KEY RESOURCES TABLE

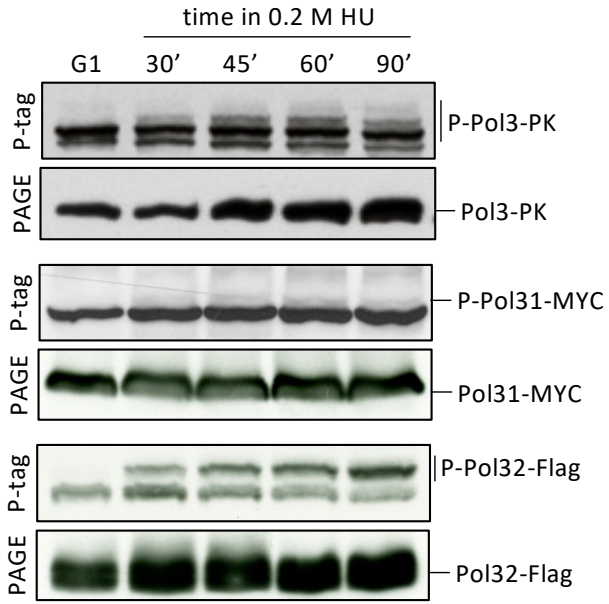
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-Flag (M2)	Sigma	F1804
anti HA (12CA5)	Roche	11666606001
Anti-Myc Tag (4A6)	Merck millipore	05-724
anti PK Anti-V5 Tag (SV5-Pk1)	Bionova	MCA1360
anti-Mouse-HRP	Taper	T03.PI2000M001
Chemicals, Peptides, and Recombinant Proteins		
RNase A	Sigma	R5503
Shortcut RNase III	NEB	M0245
Proteinase K	Roche	03115852001
Hydroxyurea	IbianTechnologies	HDU0250
Metyl methanesulfonate	Sigma	129925
Camptothecin	Sigma	C9911
Propidium iodide	Sigma	P4170
Trimetilpsoralen	Sigma	P8399
Phos-tag acrylamide	Rafer	300-93523
Complete Protease Inhibitor-EDTA free	Roche	11873580001
Alfa-factor Mating Pheromone	Insight Biotechnology	N/A
Spermine	Sigma	S1141
Spermidine	Sigma	S2501
QBT	QIAGEN	19054
QC	QIAGEN	19055
QF	QIAGEN	19056
QIAGEN Genomic-Tips 100/G	QIAGEN	10243
QIAGEN Genomic-Tips 20/G	QIAGEN	10223
Benzoylated Naphthoylated DEAE-Cellulose	Sigma	B6385
Poly-Prep chromatography column	Biorad	7311550
Amicon Ultra-0.5 ml 100K	Merck millipore	UFC510096
Deposited Data		
CGS data	GEO	GSE156480
Experimental Models: Organisms/Strains		
A full list of yeast strains used in this study is provided in Table S1	This paper	N/A
Oligonucleotides		
5'-GGGTGAAGCGTGATGACTATTTACCACAAGG-3'	This paper	pol2-S430DFw
5'-CCTTGTGGTAAATAGTCATCACGCTTCACCC-3'	This paper	pol2-S430DRev
5'-GGGTGAAGCGTGATGCTTATTTACCACAAGG-3'	This paper	pol2-S430AFw
5'-CCTTGTGGTAAATAAGCATCACGCTTCACCC-3'	This paper	pol2-S430ARev
5'-GGGTTAAGAGAGACGACTACTTGCCACAAGG-3'	This paper	pol2-S430DFw-oc
5'-CCTTGTGGCAAGTAGTCGTCTCTCTTAACCC-3'	This paper	pol2-S430DRev-oc
5' GGGTTAAGAGACGACGCTTACTTGCCACAAGG 3'	This paper	pol2-S430AFw-oc
5' CCTTGTGGCAAGTAAGCGCAGTCTCTTAACCC 3'	This paper	pol2-S430ARev-oc
Recombinant DNA		
pRS415-POL2	Herr lab	N/A
pRS415-pol2-S430D	This paper	N/A
pRS415-pol2-S430A	This paper	N/A
pAJ6	Yeeles lab	N/A
pAJ6-pol2-S430D	This paper	N/A

