iYDL139C	409	IV	212119	213352
iYDL116W	410	IV	253745	253994
iYDL114W	411	IV	256529	256632
iYDL078C	412	IV	316387	316970
YDL072C	413	IV	330446	329835
iYDL025C	414	IV	407247	408492
iYDLCdelta1-0	415	IV	434780	435985

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
iYDR008C	416(ARS1)	IV	462643	463475
iYDR018C	417	IV	483900	484203
itV(UAC)D	Early/Early	IV	488910	489548
iYDR026C	Early/Early	IV	494308	494689
YDR028C	Early/Early	IV	500874	497830
iYDR031W	Early/Early	IV	503891	504138
iYDR033W[b]	Early/Early	IV	509148	509776
iYDRCdelta3[b]	Early/Early	IV	513629	513731
itL(UAA)D[b]	Early/Early	IV	519865	520212
iYDR036C[b]	Early/Early	IV	524752	525479
YDR038C	Early/Early	IV	527801	528809
iYDR039C	Early/Early	IV	534621	535231
iYDR040C	Early/Early	IV	538506	539842
iYDR043C-1	Early/Early	IV	544500	545591
YDR046C	Early/Early	IV	550571	548757
iYDR049W	418	IV	555192	555766
YDR058C	419	IV	568698	567718
iYDR091C	420	IV	628572	629914
iYDR096W	421	IV	639861	640147
iYDR125C	422	IV	702796	703272
iYDR147W	423	IV	753273	753713
iYDR150W	424	IV	763912	764216
iYDR170W-A-1	425	IV	805607	806659
YDR186C	426	IV	835484	832851
iYDR216W	427	IV	899042	899587
iYDR223W	428	IV	913538	914353
iYDR227W	429	IV	921683	921962
iYDR277C	430	IV	1015738	1017037
iYDR297W	431	IV	1057636	1058212
iYDR342C-2	432	IV	1158415	1159643
YDR344C	Chr4E/LExtra	IV	1162439	1161996
YDR346C	Chr4E/LExtra	IV	1168649	1167204
iYDR347W	Early/Late	IV	1170178	1170361
YDR349C	Chr4E/LExtra	IV	1174166	1172376

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
iYDRWdelta26	Early/Late	IV	1175682	1175864
iYDR350C	Chr4E/LExtra	IV	1178208	1178700
iYDR351W	Early/Late	IV	1181294	1181835
YDR353W	Chr4E/LExtra	IV	1183288	1184247
iYDR354W	Early/Late	IV	1185923	1186101
YDR356W	Chr4E/LExtra	IV	1186596	1188430
YDR358W[b]	Early/Late	IV	1190048	1191721
YDR359C	Chr4E/LExtra	IV	1194374	1192495
YDR361C	Early/Late	IV	1195502	1196201
iYDR362C	Chr4E/LExtra	IV	1198731	1199217
iYDR363W	Early/Late	IV	1200587	1201784
YDR364C	Chr4E/LExtra	IV	1204199	1202832
iYDR365C	Early/Late	IV	1206417	1206738
YDR367W	Chr4E/LExtra	IV	1212837	1213603
iYDR383C	433	IV	1240752	1241238
iYDR388W	Late/Late	IV	1251668	1252571
iYDR390C	Late/Late	IV	1256881	1257042
iYDR393W[b]	Late/Late	IV	1261305	1261715
iYDR395W	Late/Late	IV	1266192	1266329
iYDR399W	Late/Late	IV	1270767	1270983
iYDR403W	434	IV	1276246	1276688
iYDR418W	435	IV	1302147	1303208
itS(AGA)D3	436	IV	1305746	1306301
iYDRWdelta30	437	IV	1353260	1353759
iYDR447C	438	IV	1355587	1356099
iYDR457W	439	IV	1379630	1380089
iYDR472W	440	IV	1404207	1404479
iYDR498C	441	IV	1447042	1447878
iYDR502C	442	IV	1454512	1455090
itL(CAA)D	443	IV	1461877	1462406
iYDR509W	444	IV	1468621	1469448
iYDR511W	445	IV	1470466	1470548
iYDR523C	446	IV	1487086	1487590
iYDR533C	447	IV	1502208	1503361

