Supplemental Figures

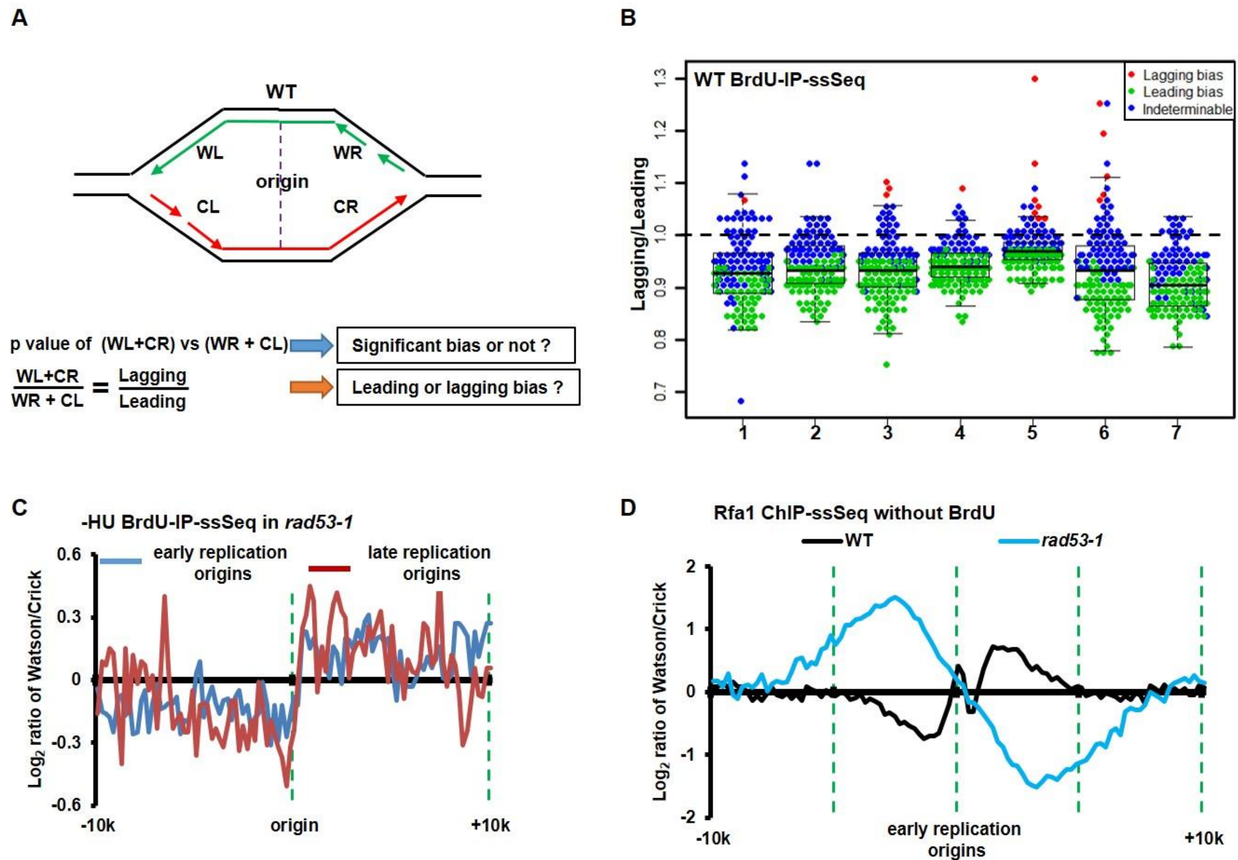


Figure S1. Related to Figure 1 and 2. **WT cells shows a small leading bias pattern with HU and *rad53-1* mutant cells do not show any lagging strand bias without HU treatment**. (**A-B**) BrdU-IP-ssSeq peaks from WT cells treated with HU shows a small leading bias pattern. (**A**) The equation to determine the bias pattern for the individual origins: sequencing reads of each BrdU-IP-ssSeq peak were split into four parts (WL, WR, CL and CR) according to Watson (W) and Crick (C) strands and the left (L) and right (R) sides of DNA replication origin. The p-value of the leading strand sequence reads (WL+CR) versus that of lagging strand (WR+CL) was used to determine whether there was a significant difference between sequence reads of leading and lagging strands at each fired origins. 10^{-5} was used as the p-value cut-off criterion. The ratio of