pAJ6-pol2-S430A	This paper	N/A
Software and Algorithms		
R version 3.6.1 (2019-07-05) -- "Action of the Toes" Platform: x86_64-apple-darwin15.6.0 (64-bit)	R Core Team 2013	N/A
Repliscope version '1.1.0'	Muller at al. 2014	N/A
Bowtie2 version 2.3.5.1	Langmead and Salzberg 2012	N/A
SAMtools version 1.9 (htslib 1.9)	Li et al. 2009	N/A
Bedtools version 2.29.0	Quinlan and Hall 2010	N/A
Deeptools version 3.4.3	Ramirez et al. 2014	N/A
Bedgraphtobigwig version 2.8 (bbi version 4)	Kent et al. 2010	N/A

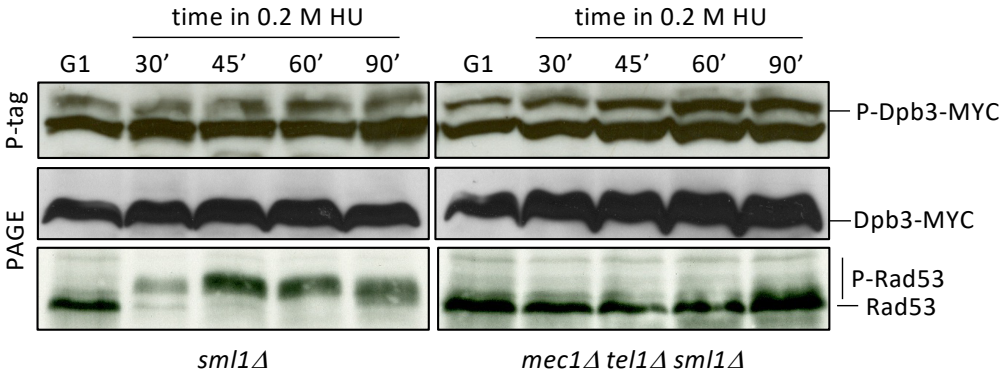
A

Subunit	*S. cerevisiae*	Function
A	Pol3	5'-3'polymerase, 3-5'exonuclease
B	Pol31	regulatory
C	Pol32	regulatory