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
iYDR534C-1	448	IV	1505800	1506653
iYDR542W	449	IV	1523652	1524675
YDR545W	450	IV	1526317	1531707
iYEL077C	502(TelV-L)	V	4097	4185
iYEL074W	503(R1)	V	6464	7230
iYEL073C-1	504(R1)	V	8786	10019
iYEL073C-3	505(R1)	V	11252	12485
iYEL071W-0	506	V	17845	18717
iYEL051W	507(R2)	V	59148	59670
iYEL031W	508(R3)	V	93905	94644
YEL023C	509(wk)	V	108977	109894
iYEL006W	510(R4)	V	145333	145906
iYER010C-0	511(R5)	V	173337	174292
iYER028C	512(R6)	V	211875	212586
iYER052C	513(R7)	V	257957	258736
iYER065C	514(R8)	V	286912	287743
iYER077C	515(R9)	V	316596	316803
iYERWdelta12	516(R10)	V	353731	354132
iYER123W	517(R11)	V	406428	407339
itV(AAC)E1	518	V	438770	439613
iSCR1	519	V	442412	442731
iYERCdelta24	520(R12)	V	498417	499343
iYER167W	521(R13)	V	520767	521025
iYER179W	522(501)	V	549513	549720
iYERWomega2-0	523(TelV-R)	V	569020	570085
YFL063W	600.1/120	VI	5113	5312
YFL062W	Chr6Extra	VI	6426	7565
iYFL059W	600.2(w)	VI	12259	12929
iYFL058W	Chr6Extra	VI	13989	14220
iYFL056C-1	Chr6Extra	VI	16217	17004
iYFL055W-0	600.3(w)	VI	18680	19763
iYFL055W-1	600.4(w)	VI	19763	20847
YFL054C	Chr6Extra	VI	22787	20847
YFL051C	Chr6Extra	VI	30489	30058

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
iYFL051C-1	601/602	VI	31906	33272
YFL050C	Chr6Extra	VI	36348	32772
iYFL049W	FALSE	VI	38674	38843
iYFL048C	Chr6Extra	VI	40180	40421
YFL034W	Chr6Extra	VI	66226	67947
iYFL034W	603	VI	68697	69114
iYFL033C	Early/Late	VI	74426	74871
iYFL031W	Chr6Extra	VI	75780	76829
YFL029C	Chr6Extra	VI	79159	78053
YFL028C	Early/Late	VI	80211	79342
YFL027C	Chr6Extra	VI	81910	80417
YFL026W	Chr6Extra	VI	82578	83873
YFL025C[b]	Early/Late	VI	87232	84143
YFL023W	Chr6Extra	VI	91384	92974
iYFL022C	Early/Late	VI	95008	95964
iYFL021W-0	Chr6Extra	VI	97496	98360
iYFLWdelta1	Early/Late	VI	100895	101370
YFL018C	Chr6Extra	VI	103121	101622
iYFL016C	Early/Late	VI	106230	106463
YFL014W	Chr6Extra	VI	107250	107579
iYFL010C	Chr6Extra	VI	115764	116149
iYFL009W	603.5	VI	118478	119424
YFL003C	605	VI	135639	136459
itN(GUU)F	Chr6Extra	VI	137553	137657
itY(GUA)F1	606	VI	167517	167880
iYFR012W	Chr6Extra	VI	168488	169913
YFR022W	Chr6Extra	VI	197070	198771
iYFR022W	607	VI	199021	199861
iYFR024C-A	FALSE	VI	203420	203730
itA(AGC)F	Chr6Extra	VI	204983	205735
YFR030W	Chr6Extra	VI	214049	215656
iYFR030W	608	VI	216406	216581
YFR053C	Chr6Extra	VI	255037	253580
iYFR053C-1	609	VI	256305	257573

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
iYFR055W-1	Chr6Extra	VI	266492	267770
YFR057W	610	VI	269049	269504
iYGL230C	702	VII	64214	64502
iYGL228W	703	VII	69330	69670
iYGLWdelta3	705	VII	115271	115490
iYGL202W	706(ARO8)	VII	117563	117856
iYGL180W	707	VII	162762	163413
iYGL179C-1	708	VII	166225	167356
YGL168W	709	VII	187468	187800
iYGL159W	710	VII	203837	204986
YGL145W	711(ARS701)	VII	230246	232351
iYGL141W	712	VII	241088	241356
YGL128C	713	VII	269379	270180
iYGL119W	714	VII	285948	287351
iYGL118C	715	VII	288452	288513
iYGL084C	716	VII	352296	353056
iYGL062W	717	VII	388730	388966
iYGL040C-0	718	VII	420552	421820
iYGL007W-1	719	VII	484774	485916
iYGR008C	720	VII	508358	509041
iYGRCdelta16	721	VII	567754	568734
iYGR039W	722	VII	575193	575392
YGR040W	723	VII	575478	576340
iYGR043C-0	724	VII	581430	582207
YGR069W	725	VII	627077	627412
iYGR087C-0	726	VII	652975	653785
YGR110W	728	VII	713976	714879
iYGR143W	729	VII	777502	778778
YGR152C	730	VII	794678	795456
iYGR168C	731	VII	834476	834683
iYGR211W	732	VII	916695	917034
iYGR243W	733	VII	977771	978031
iYGR253C-0	734	VII	999135	1000028
iYGR254W	735	VII	1002235	1002518