sequence reads at leading over lagging was used to measure the leading or lagging bias pattern. BrdU-IP-ssSeq peaks at 134 origins were separated into three categories (leading, lagging and indeterminable) based on p-values and $\log_2$ ratio of sequence reads of leading over lagging. (**B**) Seven repeats of BrdU-IP-ssSeq of wild type cells were shown. Please note that while there were variations among experiments performed in a 5-year span, and results from all these independent repeats show that newly synthesized lagging strand DNA was less than leading strand in wild type cells. **(C)** BrdU-IP-ssSeq peaks in *rad53-1* mutant cells without HU treatment do not show any lagging strand bias. G1-arrested cells were released fresh YPD media at 25°C containing 400 µg/ml bromodeoxyuridine (BrdU). Cells were collected after 30 minutes' release cells for BrdU-IP-ssSeq. (**D**) RPA proteins are enriched at leading strand templates in *rad53-1* mutant cells without the incorporation of BrdU. The average bias at early replication origins for Rfa1 ChIP-ssSeq peaks in wild type and *rad53-1* mutant cells was calculated and shown. The experiment was performed according to the same procedure as described in Figure 2 except that BrdU was not used in the experiment. The purpose of the experiment was to test whether BrdU incorporation leads to the long ssDNA in *rad53-1* mutant cells. The long ssDNA of *rad53-1* mutant cells may be due to different BrdU incorporation efficiency at leading and lagging strand DNA. Alternatively, the incorporated BrdU will induce DNA damage/DNA processing at leading and lagging strand differently. The results show that RPA still bound long stretches of ssDNA of leading template strand in *rad53-1* mutant cells without BrdU incorporation, ruling out complication of BrdU incorporation.

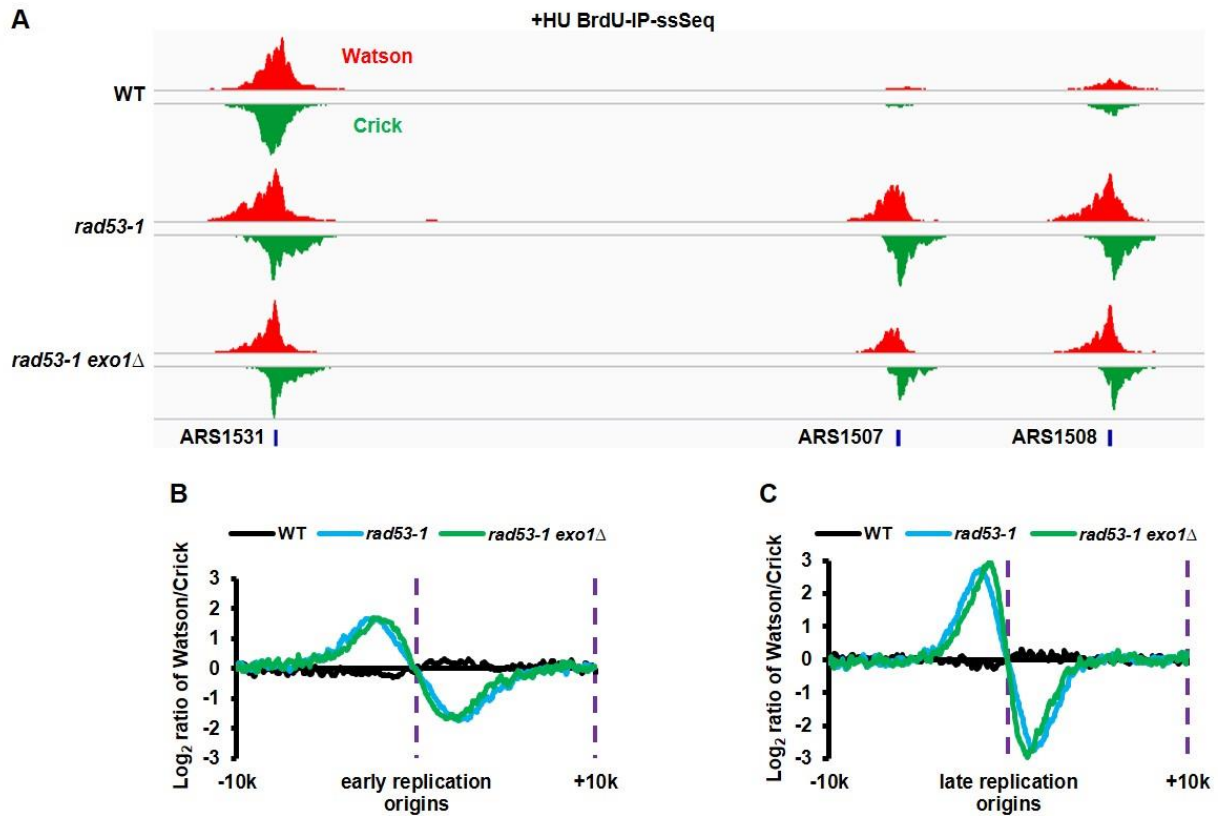
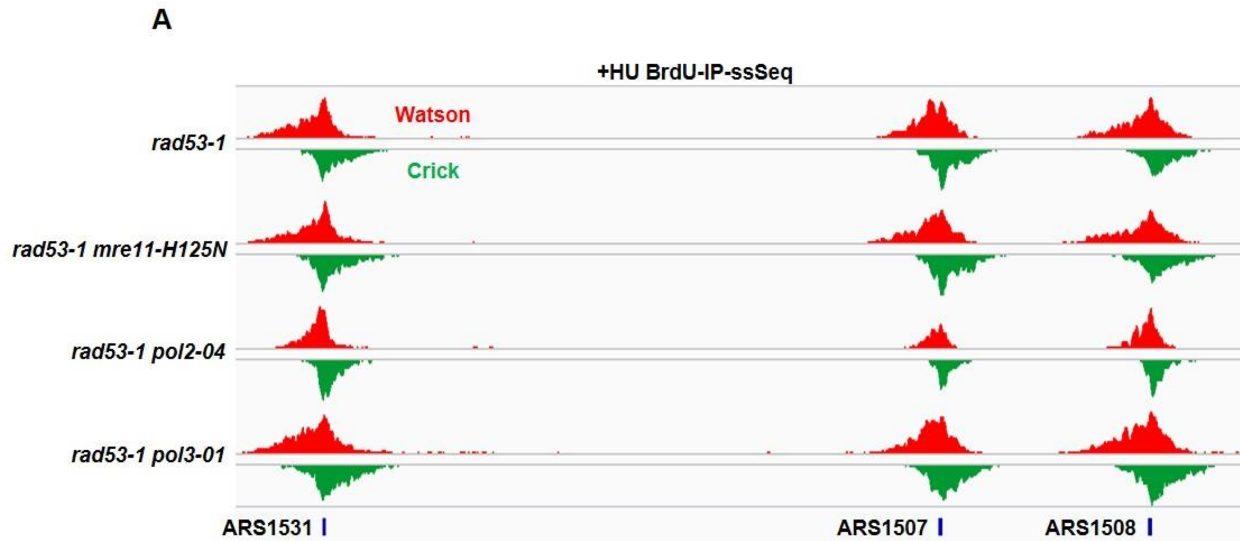