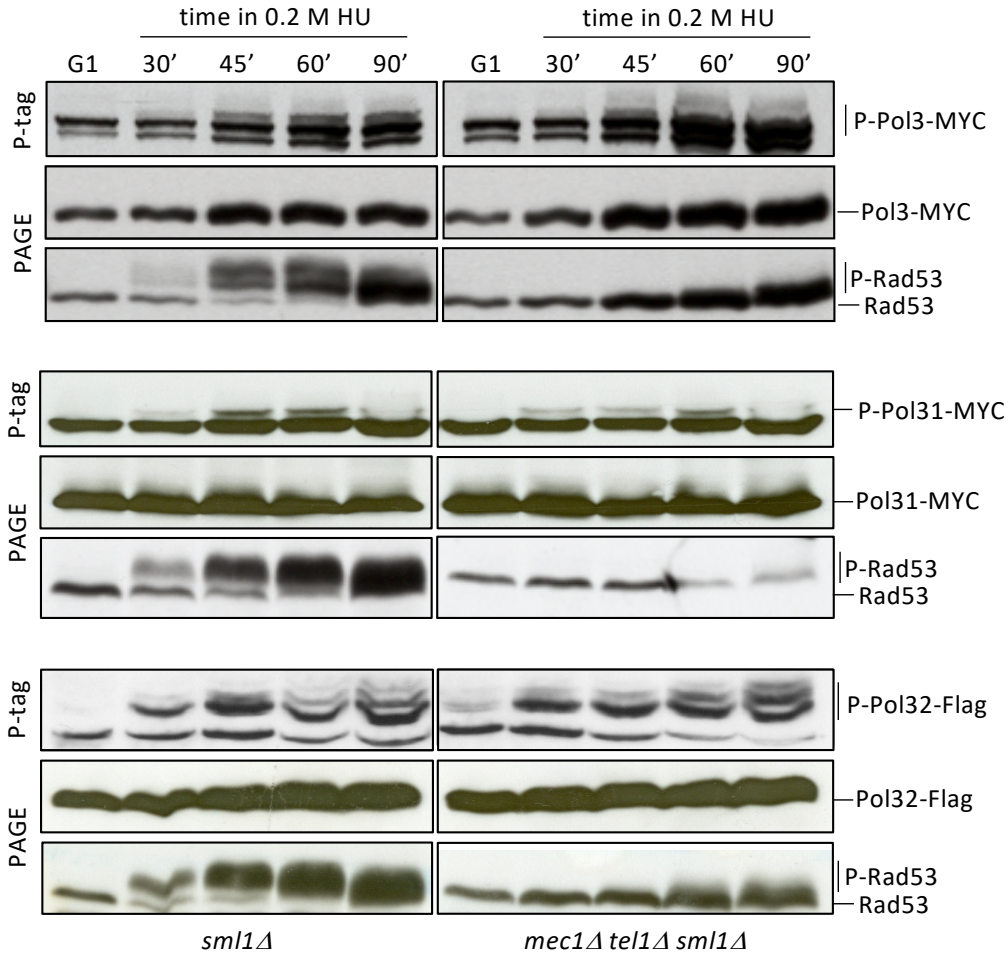
B



C



D



E

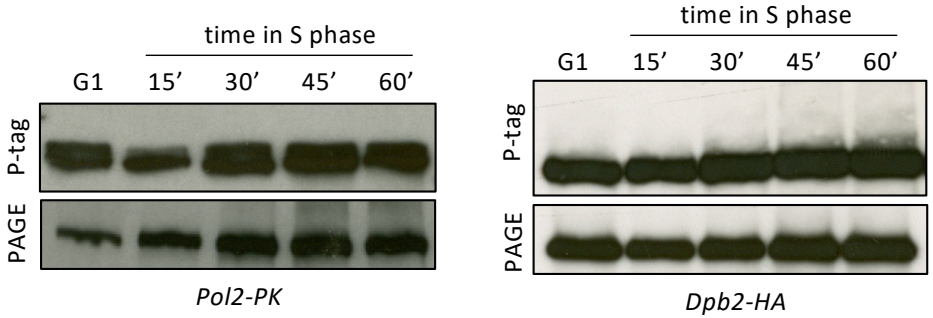
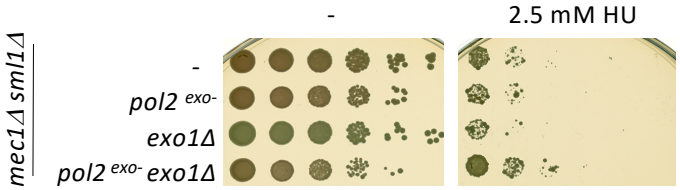