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
iYGR257C	Late/Late	VII	1007301	1007667
iYGR258C-1	Late/Late	VII	1011619	1012477
iYGR262C	Late/Late	VII	1017755	1017963
iYGR269W[b]	Late/Late	VII	1026958	1027366
iYGR270W[b]	Late/Late	VII	1031505	1031787
iYGR271W[b]	Late/Late	VII	1037690	1038038
iYGR274C	Late/Late	VII	1043091	1043185
iYGR278W[b]	Late/Late	VII	1048460	1048794
iYGR280C[b]	Late/Late	VII	1051721	1052820
iYGR282C	Late/Late	VII	1058720	1059011
iYGR285C[b]	Late/Late	VII	1063149	1063809
iYGR287C	Late/Late	VII	1068987	1070289
iYGR288W-1	Late/Late	VII	1072834	1073959
iYGR292W-0	Late/Late	VII	1078349	1079115
iYGR295C-1	736	VII	1083792	1084860
iYHL049C-1	801	VIII	5470	6400
iYHLComega1	802	VIII	8224	8298
iYHL043W	Late/Late	VIII	15411	15665
iYHL038C	Late/Late	VIII	25506	25698
YHL033C	Late/Late	VIII	36023	35253
iYHL031C	Late/Late	VIII	39484	40082
iYHL030W	804	VIII	45688	45927
iYHL022C	805(SPO11)	VIII	64154	64458
itT(AGU)H	806	VIII	116172	116414
itQ(UUG)H	807	VIII	134383	134545
YHR018C	808	VIII	141393	140002
iYHR029C	809	VIII	168552	168881
iYHR053C	810	VIII	212720	213185
iYHR055C	811	VIII	214718	215183
iYHR057C	812	VIII	218844	218998
iYHR073W	813	VIII	245573	246194
iYHR091C	814	VIII	286771	287131
iYHR096C	815	VIII	296449	297387
YIL030C	Late/Late	VIII	300008	296049

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
iYHRWdelta9	816	VIII	359374	359704
YHR140W	817	VIII	380625	381171
iYHR146W	818	VIII	391697	392639
YHR147C	819	VIII	392741	393246
iYHR184W	821	VIII	474454	474622
iYHR201C	822	VIII	501138	502383
YHR209W	823	VIII	519557	520082
YHR212C	Late/Late	VIII	538090	537755
iYHR213W-0	Late/Late	VIII	539742	540694
iYHRCdelta16-0	Late/Late	VIII	549632	550863
YHR217C	824	VIII	556619	557030
iYIL177C-1	902	IX	7470	8793
iYIL174W-1	903	IX	10594	11492
iYIL173W	904	IX	16141	16784
iYIL166C	905	IX	32566	33718
iYIL156W	906	IX	51306	51759
iYIL147C	907	IX	73453	74184
iYIL130W	909	IX	105676	106107
iYIL129C	910	IX	113237	113806
iYIL120W	911	IX	136105	136651
YIL101C	912	IX	175673	176612
iYIL078W	913(901)	IX	214700	214988
iYIL058W	914	IX	247195	247899
iYIL057C	915	IX	248393	248847
iYIL035C	Late/Late	IX	288907	289225
iYIL032C	Late/Late	IX	292316	292632
iYIL028W	Late/Late	IX	302496	303677
YIL025C	916	IX	308199	308573
iYIL023C	917	IX	310424	311163
iYIL021W	918	IX	313859	314033
iYIL009W	919	IX	341425	342533
iYIR001C	920	IX	356892	357412
iYIR003W	921	IX	362921	363218
iYIR029W	922	IX	411835	412033