Figure S2: Related to Figure 3 and 4. Deletion of *EXO1* does not affect the lagging-strand bias pattern of *rad53-1* mutant. (**A**) A snap shot of BrdU-IP-ssSeq in wild type, *rad53-1*, and *rad53-1 exo1*Δ mutation cells treated with HU. (**B-C**) The average of $\log_2$ ratios of sequence reads of Watson over Crick strand surrounding all early (B) or late (C) replication origins were calculated using a 200 bp sliding window.


A
+HU BrdU-IP-ssSeq
Watson
Crick
rad53-1
rad53-1 mre11-H125N
rad53-1 pol2-04
rad53-1 pol3-01
ARS1531
ARS1507
ARS1508

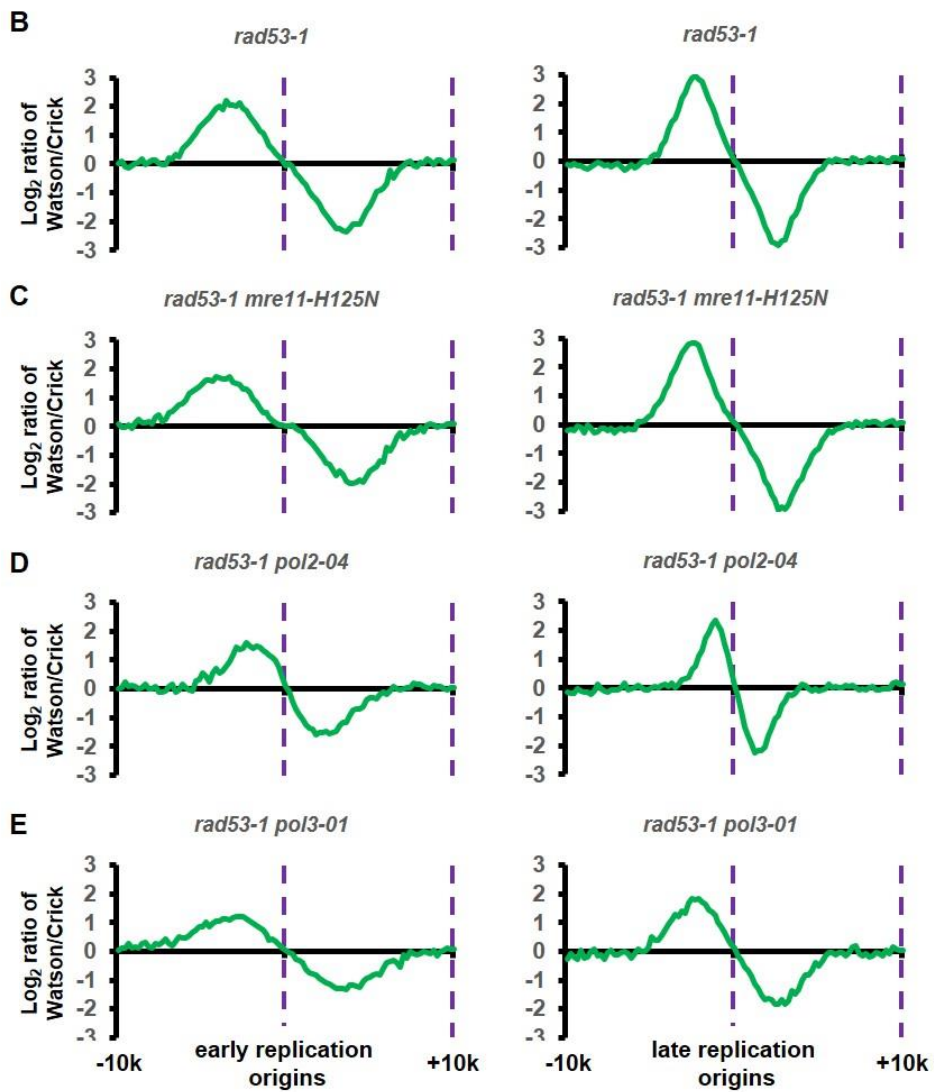

B
rad53-1
rad53-1
C
rad53-1 mre11-H125N
rad53-1 mre11-H125N
D
rad53-1 pol2-04
rad53-1 pol2-04
E
rad53-1 pol3-01
rad53-1 pol3-01
Log2 ratio of Watson/Crick
3
2
1
0
-1
-2
-3
-10k
early replication origins
+10k
-10k
late replication origins
+10k

Figure S3. Related to Figure 3 and 4. Inactivation of the exo-nuclease activity of Mre11, Pol2 (catalytic subunit of Polε and Pol3 (catalytic subunit of Polδ) in *rad53-1* mutant cells does not affect the bias pattern of BrdU-IP-ssSeq peaks dramatically. (**A**) A snap shot of BrdU-IP-ssSeq peaks at three replication origins in *rad53-1*, *rad53-1 mre11-H125N*, *rad53-1 pol2-04* and *rad53-1 pol3-01* mutation cells treated with HU. The *mre11-H125N* (Moreau et al., 2001), *pol2-04* (Morrison et al., 1991), and *pol3-01 (Simon et al., 1991)* are 3′ to 5′ exonuclease-deficient mutants. (**B-E**) The average bias pattern of BrdU-IP-ssSeq at early (left panel) and late (right panel) replication origins in *rad53-1* (B), *rad53-1 mre11-H125N* (C), *rad53-1 pol2-04* (D) and *rad53-1 pol3-01* (E) cells.

