Supplemental information

Both DNA Polymerases δ and ε Contact Active and Stalled Replication Forks differently

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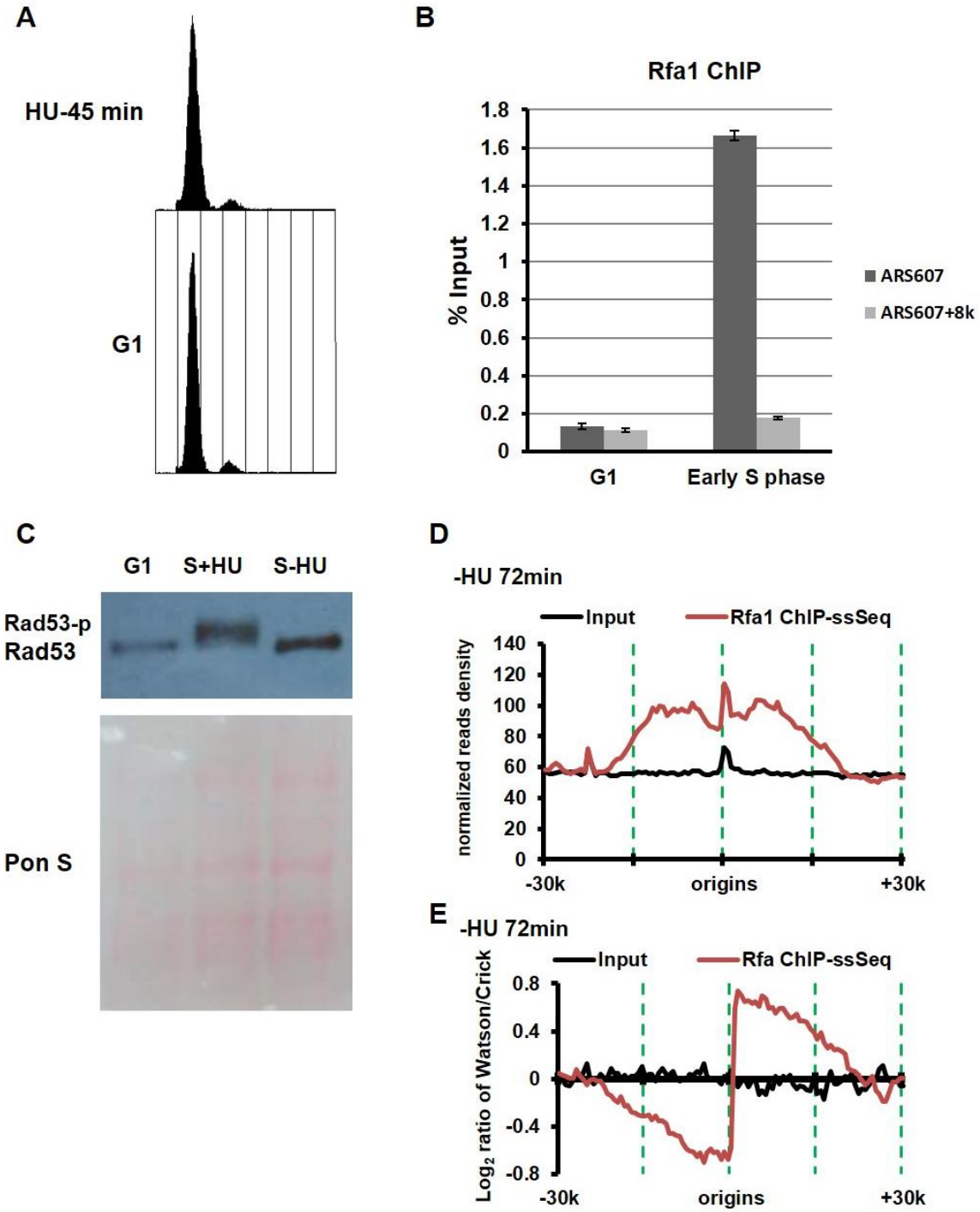
Supplemental Figure 1