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
YJL225C	1001(+)	X	543	1373
iYJL225C-1	1002(+)	X	7453	8776
iYJL222W	1003(+)	X	16124	16767
iYJL217W	1004(+)	X	23729	24341
iYJL215C	Questionable	X	26771	26887
iYJL201W	Non-origin	X	56178	56444
iYJL197W	1005(+)	X	67568	67849
iYJL172W	1006(+)	X	99460	99697
iYJL163C	1007(+)	X	113327	114175
iYJL157C	Non-origin	X	126025	126587
iYJL146W	Non-origin	X	144399	144858
YJL119C	Non-origin	X	191272	191595
itD(GUC)J1	1008(+)	X	204503	205002
isnR37	1009(+)	X	228297	228722
YJL092W	Non-origin	X	258972	260027
iYJL075C	1010(+)	X	298572	298853
YJL068C	Non-origin	X	312908	313407
iYJL053W	1011(+)	X	336733	337967
itR(ucu)J2	1012(+)	X	374273	374540
iYJL038C	1013(wk)	X	375470	376358
iYJL037W	Non-origin	X	377032	377294
YJL034W	Early/Early	X	381023	383071
iYJL031C	Early/Early	X	386936	387353
iYJL017W	Early/Early	X	406256	406448
iYJL013C	Early/Early	X	410729	411141
iYJLWdelta9	1014(+)	X	416714	417250
iYJR003C	1015(+)	X	442300	442596
iYJR009C-0	1016(false)	X	454368	455147
iYJR011C	1017(false)	X	459113	459482
iYJR044C	Questionable	X	518873	519326
iYJR055W	1018(+)	X	538954	540454
iYJR058C	Early/Early	X	544863	545475
iYJR062C	Early/Early	X	554537	554883
iYJR065C	Early/Early	X	558846	559104

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
YJR069C[b]	Early/Early	X	569087	568494
YJR075W[b]	Early/Early	X	573669	574859
YJR078W[b]	Early/Early	X	578548	579909
iYJR084W[b]	Early/Early	X	584692	584808
YJR089W[b]	Early/Early	X	587406	590270
iYJR090C	Early/Early	X	594015	594749
YJR092W	Early/Early	X	598807	602769
YJR094C	Early/Early	X	605342	604260
YJR095W	Early/Early	X	609464	610432
iYJR097W	1019(+)	X	612622	612877
YJR115W	Questionable	X	639743	640143
YJR118C	Questionable	X	643183	643794
iYJR124C	1020(+)	X	653927	654426
iYJR137C	1021(121)	X	682974	684256
iYJR139C	Questionable	X	690213	690439
iYJR144W	Questionable	X	701380	701716
iYJR150C-1	1022(+)	X	710670	711944
iYJR156C	1023(+)	X	729285	730204
iYJR159W	1024(wk)	X	736806	737697
iYJR161C	1025(+)	X	743688	744600
iYKL225W	1101	XI	799	1811
iYKL221W-0	1102	XI	7529	8310
iYKL204W	1103	XI	55602	55935
iYKL188C	1104	XI	88791	89289
YKL178C	1105	XI	114629	113217
YKL160W	1106	XI	153271	153708
iYKL098W	1107	XI	257488	258148
iYKLWdelta7	1108	XI	314169	314457
iYKL059C	1109	XI	329088	329811
iYKL057C	1110	XI	333614	334057
YKL031W	1111	XI	381710	382123
iYKL027W	1112	XI	388546	389021
iYKL014C	1113	XI	416554	417149
iYKR004C	1114	XI	447318	448162

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
iYKR011C	1115	XI	462337	463380
iYKR039W	1116	XI	516511	517093
iYKR050W	1117	XI	530124	530586
iYKR075C-1	1118	XI	581155	581922
iYKR077W	1119	XI	584383	584591
iYKR091W	1120	XI	611902	612302
iYKR093W	1121	XI	617172	617633
YKR098C	1122	XI	634809	632656
iYKR101W-0	1123	XI	642136	643420
YKR105C	1124	XI	660093	658345
YLL065W	1201	XII	11771	12028
YLL055W	1202	XII	30209	31384
YLL042C	1203	XII	52086	52589
YLL034C	1204	XII	71074	71892
iYLL032C	1205	XII	76746	77151
iYLL026W	1206	XII	91348	92049
YLL005C	1207	XII	138184	139163
iCEN12	1208	XII	150946	151388
iYLR002C	1209	XII	156333	156854
iYLR036C	1210	XII	222133	222686
iYLR042C	1211	XII	230452	231703
iYLR080W	1212	XII	289251	290213
YLR084C	Early/Late	XII	297483	298293
YLR086W	Early/Late	XII	302244	306500
iYLR087C[b]	Early/Late	XII	315732	316108
YLR090W[b]	Early/Late	XII	320702	322081
YLR093C[b]	Early/Late	XII	327269	326514
iYLR095C[b]	Early/Late	XII	332116	332591
iYLR097C[b]	Early/Late	XII	337266	337528
YLR100W[b]	Early/Late	XII	341811	342854
YLR105C	Early/Late	XII	347063	348173
YLR106C	Early/Late	XII	351146	352335
iYLR106C	Early/Late	XII	363739	364117
YLR109W	Early/Late	XII	368782	369312

