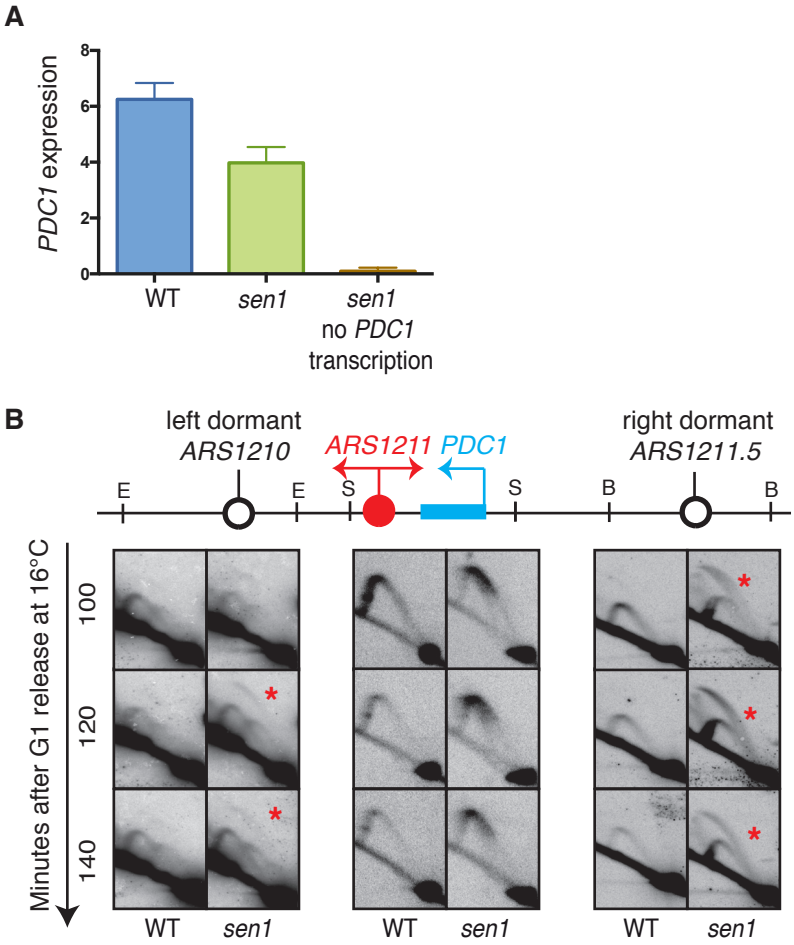
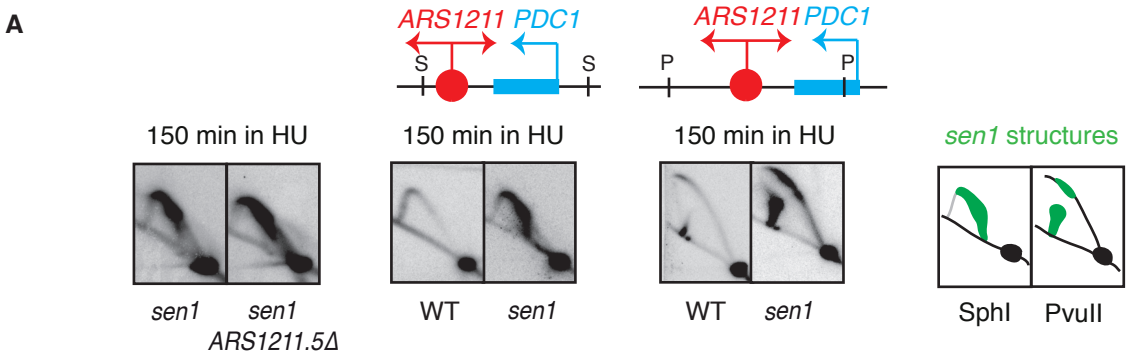
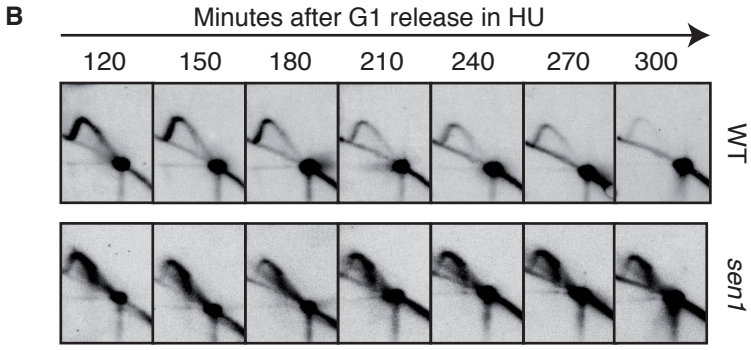
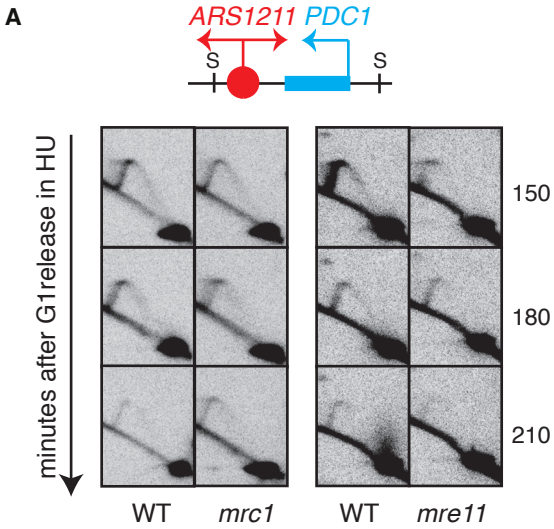
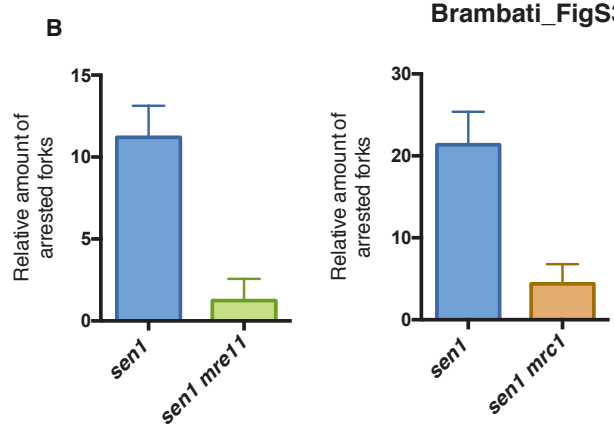
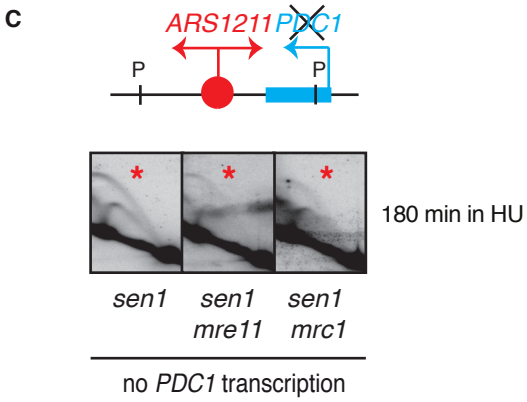