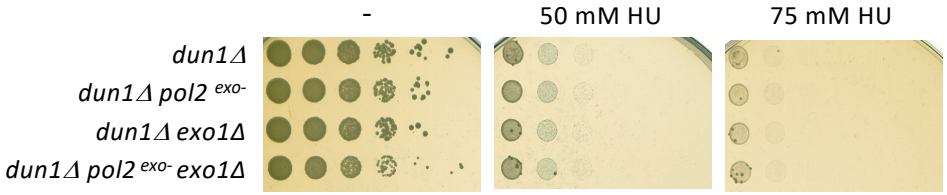
Figure S1

Figure S1. DNA Polymerase δ and DNA polymerase ε subunit phosphorylation in cells experiencing replication stress, Related to Figure 1. (A) DNA polymerase δ subunits. (B) Western blot analysis of the phosphorylation of Polδ subunits. Proteins were extracted from cells expressing epitope-tagged Pol3, Pol31 or Pol32 released from an alpha-factor induced block (G1) into a synchronous S-phase in the presence of 0.2 M hydroxyurea (HU) and subject to electrophoresis in the presence (P-tag) or absence (PAGE) of PhosTag reagent. (C) Western blot analysis of the phosphorylation of Dpb3 in *sml1Δ* and *mec1Δ tel1Δ sml1Δ* cells. (D) Western blot analysis of the phosphorylation of Pol3, Pol31 and Pol32 in *sml1Δ* and *mec1Δ tel1Δ sml1Δ* cells. Checkpoint activation can be inferred from Rad53 phosphorylation status assayed by western blotting with EL7 antibodies. (E) Western blot analysis of the phosphorylation of Pol2 and Dpb2 in cells released from a G1 block into an unperturbed S-phase.