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
iYLR113W	1213	XII	372928	374000
iYLR117C	1214	XII	384535	384726
iYLR134W	1215	XII	412415	413282
iYLRWdelta6-0	1216	XII	449029	450404
YLR178C	1217	XII	513256	513786
iYLR228C-0	1218	XII	602463	603337
YLR242C	1219	XII	623198	623861
iYLR257W	1220	XII	659791	660716
YLR293C	1221	XII	720784	721418
iYLR300W	1222	XII	730301	730825
iYLR306W	1223	XII	744851	745620
iYLR308W	1224	XII	748875	749034
YLR319C	1225	XII	769482	770633
itD(GUC)L2	1226	XII	793988	794485
iYLR345W	1227	XII	822039	822286
YLR399C	1228	XII	919799	920993
iYLR403W	1229	XII	927615	928739
iYLR405W	1230	XII	930888	931061
iYLR411W	1231	XII	947973	948363
iYLR435W	1232	XII	1006989	1007415
YLR438W	1233	XII	1012504	1013399
iYLR444C	1234	XII	1023982	1024183
iYLR459W	1235	XII	1058513	1059752
iYLR461W-0	1236	XII	1063276	1064613
iYLR466W	1237	XII	1071230	1072503
iYML133C-1	1301	XIII	5964	7244
iYML132W-1	1302	XIII	9291	10199
iYML119W	1303	XIII	31684	32334
iYML116W	1304	XIII	39824	40187
YML087C	1305	XIII	94602	95145
iYML077W	1306	XIII	112344	112513
iYML069W	1307	XIII	137158	137550
itG(GCC)M	1308	XIII	183968	184170
iYMLWdelta4	Early/Late	XIII	190083	190244

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
YMR045C	Early/Late	XIII	195006	195649
YML041C	Early/Late	XIII	195006	195649
YML039W	Early/Late	XIII	196628	201896
YML036W[b]	Early/Late	XIII	205642	206211
YML034W[b]	Early/Late	XIII	209525	212155
iYML031W	Early/Late	XIII	216156	216435
YML027W	Early/Late	XIII	221406	222563
YML023C	Early/Late	XIII	228664	226994
iYML004C	1309	XIII	262685	263483
iYMR010W-0	1310	XIII	286316	287197
iYMR011W	1311	XIII	289703	290048
YMR021C	Early/Late	XIII	318417	317164
iYMR024W	Early/Late	XIII	323046	323299
YMR028W	Early/Late	XIII	327481	328581
YMR031C	Early/Late	XIII	334742	332211
YMR033W[b]	Early/Late	XIII	337787	339276
iYMR036C[b]	Early/Late	XIII	343519	344402
YMR039C	Early/Late	XIII	349521	348643
YMR043W	Early/Late	XIII	353870	354730
itH(GUG)M	Early/Late	XIII	363134	363363
iYMR049C-0	1312	XIII	370516	371480
itV(AAC)M1	1313	XIII	372517	372696
itV(AAC)M2	1314	XIII	420660	421483
iYMR082C-0	1315	XIII	432124	433455
iYMR100W	1316	XIII	468161	468444
iYMR105C	1317	XIII	477605	478300
YMR110C	1318	XIII	490450	491460
iYMRWdelta16	1319	XIII	503388	503770
iYMR132C	1320	XIII	535569	536206
iYMR136W	1321	XIII	542880	542977
YMR144W	1322	XIII	553421	554330
iYMR175W	1323	XIII	611254	611739
iYMR186W	1324	XIII	634471	634688
iYMR192W	1325	XIII	649279	650035

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
iYMR210W	1326	XIII	688864	689082
YMR244W	1327	XIII	757286	758314
iYMR250W	1328	XIII	772557	772914
iYMR269W	1329	XIII	805091	805326
iYMR274C	1330	XIII	815311	815651
iYMRWdelta21	1331	XIII	837764	837928
iYMR311C	1332	XIII	897603	898405
iYMR321C	1333	XIII	917895	918365
YMR323W	1334	XIII	920087	921400
iYMR325W	1335	XIII	923014	923493
YNL339C	1401	XIV	371	6098
iYNL337W	1402	XIV	7419	8330
iYNL336W-1	1403	XIV	10463	11452
iYNL333W	1404	XIV	14163	14832
YNL326C	1405	XIV	27353	28318
YNL303W	1406	XIV	61509	61856
iYNL289W-1	1407	XIV	89517	90301
iYNL287W	1408	XIV	94799	95221
YNL285W	1409	XIV	96245	96461
iYNL273W	1410	XIV	126597	126803
iYNL254C	1411	XIV	169247	170017
iYNL242W	1412	XIV	196101	196425
iYNL211C	1413	XIV	250314	250930
iYNL192W	1414	XIV	279893	280428
iYNL167C	1415	XIV	321358	322218
iYNL165W	1416	XIV	325051	325264
iYNL115C	1417	XIV	412052	412683
iYNL112W	1418	XIV	416280	416939
iYNL094W	1419	XIV	449373	449867
iYNL068C	1420	XIV	498288	499680
iYNL043C	1421	XIV	545907	546421
itT(AGU)N2	1422	XIV	560763	561605
itP(UGG)N2	1423	XIV	568215	568520
iYNL013C	1424	XIV	609508	610053