A
PDC1 expression
8
6
4
2
0
WT
sen1
sen1
no PDC1
transcription
B
left dormant
ARS1210
ARS1211 PDC1
right dormant
ARS1211.5
E
E
S
S
B
B
Minutes after G1 release at 16°C
100
120
140
WT
sen1
WT
sen1
WT
sen1

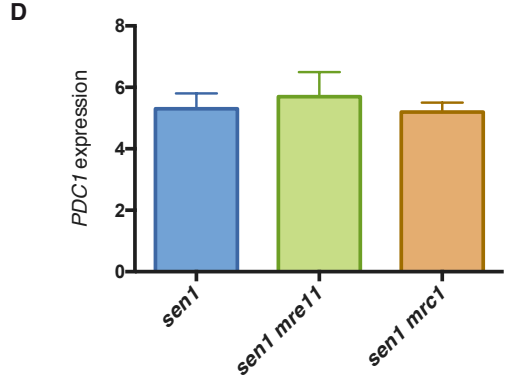

A
ARS1211 PDC1
S
S
ARS1211 PDC1
P
P
150 min in HU
150 min in HU
150 min in HU
sen1 structures
sen1
sen1
ARS1211.5Δ
WT
sen1
WT
sen1
SphI
PvuII


B
Minutes after G1 release in HU
120
150
180
210
240
270
300
WT
sen1


A
ARS1211 PDC1
S
S
minutes after G1release in HU
150
180
210
WT
mrc1
WT
mre11


B
Relative amount of arrested forks
15
10
5
0
sen1
sen1 mre11
Relative amount of arrested forks
30
20
10
0
sen1
sen1 mrc1