RPA is enriched at replication forks. (A) FACS analysis of DNA contents of G1 cells (arrested with α factor) as well as cells that were released into medium containing HU for 45 minutes from the G1 block. **(B)** Rfa1 ChIP-PCR analysis shows that RPA binds to the early replication origin *ARS607*. ChIP assays were performed using antibodies against FLAG-tagged Rfa1, and the ChIP DNA was analyzed by real-time PCR using primers amplifying replication origin (*ARS607*) and a distal site (ARS607+8kb). **(C)** Rad53 is activated upon HU-induced replication stress. G1 cells were released into S phase with/without HU condition. The 5 ml cells samples (G1, S phase with HU for 45mins and S phase without HU for 45mins) were collected. Total proteins were prepared using the TCA methods and analyzed by Western blot. **(D)** Normalized reads plots of Rfa1 ChIP-ssSeq signals within a 30Kb window centered on early replication origins without HU conditions. Log-phase cells were synchronized to G1 with α factor and then released into fresh YPD medium for 72 minutes at 16ºC. Cells were collected for Rfa1 ChIP-ssSeq. **(E)** The average bias at early replication origins for Rfa1 ChIP-ssSeq without HU conditions. The average log2 ratios of Watson strand over Crick strand surrounding all early replication origins were calculated as described in Fig. 2B.