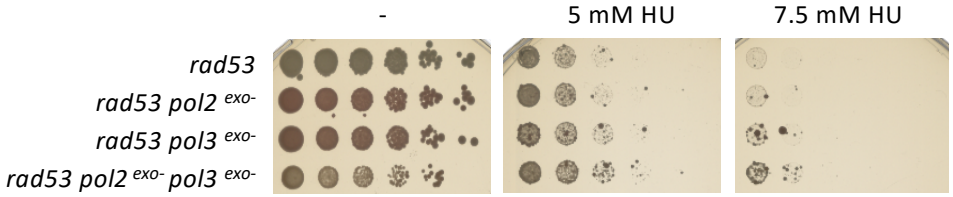
A



B



C



D

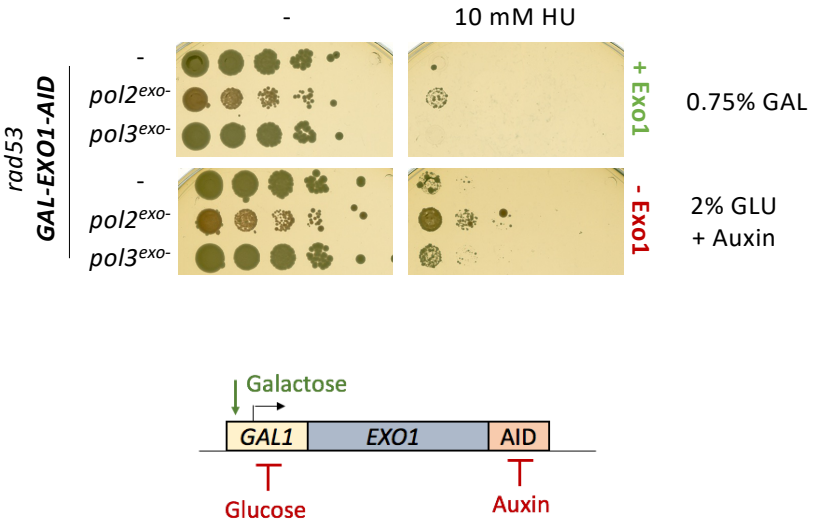


Figure S2

Figure S2. Contribution of the exonuclease activities of DNA Polymerase ε and DNA polymerase δ to the HU sensitivity of checkpoint-deficient cells, Related to Figure 3. (A) Serial dilutions of *mec1Δ sml1Δ, mec1Δ sml1Δ pol2-4*, *mec1Δ sml1Δ exo1Δ* and *mec1Δ sml1Δ pol2-4 exo1Δ* cells plated in absence (-) or presence of 2.5 mM HU. (B) Serial dilutions of *dun1Δ, dun1Δ pol2-4*, *dun1Δ exo1Δ* and *dun1Δ pol2-4 exo1Δ* cells plated in absence (-) or presence of 50 or 75 mM HU. (C) Serial dilutions of *rad53, rad53-K227A pol2-4*, *rad53-K227A pol3-01* and *rad53-K227A pol2-4 pol3-01* cells plated in absence (-) or presence of 5 or 7.5 mM HU. (D) Serial dilutions of *rad53 GAL1-HA-exo1-AID, rad53-K227A GAL1-HA-exo1-AID pol2-4* and *rad53-K227A GAL1-HA-exo1-AID pol3-01* cells plated on YP supplemented with 0.025% raffinose and 0.004% ethanol in absence or presence of 10 mM HU and 0.75% Galactose (Exo1 expression) or 2% Glucose and 500 μM Auxin (Exo1 repression). A schematic diagram of the genetic strategy used to modulate Exo1 expression levels is shown.