C
ARS1211 PDC1
P
P
180 min in HU
sen1
sen1 mre11
sen1 mrc1
no PDC1 transcription


D
PDC1 expression
8
6
4
2
0
sen1
sen1 mre11
sen1 mrc1

**SUPPLEMENTARY FIGURE LEGENDS**

**Figure S1.** Analysis of *PDC1* levels and replicon dynamic in unperturbed conditions. (A) *PDC1* levels in WT, *sen1* and *sen1 no PDC1 transcription* strains were measured by qPCR in cells grown in exponential phase under untreated condition. Data are represented as mean ± SD on the basis of three independent experiments (B) WT and *sen1* cells were synchronized in G1 and released into the cell cycle at 16°C. Genomic DNA was digested with EcoRI (E), SphI (S) or BclI (B) to monitor replication intermediates, respectively, at *ARS1210*, *ARS1211-PDC1* and *ARS1211 loci*. Asterisks indicate replication initiation events.

**Figure S2.** *sen1* 2D gel structures accumulation is not influenced by right dormant origin activation and prolonged HU-treatment. (A) 2D gel analysis of replication intermediates at the *ARS1211-PDC1 locus* in *sen1* and *sen1* carrying mutated right dormant origin (*sen1 ARS1211.5Δ*) upon SphI digestion (S). On the left, 2D gel samples from Figure 1 and 2 with their schematic representation indicate migration of replication intermediates and *sen1* specific structures upon digestion with SphI (S) or PvuII (P). (B) WT and *sen1* mutants were synchronized in G1 and released into 0.2M HU. Genomic DNA was digested with SphI to monitor replication intermediates by 2D gels at the *ARS1211-PDC1 locus*.

**Figure S3.** Analysis of replication *status* at the *ARS1211* origin and *PDC1* expression in *mrc1* and *mre11* mutants. (A) WT, *mrc1* and *mre11* strains were synchronized in G1 and released into 0.2M HU. Genomic DNA was digested with SphI (S) to monitor replication intermediates by 2D gels at the *ARS1211-PDC1 locus*.

(B) Autoradiogram signals obtained from three independent experiments were quantified to assess the levels of the arrested forks at the *PDC1 locus* in *sen1*, *sen1 mre11*, and *sen1 mrc1* mutants (C) *sen1*, *sen1 mre11* (GH688) and *sen1 mrc1* (GH690) carrying a deletion of the *PDC1* promoter were synchronized in G1 and released into 0.2M HU. Genomic DNA was digested with PvuII (P) to monitor replication intermediates at the *ARS1211-PDC1 locus*. Asterisks indicate replication initiation events. (D) *PDC1* levels in *sen1, sen1 mre11* and *sen1 mrc1* strains were measured by qPCR in cells treated for 150 minutes in HU after synchronization in G1. Data are represented as mean ± SD on the basis of three independent experiments.

Table S1. *Saccharomyces cerevisiae* strains used in this study.

| | | |
|---|---|---|
| GF8 | *MATa, ade2-1 trp1-1 leu2-3 112 his3-11,15 ura3 can1-100 GAL PSI⁺RAD5+* | R.Rothstein/ H. Klein |
| GF81 | *W303 MATa, mrc1-AQ::HIS3MYC13* | S. Elledge |
| GH100 | *W303 MATa, ura3::URA3/GPD-TK(7x), rad53K227A-KanMX6* | Lab Stock |
| GH123 | *W303 MATa sen1-G1747D-HIS3MX6* | Lab Stock |
| GH132 | *W303 MATa, ura3::URA3/GPD-TK(7x)* | Lab Stock |
| GH169 | *W303 MATa, NAT1-pGAL1-URL-3HA-SEN1, mre11::KanMX6* | This study |
| GH172 | *W303 MATa, mre11::KanMX6* | Lab Stock |
| GH320 | *W303 MATa, mrc1::KANMX6* | Lab Stock |
| GH344 | *W303 MATa, ura3::URA3/GPD-TK(7x), KanMX6-pGAL1-URL-3HA-SEN1* | Lab Stock |
| GH455 | *W303 MATa, KanMX6-pGAL1-URL-3HA-SEN1* | Lab Stock |
| GH469 | *W303 MATa, NAT1-pGAL1-URL-3HA-SEN1, mrc1::KanMX6* | This study |
| GH472 | *W303 MATa, NAT1-pGAL1-URL-3HA-SEN1, ctf4::TRP1* | This study |
| GH531 | *W303 MATa, KanMX6-pGAL1-URL-3HA-SEN1, mre11D56N-HIS3MX* | L.Symington /This study |
| GH535 | *W303 MATa, NAT1-pGAL1-URL-3HA-SEN1, rad50::KanMX6* | This study |
| GH538 | *W303 MATa, NAT1-pGAL1-URL-3HA-SEN1, xrs2::KanMX6* | This study |
| GH541 | *W303 MATa, mre11D56N-HIS3MX6* | L.Symington /This study |
| GH551 | *W303 MATa, ars1211.5Δ (deletion from coordinates 243503 to 243813)* | This study |