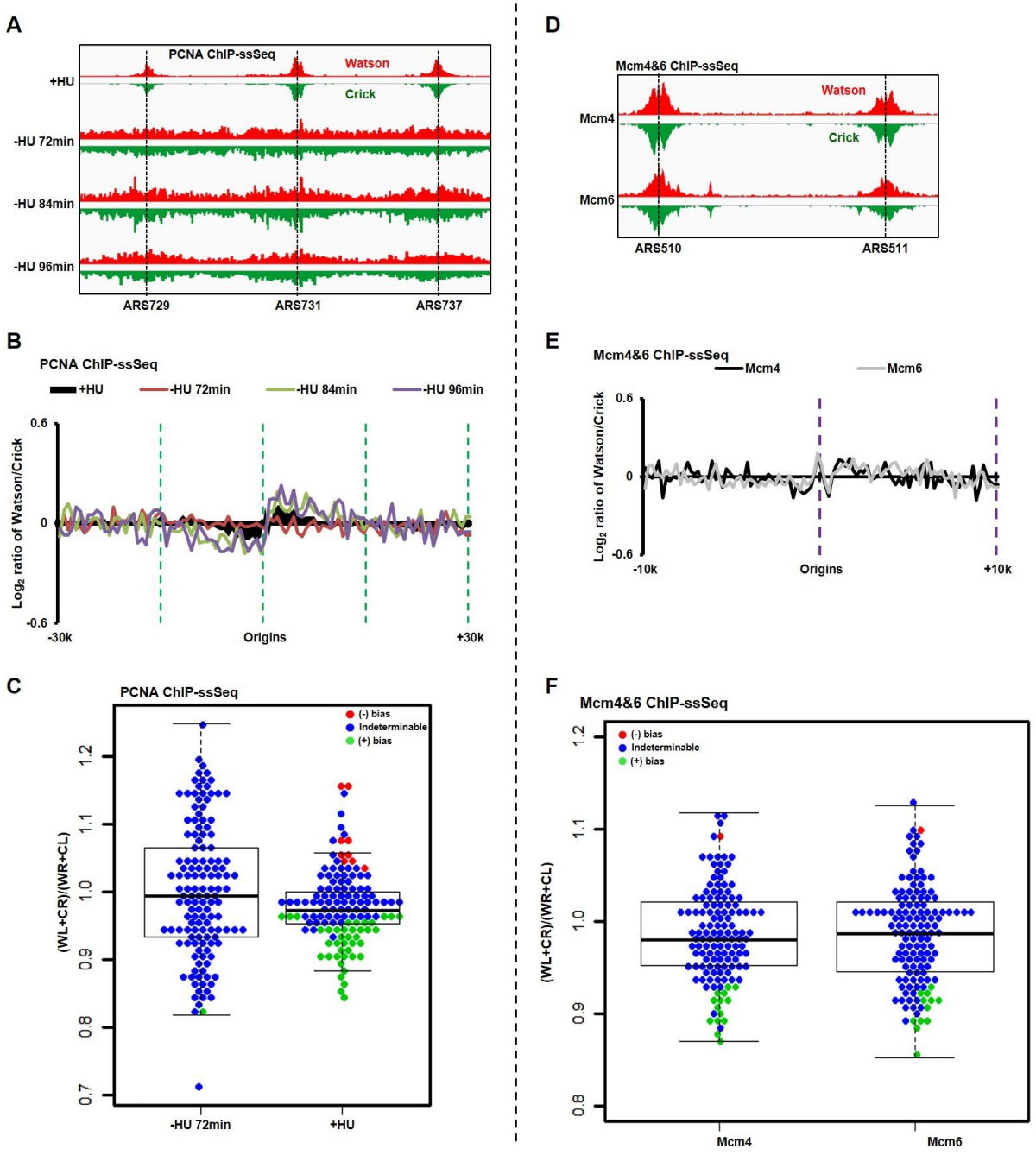

A
HU-45 min
G1
B
Rfa1 ChIP
% Input
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
G1
Early S phase
ARS607
ARS607+8k
C
G1
S+HU
S-HU
Rad53-p
Rad53
Pon S
D
-HU 72min
Input
Rfa1 ChIP-ssSeq
normalized reads density
140
120
100
80
60
40
20
0
-30k
origins
+30k
E
-HU 72min
Input
Rfa ChIP-ssSeq
Log2 ratio of Watson/Crick
0.8
0.4
0
-0.4
-0.8
-30k
origins
+30k