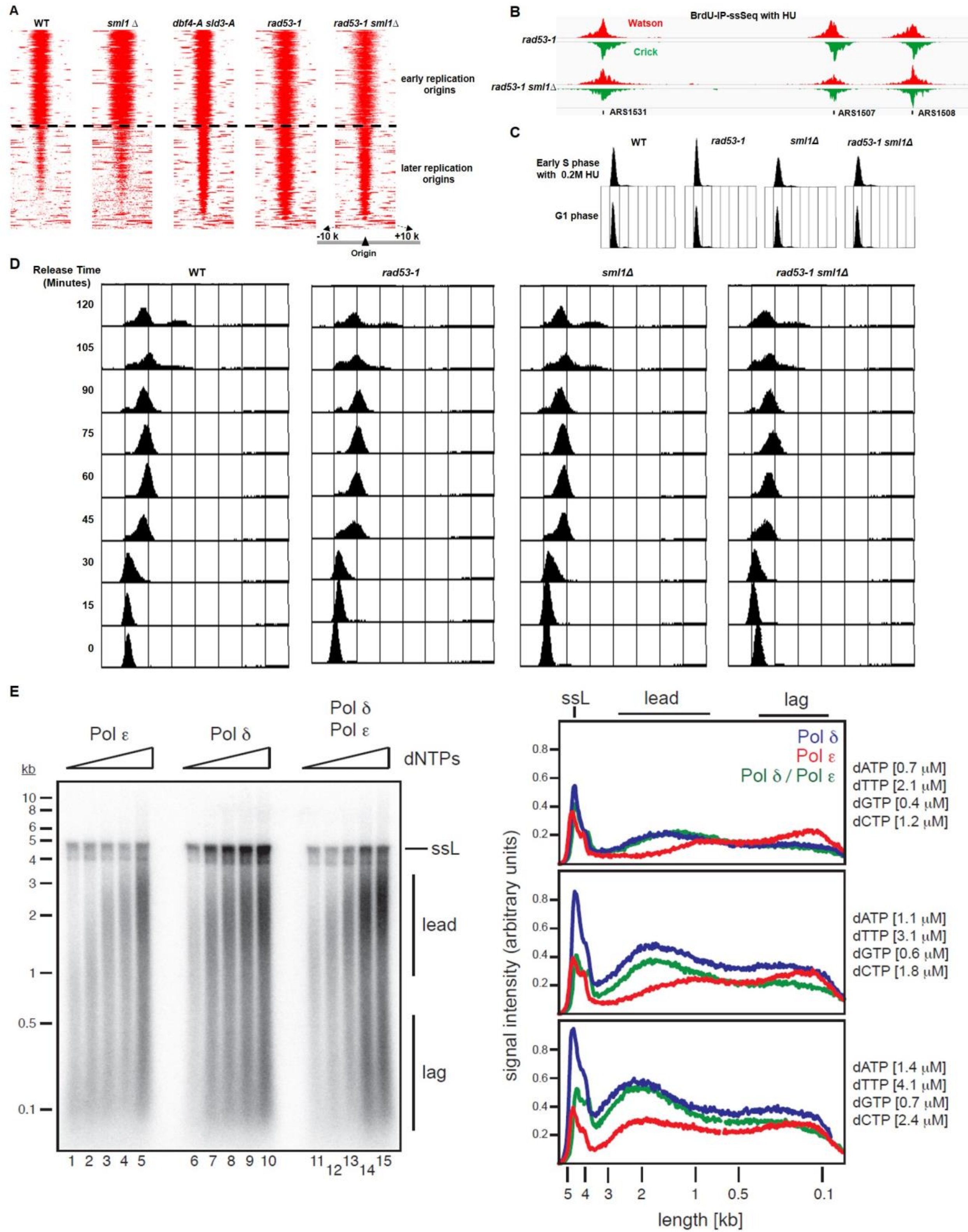

A
WT
sml1 Δ
dbf4-A sld3-A
rad53-1
rad53-1 sml1Δ
early replication origins
later replication origins
-10 k
+10 k
Origin
B
BrdU-IP-ssSeq with HU
Watson
Crick
rad53-1
rad53-1 sml1Δ
ARS1531
ARS1507
ARS1508
C
WT
rad53-1
sml1Δ
rad53-1 sml1Δ
Early S phase with 0.2M HU
G1 phase
D
Release Time (Minutes)
WT
rad53-1
sml1Δ
rad53-1 sml1Δ
120
105
90
75
60
45
30
15
0
E
Pol ε
Pol δ
Pol δ
Pol ε
dNTPs
kb
10
8
6
5
4
3
2
1
0.5
0.1
ssL
lead
lag
1 2 3 4 5
6 7 8 9 10
11 12 13 14 15
ssL
lead
lag
Pol δ
Pol ε
Pol δ / Pol ε
dATP [0.7 μM]
dTTP [2.1 μM]
dGTP [0.4 μM]
dCTP [1.2 μM]
dATP [1.1 μM]
dTTP [3.1 μM]
dGTP [0.6 μM]
dCTP [1.8 μM]
dATP [1.4 μM]
dTTP [4.1 μM]
dGTP [0.7 μM]
dCTP [2.4 μM]
signal intensity (arbitrary units)
0.8
0.6
0.4
0.2
5 4 3 2 1 0.5 0.1
length [kb]