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
YNL008C	1425	XIV	616307	617121
iYNR003C	1426	XIV	635296	635942
iYNR034W-0	1427	XIV	691285	692151
YNR049C	1428	XIV	713136	713640
iYNR069C-1	1429	XIV	763980	765372
iYNR076W	1430	XIV	782277	783284
iYOL165C	1503	XV	2078	2894
iYOL162W	1504	XV	10765	11548
iYOL161C-1	1505	XV	13111	14312
iYOLCdelta2	1506	XV	18560	19490
iYOL132W	1507	XV	72714	73030
iYOL125W	1508	XV	85263	85455
itT(AGU)O1	1509	XV	113873	114137
iYOL104C	Early/Early	XV	117453	117702
YOL103W	Early/Early	XV	123991	125829
iYOL101C	Early/Early	XV	127919	129236
iYOL098C	Early/Early	XV	135837	136087
iYOL096C	Early/Early	XV	139056	139226
iYOL093W	Early/Early	XV	143695	144203
YOL090W	Early/Early	XV	148410	149596
YOL088C	Early/Early	XV	154744	153911
YOL086C	Early/Early	XV	159690	160563
iYOL083W-0	1510	XV	166951	167838
iYOL024W	1511	XV	277604	278056
YOL009C	1512	XV	309359	309925
iYOR005C	1513	XV	337343	337680
iYOR058C-0	1514	XV	436346	437261
iYOR128C	1516(ADE2)	XV	566189	566875
iYORWdelta14	1517(1502)	XV	600731	601381
iYOR151C	1518	XV	616669	617516
iYOR172W	1519	XV	656568	657130
iYOR184W	1520	XV	680542	681442
iYOR206W	1521	XV	729642	730006
iYOR227W	1522	XV	766563	766867

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
YOR228C	1523	XV	766904	767435
iYOR237W	1524	XV	783296	783666
iYORWsigma3	1525	XV	854609	855142
iYOR296W	1526	XV	874066	874738
iYOR316C-0	1528	XV	907546	908440
YOR345C	1529	XV	981804	982154
iYOR390W	1530	XV	1077903	1078537
iYPL283C-1	1601	XVI	6970	7933
iYPL280W	1602	XVI	12600	13228
YPL275W	1603	XVI	18369	19079
iYPL265W	1604	XVI	42869	43283
iYPL253C	1605	XVI	73006	73363
iYPL243W	1606	XVI	90316	90622
iYPL230W	1607	XVI	116487	117067
iYPL207W	1608	XVI	162340	162631
iYPLWdelta7	1609	XVI	210770	211576
iYPL177C-1	1610	XVI	214986	216011
iYPL175W	1611	XVI	220087	220166
iYPL154C	1612	XVI	260930	261726
iYPL152W	1613	XVI	266103	266179
iYPL140C	1614	XVI	289033	289668
iYPL131W	1615	XVI	304013	304386
YPL124W	1616	XVI	316754	317515
iYPL116W	1617	XVI	331698	332099
iYPL087W	1618	XVI	384403	384768
iYPL074W	1619	XVI	418022	418494
iYPL070W	1620	XVI	422781	422880
iYPL021W	1621	XVI	511660	512309
iYPR003C	1622	XVI	563763	564002
iYPR031W	1623	XVI	633756	634118
iYPR068C	1624	XVI	684348	684551
iYPR074C	1625	XVI	694833	695732
iYPR111W	1626	XVI	748997	749253
iYPR143W	1627	XVI	819070	819524

Supplementary Table I. Continued from previous page.