37

38

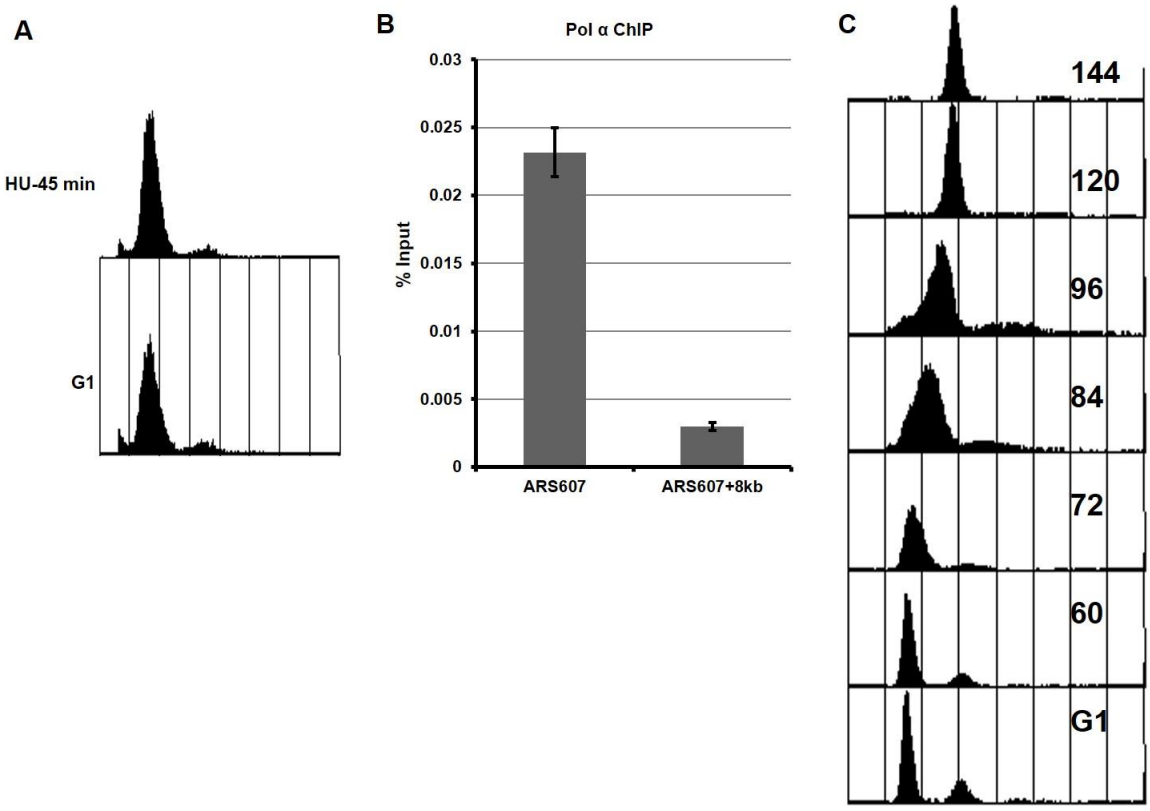
Supplemental Figure 2

PCNA, Mcm4 and Mcm6 ChIP-ssSeq peaks do not show strand bias. (A-C) PCNA ChIP-ssSeq peaks at active and stalled replication forks show no strand bias pattern. PCNA ChIP-ssSeq was performed as described in experimental procedures using early S phase cells in the presence of HU (+HU) at 30 °C for 45 min or without HU at 16 °C at indicated time points. **(A)** A snap shot of PCNA ChIP-ssSeq at three indicated origins. **(B)** Analysis of PCNA ChIP-ssSeq peaks using a 200bp sliding window. **(C)** Analysis of PCNA ChIP-ssSeq peaks at individual replication origins. **(D-F)** Mcm 4 and Mcm6 ChIP-ssSeq peaks at stalled replication forks show no strand bias. **(D)** A snap shot of Mcm4 and Mcm6 ChIP-ssSeq at the indicated origins. **(E)** Analysis of the average bias pattern of Mcm4 and Mcm6 ChIP-ssSeq peaks using a 200bp sliding window. **(F)** Analysis of Mcm4 and Mcm6 ChIP-ssSeq peaks at individual replication origins. The colors in dot and box plots represent 3 bias patterns: red for positive bias (+); green for negative bias (−); and blue for indeterminable.


A
PCNA ChIP-ssSeq
Watson
Crick
+HU
-HU 72min
-HU 84min
-HU 96min
ARS729
ARS731
ARS737
B
PCNA ChIP-ssSeq
+HU
-HU 72min
-HU 84min
-HU 96min
Log2 ratio of Watson/Crick
0.6
0
-0.6
-30k
Origins
+30k
C
PCNA ChIP-ssSeq
(-) bias
Indeterminable
(+) bias
(WL+CR)/(WR+CL)
-HU 72min
+HU
D
Mcm4&6 ChIP-ssSeq
Watson
Crick
Mcm4
Mcm6
ARS510
ARS511
E
Mcm4&6 ChIP-ssSeq
Mcm4
Mcm6
Log2 ratio of Watson/Crick
-10k
Origins
+10k
F
Mcm4&6 ChIP-ssSeq
(-) bias
Indeterminable
(+) bias
(WL+CR)/(WR+CL)
Mcm4
Mcm6