Figure S4. Related to Figure 4. *SML1* deletion in *rad53-1* mutant cells does not affect cell cycle progression. (**A**) Late replication origins fire in *dbf4-A sld3-A*, and *rad53-1* mutant cells. Heatmaps representing the signal of BrdU-IP-ssSeq at 310 non-telomere origins in Wild type (WT), *sml1*Δ, *dbf4-A sld3-A*, and *rad53-1* mutant cells. The early and later replication origins are defined in (Yu et al., 2014). The *dbf4-A sld3-A* mutant was from the Dr. Diffley's laboratory. (**B**) A snap shot of BrdU-IP-ssSeq peaks in *rad53-1* and *rad53-1* mutant cells released from G1 into HU. This is an independent repeat of Figure 4A. (**C**) FACS analysis of Wild type (WT), *rad53-1*, *sml1*Δ, *and rad53-1 sml1*Δ mutant cells released from G1 block into early S phase in the presence of HU. (**D**) FACS analysis of Wild type (WT), *rad53-1*, *sml1*Δ, *and rad53-1 sml1*Δ mutant cells released from G1 block into the cell cycle without HU. Yeast cells were synchronized at G1 using alpha factor. Then G1 cells were released into fresh YPD medium at 25 °C and samples were taken every 15mins. (**E**) Impaired leading strand synthesis by Pol ε at low dNTP concentrations. Reconstituted DNA replication reactions were carried out on chromatinized pARS1 (4.8 kb) in the presence of Pol ε and absence of Pol δ (lanes 1-5), in the presence of Pol δ and the DNA polymerase-deficient Pol ε mutant Pol ε^{pol-} (lanes 6-10), or in the presence of both Pol δ and Pol ε (lanes 11-15). dNTPs were included at 4.9 µM dCTP, 8.3 µM dTTP, 2.8 µM dATP, and 1.4 µM dGTP (lanes 5, 10, 15), or at 1.5-fold (lanes 4, 9, 14), two-fold (lanes 3, 8, 13), three-fold (lanes 2, 7, 12), or four-fold (lanes 1, 6, 11) reduced concentrations. DNA products were analyzed by alkaline agarose gel-electrophoresis and autoradiography in the panel on the left; the gel positions of full-length single-stranded DNA (ssL; resulting from nick-labeling), as well as leading (lead) and lagging (lag) strand products are indicated. Traces of signal intensities for lanes 1, 6, and 11 (top panel), lanes 2, 7, and 12 (middle panel), or lanes 3, 8, and 13 (bottom panel) are shown on the right.

Table S1. Related to STAR method. Yeast strains used in this study.

Strains	Genotype	Reference
cvy43	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hisG + URA3::BrdU–Inc*	(Knott et al., 2009; Viggiani and Aparicio, 2006)
zgy2566	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hisG POL2-5flag::kanMX6+ URA3::BrdU–Inc*	(Yu et al., 2014)
cyc209	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 Pol2-5flag::kanMX6 + URA3::BrdU–Inc*	(Yu et al., 2014)
zgy3112	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 POL2-5flag::kanMX6 rad53-1+ URA3::BrdU–Inc*	This study
cyc215	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hisG RFA1-flag::KANMX6 +URA3::BrdU–Inc*	(Yu et al., 2014)
zgy3171	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 RFA1-flag::KANMX6 rad53-1+URA3::BrdU–Inc*	This study
cyc126	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1- MCM6-3HA::LEU +URA3::BrdU–Inc*	(Yu et al., 2014)
zgy3134	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 MCM6-3HA::LEU rad53-1 + URA3::BrdU–Inc*	This study
zgy3189	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 dbf4Δ::TRP1 his3::PDBF4-dbf4 19A::HIS3 sld3-38A-10his-13myc::KanMx + URA3::BrdU–Inc*	This study
cyc311	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 sml1::HIS, + URA3::BrdU–Inc*	This study
zgy3182	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 sml1::HIS rad53-1 + URA3::BrdU–Inc*	This study
zgy3111	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hisG rad53-1+ URA3::BrdU–Inc*	This study
zgy3217	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 rad53-1 exo1Δ::kanMX6+ URA3::BrdU–Inc*	This study
zgy3379	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 rad53-1 mre11-H125N + LEU2::BrdU–Inc*	This study
zgy3381	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 rad53-1 pol2-04 LEU2::BrdU–Inc*	This study
zgy3383	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 rad53-1 pol3-01 LEU2::BrdU–Inc*	This study