Gene ID	proARS/ Inter-origin locus[a]	Chr	Start (bp)	End (bp)
iYPR157W	1628	XVI	842664	843257
iYPRCdelta22	1629	XVI	850507	850624
iYPRWsigma4	1630	XVI	880721	881482
iYPRWtau4	1631	XVI	933399	933893
iYPR201W-1	1632	XVI	942079	943027

[a] The table lists the chromosomal coordinates and gene IDs of potential replication origins (pro-ARSs) and inter-origin loci. "Early/Early", "Early/Late" or "Late/Late" was assigned to the inter-origin loci based on the replication times (Raghuraman *et al*, 2001) of the two pro-ARSs that flank these regions. Names of known ARSs are indicated in parentheses next to the pro-ARS name. The positive sign (+) next to the pro-ARSs on chromosome X indicates that these ARSs tested positive in an ARS assay (Wyrick *et al*, 2001).

[b] Inter-origin loci (negative controls) used for normalization.

Supplementary Table II. Origins activated in wild-type cells.

Chr	80 min in HU (Total 66)[a]	120 min in HU (Total 180)[a]
I	-	108,109
II	208,212	202,208,209,212,213,215,220,221,228
III	305,306,310,315	304,305,306,307,309,310,313,315
IV	409,410,411,416,417,418	406,409,411,412,413,414,415,416,417,418,419,420,425,427,429,432,435, 436,439,442
V	508	502,508,510,511,514,516,517,518,519,520
VI	606	605,606,607,610
VII	710,712,717,718,721,722,724,728	709,710,712,714,715,717,718,719,720,721,722,723,724,728,729,733,735, 734,736
VIII	806,809,818	801,802,805,806,807,808,809,810,811,814,815,818,824
IX	904,909,910,918,919,920	902,909,913,914,915,918,919,920,921,922
X	1008,1012,1014,1021	1003,1004,1008,1009,1012,1013,1014,1018,1019,1021
XI	1104,1114,1116	1102,1103,1106,1114,1115
XII	1213,1214,1215,1227,1230,1232	1202,1207,1213,1214,1215,1216,1217,1218,1219,1220,1221,1223, 1228,1230,1232,1233
XIII	1304,1305,1308,1309,1310,1319, 1329	1303,1304,1305,1307,1308,1309,1310,1316,1317,1319,1320,1321,1322, 1323,1324,1325,1329,1330,1331,1332,1333,1335
XIV	1407,1412,1416,1419,1426	1401,1409,1410,1411,1415,1416,1421,1423,1424,1425,1426
XV	1510,1511,1512,1513,1514,1524, 1528	1509,1510,1511,1512,1513,1514,1517,1518,1529
XVI	1608,1622,1629	1603,1605,1608,1611,1612,1614,1617,1619,1620,1622,1626,1629,1632

[a] Replication origins with a copy number change above significance level 1 (one standard deviation above background) and a p-value < 0.05 were considered activated.

Supplementary Table III. List of all origins activated in *mec1-1* cells.

Chr	Activated origins[a]
I	102,108,111
II	201,210,211,212,213,223,224,225
III	301,303/320,304,305,306,313,316,318
IV	406,407,410,411,413,416,417,418,419,421,422,423,424,426,432,438,443,445
V	503,505,507,508,509,510,523
VI	600.1,600.2,600.4,607,608,609,610
VII	705,706,707,708,709,710,711,712,713,715,717,719,721,722,724,725,726,727,733,734,736
VIII	802,808,809,814,815,817,821,823,824
IX	902,903,904,905,906,910,913,914,917,918,919,920,922
X	1001,1004,1007,1009,1010,1011,1014,1018,1021,1023
XI	1104,1105,1113,1114,1115,1116,1117,1118,1120,1121,1122,1123,1124
XII	1204,1206,1212,1213,1214,1215,1218,1219,1221,1224,1225,1227,1228,1230,1231,1232,1233,1236,1237
XIII	1302,1303,1304,1306,1307,1308,1310,1311,1316,1320,1321,1322,1324,1328,1330,1331,1334,1335
XIV	1406,1412,1413,1417,1418,1419,1421,1425,1427,1430
XV	1503,1504,1505,1509,1511,1513,1514,1515,1516,1517,1518,1522,1524,1528,1529
XVI	1604,1605,1606,1609,1610,1611,1612,1617,1620,1621,1622,1626,1629,1630,1631,1632

[a] Replication origins with a copy number change above significance level 1 (one standard deviation above background) and a p-value < 0.05 were considered activated.

Supplementary Table IV. List of all origins activated in *rad53-1* cells.