| | | |
|---|---|---|
| GH557 | *W303 MATa, KanMX6-pGAL1-URL-3HA-SEN1, ars1211.5Δ (deletion from coordinates 243503 to 243813)* | This study |
| GH560 | *W303 MATa, NAT1-pGAL1-URL-3HA-SEN1, mrc1-AQ::HIS3MYC13* | This study |
| GH566 | *W303 MATa, KanMX6-pGAL1-URL-3HA-SEN1, ars1211.5Δ (deletion from coordinates 243503 to 243813), mre11::HIS3MX6* | This study |
| GH574 | *W303 MATa, NAT1-pGAL1-URL-3HA-SEN1, hog1::HIS3MX6* | This study |
| GH578 | *W303 MATa, NAT1-pGAL1-URL-3HA-SEN1, tof1::KanMX6* | This study |
| GH586 | *W303 MATa, KanMX6-pGAL1-URL-3HA-SEN1, ars1211.5Δ (deletion from coordinates 243503 to 243813), mrc1::HIS3MX6* | This study |
| GH612 | *W303 MATa, KanMX6-pGAL1-URL-3HA-SEN1, PDC1promoterΔ (deletion from coordinates 234406 to 235141)* | This study |
| GH688 | *W303 MATa, KanMX6-pGAL1-URL-3HA-SEN1, PDC1promoterΔ (deletion from coordinates 234406 to 235141), mre11::HIS3MX6* | This study |
| GH688 | *W303 MATa, KanMX6-pGAL1-URL-3HA-SEN1, PDC1promoterΔ (deletion from coordinates 234406 to 235141), mrc1::HIS3MX6* | This study |
| GH690 | *W303 MATa, KanMX6-pGAL1-URL-3HA-SEN1, PDC1promoterΔ (deletion from coordinates 234406 to 235141), mrc1::HIS3MX6* | This study |
| GH718 | *W303 MATa, NAT1-pGAL1-URL-3HA-SEN1, mrc1::KANMX6, rad51::LEU2* | This study |
| GH790 | *W303 MATa, NAT1-pGAL1-URL-3HA-SEN1, exo1::HIS3MX6, mrc1::KanMX6* | This study |
| GH793 | *W303 MATa, NAT1-pGAL1-URL-3HA-SEN1, exo1::HIS3MX6* | This study |
| GH869 | *W303 MATa, NAT1-pGAL1-URL-3HA-SEN1, mre11::HIS3MX6, KanMX6-pGAL1-URL-3HA-EXO1* | This study |
| GH876 | *W303 MATa, ars1211Δ (deletion from coordinates 231248 to 231292)* | This study |
| GH977 | *W303 MATa, ura3::URA3/GPD-TK(7x), ars1210::HIS3MX6* | This study |
| GH979 | *W303 MATa, ura3::URA3/GPD-TK(7x), NAT1-pGAL1-URL-3HA-SEN1, ars1210::HIS3MX6* | This study |

Table S2. List of primers used in this study

| **Name** | **Sequence (5'-3')** |
|---|---|
| ARS1211Fa | TCTTCGGCTTACCGGTCTTG |
| ARS1211Rb | CGCAACCTTTCAGTTGGGC |
| STU2 Fa | GCACATCACATCAGCGGAAC |
| STU2 Rb | ATGCAAAGAGGTGGTACCCG |
| RIC1 Fe | AACCAACGAGCTCTTGCTAAC |
| RIC1 Rf | TTTCCAAAGTCGGGGATGGG |
| PAU23 Fa | TGGGCTCCCCTATCCCATAC |
| PAU23 Rb | ACCGAACATTCCTGTGCTCC |
| 15 kb Fa | CGCATGACCATCCACGAACT |
| 15 kb Rb | ACAAAGTGGAGCGAACTGGT |
| PDC1 Fa | CAGCAACTGGCTTGTAACCC |
| PDC1 Rb | CCCCAATGGGTAAGGGTTCC |
| ACT1 Fa | TGAAGAAGATTGAGCAGCGG |
| ACT1 Rb | TTCTACGTTTCCATCCAAGCCG |