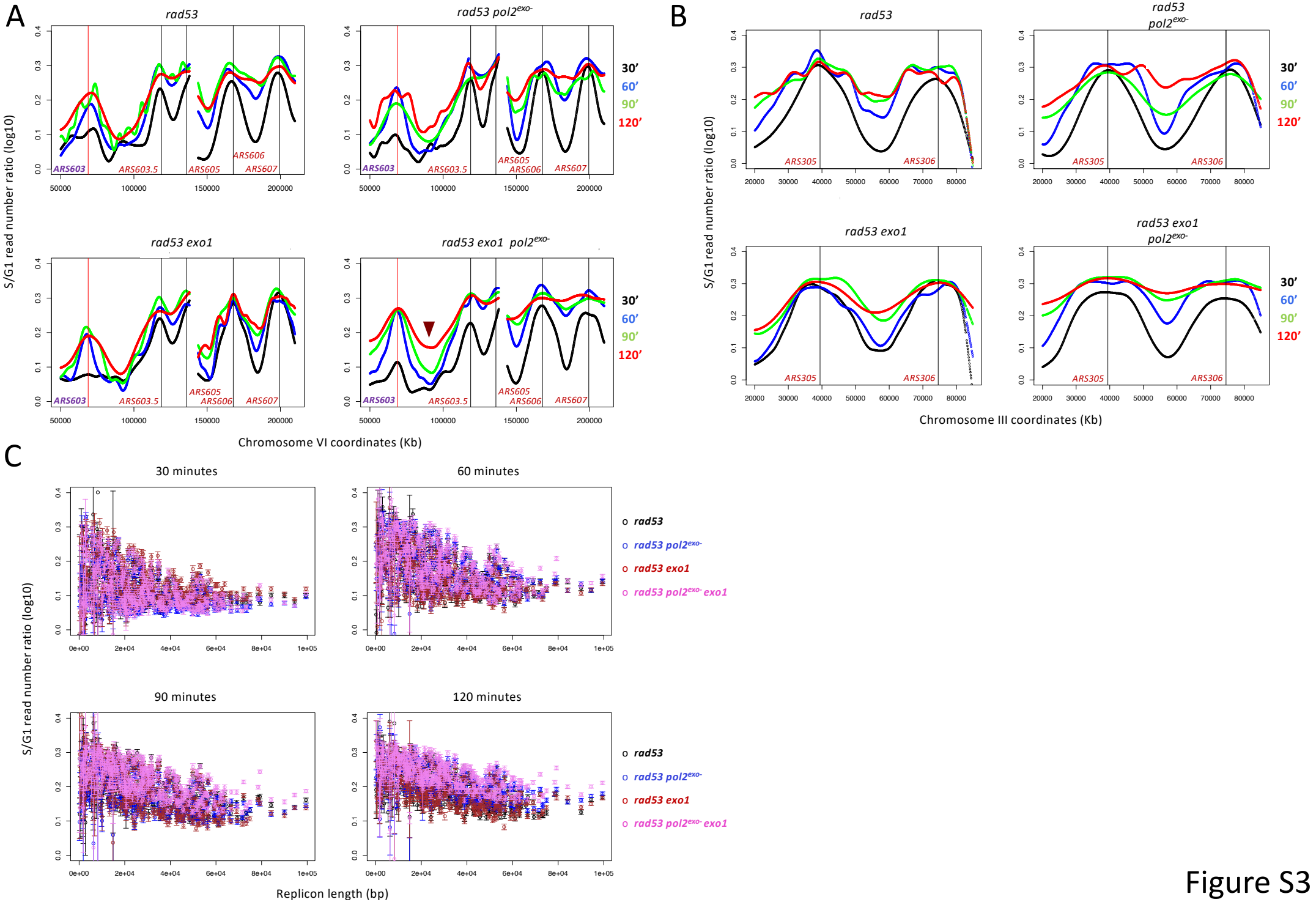

A
rad53
rad53 pol2exo-
rad53 exo1
rad53 exo1 pol2exo-
S/G1 read number ratio (log10)
ARS603
ARS603.5
ARS605
ARS606
ARS607
30'
60'
90'
120'
Chromosome VI coordinates (Kb)
B
rad53
rad53 pol2exo-
rad53 exo1
rad53 exo1 pol2exo-
ARS305
ARS306
Chromosome III coordinates (Kb)
C
30 minutes
60 minutes
90 minutes
120 minutes
rad53
rad53 pol2exo-
rad53 exo1
rad53 pol2exo- exo1
Replicon length (bp)

Figure S3

Figure S3. Contribution of the exonuclease activity of Polε and Exo1 to stalled fork collapse, Related to Figure 3. (A) CGS analysis of fork progression as in Figure 3C. A 150-Kb genomic region on chromosome VI containing early origins *ARS603.5*, *ARS605*, *ARS606* and *ARS607* and the late origin *ARS603* (in purple color) is shown. A red arrowhead evidences increased replication completion at the 45-Kb segment between *ARS603* and *ARS603.5* in *rad53-K227A exo1Δ pol2-4* cells. (B) CGS analysis of fork progression as in the experiment shown in Figure 3C. A 60-Kb region genomic region on chromosome III containing the early origins *ARS305* and *ARS306* is shown. (C) Plots representing mean and standard error of S/G1 read enrichment ratios (log10) of regions shown in Figure 3D ordered by replicon length. Ratios are shown for the different time points after release in HU for *rad53-K227A, rad53-K227A pol2-4*, *rad53-K227A exo1Δ* and *rad53-K227A pol2-4 exo1Δ* cells.

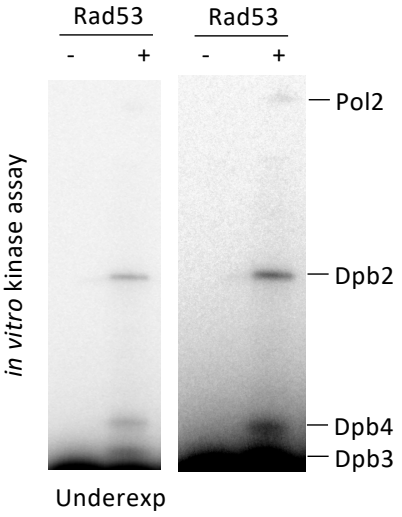
A

Rad53 consensus motif (+1 or +2 Ψ)	
Pol2 Phosphopeptides	a.a. position
YNTphosLSNNYALSAQQLLNASK	25
YNTLSphosNNYALSAQQLLNASK	27
YNTLSNNYALSphosAQQLLNASK	33
RDSphosYLPQGSQGLK	430
DCASphosCDFNRPGK	667
VKVSphosEIVER	753
AMILPSSphosKEEGK	957 or 958
STSphosITTAR	1066
LGSphosAIQK	1152
IITIPAALQGVSphosNPVPR	1168
DQLFGNTNSSphosR	1299 or 1300
SphosALGSMIR	1304
SALGSphosMIR	1308
TSphosNPAGGQLFK	1390
DAVINSphosPSEFVHDAFSNDALNVLR	1774
LNSphosGTQRPTQIVNVK	1976