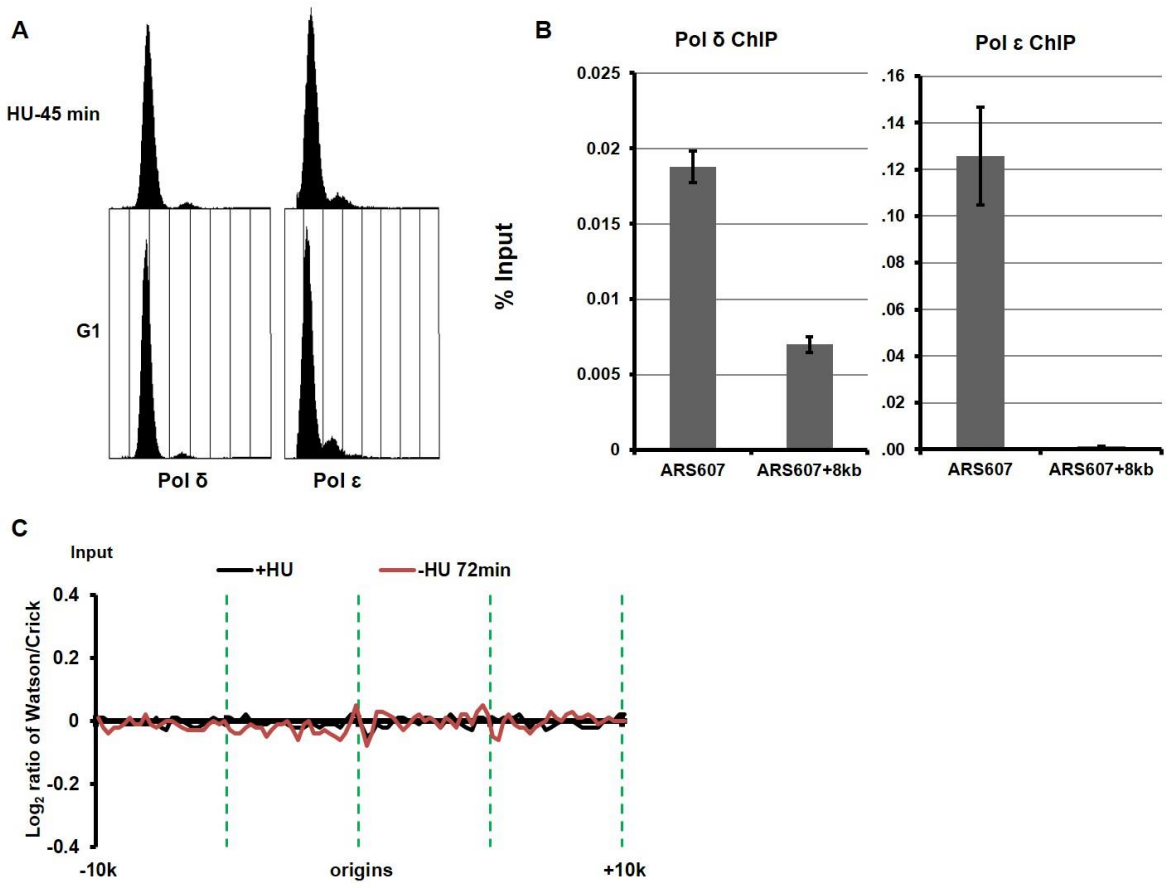
Supplemental Figure 3

Pol α is enriched at replicating DNA. (A) FACS analysis of G1 cells as well as cells released from G1 into medium containing HU for 45 minutes. **(B)** ChIP experiments show that Pol α associates early replication origin *ARS607*. ChIP assays were performed using antibodies against the FLAG-tagged *Pol1* gene and ChIP DNA was analyzed as described above. **(C)** Cell cycle analysis of yeast cells used for Pol α ChIP experiments described in Figure 3 (C-E). G1-synchronized cells were released into S phase at 16°C in the presence of BrdU. Cells were collected at the indicated time points (in minutes) for analysis of DNA content by flow cytometry.



Supplemental Figure 4

Pol δ and Pol ε are enriched at replicated loci. (A) FACS analysis of DNA content of cells used for Pol ε and Pol δ ChIP. **(B)** ChIP experiments show that Pol ε and Pol δ associates early replication origin *ARS607*. ChIP assays were performed using antibodies against the FLAG-tagged *Pol2* and *Pol3*, and the immunoprecipitated DNA was analyzed by using real time PCR. **(C)** Average bias at early replication origins of the input sample with HU or without HU treatment. Pol δ input reads were used for calculation.



Supplemental Table 1 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*	(1, 2)
zgy2565	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hisG POL1-5flag::kanMX6+ URA3::BrdU–Inc*	(3)
zgy2566	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hisG POL2-5flag::kanMX6+ URA3::BrdU–Inc*	(3)
zgy2567	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hisG POL3-5flag::kanMX6+ URA3::BrdU–Inc*	(3)
cyc209	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 Pol2-5flag::kanMX6 + URA3::BrdU–Inc*	(3)
cyc215	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hisG RFA1-flag::KANMX6 +URA3::BrdU–Inc*	(3)

Supplemental Table 2 qPCR primers used in this study.

Name	Forward Primer	Reverse Primer
ARS607	cggctcgtgcattaagcttg	tgccgcacgccaaacattgc
ARS607+8kb	tcacctattttcccatcataccg	aggatgatcaaggcggcag

Supplemental references

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3. **Yu, C., H. Gan, J. Han, Z. X. Zhou, S. Jia, A. Chabes, G. Farrugia, T. Ordog, and Z. Zhang.** 2014. Strand-Specific Analysis Shows Protein Binding at Replication Forks and PCNA Unloading from Lagging Strands when Forks Stall. Mol Cell **56:**551-563.