Chr	Activated origins[a]
I	102,108,110,111
II	201,203,212,213,214,218,219,224,225,226,227
III	300.5,301,302,303/320,305,306,313,314,316,318
IV	405,406,407,408,409,410,411,412,413,416,418,419,420,424,426,431,432,434,436,438, 442,443,444,445,446,447,448
V	502,503,504,505,506,507,508,509,510,511,513,515,516,517,518,519,520,521,522(501),523
VI	600.1/120,600.2,600.3,600.4,603.5,607,608,609,610
VII	702,704,705,706,707,708,709,710,711,712,714,715,716,717,718,719,720,721,722,724,725, 726,727,728,731,732,733,734
VIII	801,802,803,804,807,808,809,811,813,815,816,817,818,819,820,821,822,823,824
IX	902,903,904,905,906,907,908,910,914,918,919,920
X	1004,1005,1008,1009,1010,1011,1012,1013,1014,1015,1018,1021,1022,1023
XI	1101,1103,1104,1105,1107,1109,1110,1111,1113,1114,1115,1116,1117,1118,1119,1123, 1120,1124
XII	1201,1202,1204,1205,1206,1208,1209,1212,1213,1214,1215,1216,1218,1219,1220,1221, 1225,1226,1227,1229,1230,1231,1233,1236
XIII	1301,1302,1303,1304,1306,1310,1311,1312,1313,1314,1315,1316,1318,1319,1322,1323, 1324,1325,1326,1327,1328,1329,1330,1333,1334,1335
XIV	1402,1403,1404,1406,1407,1408,1410,1411,1412,1415,1416,1417,1418,1419,1420, 1421,1422,1423,1424,1426,1427,1428,1429,1430
XV	1503,1504,1505,1509,1510,1511,1512,1513,1515,1516,1517,1518,1522,1525,1526, 1527,1528,1529,1530
XVI	1605,1608,1609,1610,1619,1620,1626,1630,1631,1632

[a] Replication origins with a copy number change above significance level 1 (one standard deviation above background) and a p-value < 0.05 were considered activated.

Supplementary Table V. List of tRNA genes, Ty elements and centromeres in the yeast strain S288C that lie within 10 kb of a compromised early origin.

ARS			**Feature**[a]			
Name	**Start (bp)**	**End (bp)**	**Name**	**Start (bp)**	**End (bp)**	**Distance to ARS (kb)**
208	237425	238165	tRNA	227078	227159	10.00
			Centromere	238209	238325	0.04
310	166050	167084	tRNA	168296	168367	1.21
			Ty1 LTR	169568	169883	2.48
315	223463	224292	tRNA	227937	228037	3.65
606	167517	167880	tRNA	167430	167518	0.00
718	420552	421820	tRNA	423098	423211	1.28
728	713976	714879	Ty3 LTR	712210	712549	1.43
			Ty1 LTR	713388	713088	0.89
806	116172	116414	tRNA	116173	116101	0.00
			Ty1 LTR	116746	116415	0.00
909	105676	106107	-			
1305	94602	95145	-			
1319	503388	503770	Ty1 LTR	503191	503388	0.00
			Ty4 LTR	503770	504140	0.00
			Ty1 LTR	504345	504676	0.58
			tRNA-Leu	504894	505007	1.12
1407	89517	90301	-			
1416	325051	325264	-			
1426	635296	635942	tRNA-Pro	631917	631846	3.45
			Ty1 LTR	632114	631998	3.30
			Ty4 LTR	632489	632119	3.18
			tRNA-Asn	632599	632672	2.62
1510	166951	167838	-			

Supplementary Table V. Continued from previous page.

ARS			**Feature**[a]			
Name	**Start (bp)**	**End (bp)**	**Name**	**Start (bp)**	**End (bp)**	**Distance to ARS (kb)**
1512	309359	309925	tRNA-Pro	301097	301198	8.16
			Centromere	326702	326584	16.78
1622	563763	564002	tRNA-Phe	560286	560195	3.48
			Ty1 LTR	560478	560809	3.29
1629	850507	850624	Full length Ty1	844408	850336	0.17
			Ty1 LTR	844408	844745	5.76
			Ty1 LTR	849999	850336	0.17
			Ty1 LTR	850508	850353	0.00
			Ty1 LTR	850962	850625	0.00
			Ty3 LTR	856541	856881	5.92
			Full length Ty1	856550	850625	0.00
			Ty1 LTR	856550	856213	5.59
			tRNA-Ala	856898	856970	6.27

[a] Based on data from the *Saccharomyces* Genome Database (`http://www.yeastgenome.org/`).

Supplementary Table VI. Summary of the total percentages of activated origins and compromised early origins that lie within 10 kb of a tRNA and/or Ty element in the yeast strain S288C.

Origin group	% of origins[a]
All activated origins	34
Activated early origins	44
Activated late origins	25
Compromised early origins	71

[a] Based on data from the *Saccharomyces* Genome Database (`http://www.yeastgenome.org/`).