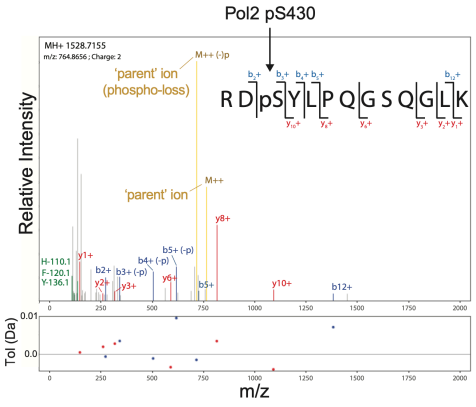
B

Rad53 consensus motif (+1 or +2 Ψ)	
Dpb2 Phosphopeptides	a.a. position
MFGSphosGNVLPVK	4
TphosDDDENNSDDEMPIAADSSLQNVSLSSPMR	115
TDDDENNSDDEMPIAADSSLQNVSLSSphosPMR	141
QNVSLSSphosPMR	141
DEYKQPFKPESphosSK	161
VINASphosQQQR	176
NENFQNSDMFNPLSSphosMVSLQNELSNTNR	246
QQQSSSphosMITPIK	265
QQQSSSMSphosITPIK	267
QQQSSSMSITphosPIK	269
VINPGSphosFIHNR	663

C

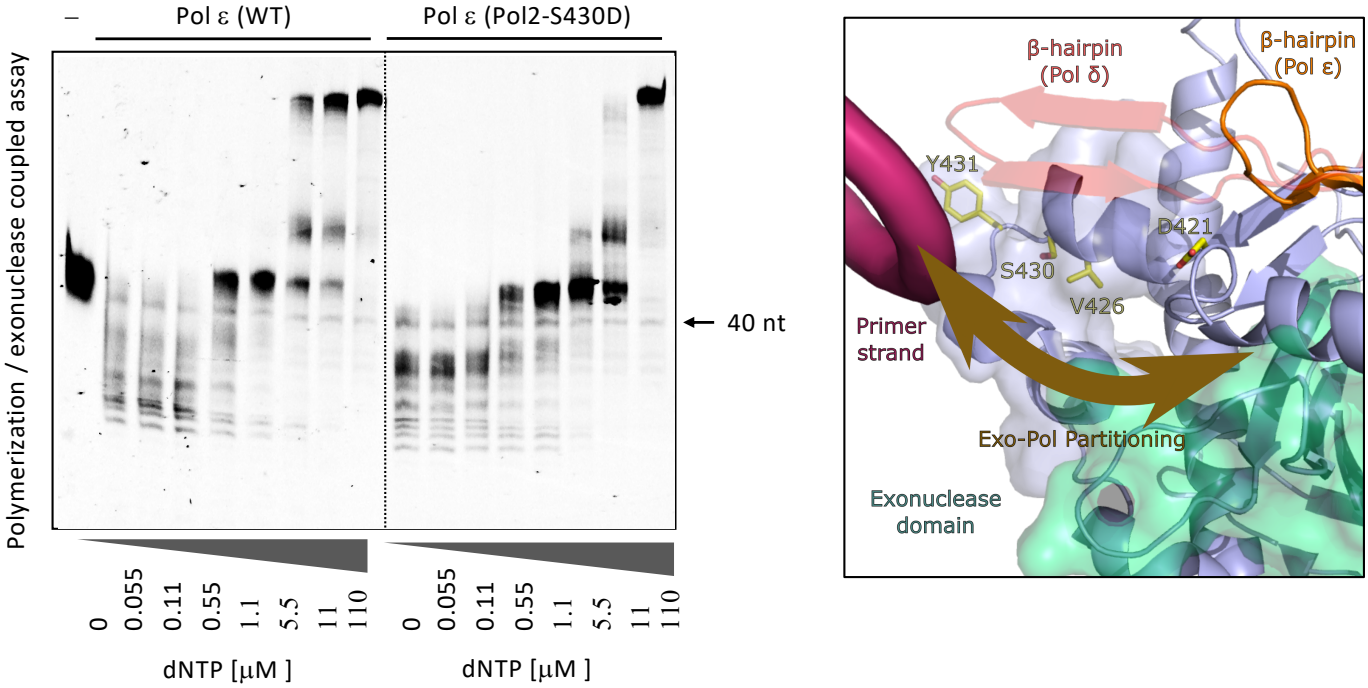


D



E

F



G

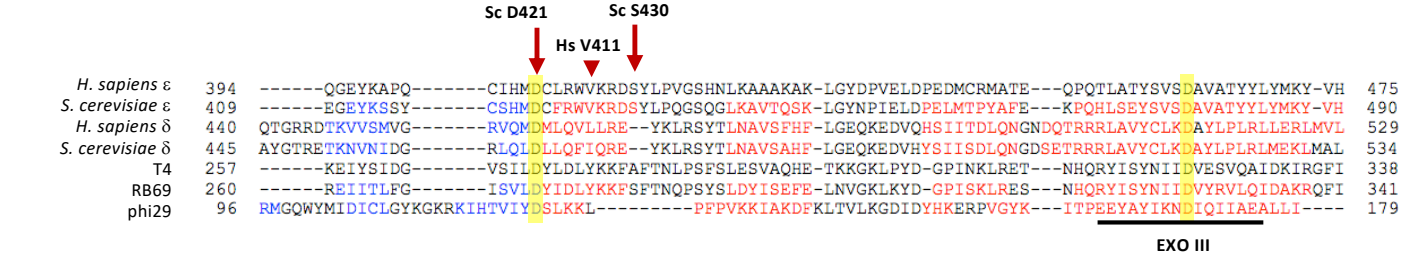


Figure S4

Figure S4. Analysis of Polε residues phosphorylated in cells experiencing replication stress, Related to Figure 4. (A-B) Phosphopeptides identified by mass spectrometry in Pol2 (A) and Dpb2 (B) immunoprecipitates from cells experiencing replication stress. Peptides containing phospho-serines or phospho-threonines within Rad53 consensus motives are highlighted in salmon. (C) *In vitro* kinase assay. Radiogram of a protein gel with reactions in which purified Polε was incubated in the absence (-) or presence (+) of Rad53 and radiolabeled ATP. Incorporation of hot ATP by Dpb3 was also observed in underexposed gels. (D) Peptide Spectrum Match (PSM) for a phosphopeptide corresponding to Pol2 phosphorylation at S430. Fragment ion match tolerance is set to 0.01 Daltons. (-p) ion fragments denote the neutral loss of the phosphogroup. (E) Polymerase/exonuclease coupled assays performed with WT and S430D Polε variants. (F) Detailed view of the location of S430 with respect to the primer strand and the exonucleolytic active site. S430 is located at the tip of an α-helix that also contains D421 (the orthologous residue influences partitioning in Phi29 DNA polymerase) and V426 (mutated in human cancer). S430 is next to Y431, a residue of unclear function that is in proximity to the major groove of the nascent DNA duplex and that could play a role during partitioning. This area of the structure is adjacent to the β-hairpin loop implicated in Polδ partitioning (shown for reference in transparent red) that is dramatically shorter in Polε (orange) and lines the channel that the primer strand (magenta) needs to traverse in order to relocate to the exonuclease active site (brown arrow). The surface of the exonuclease domain is shown in transparent green. Relevant residues are shown as sticks. (G) Sequence alignment of a portion of the exonuclease domain of B family polymerases. Exonuclease domains were aligned using Clustal Omega 1.2.0. Conserved non-catalytic (Sc D421) and catalytic aspartates are evidenced by yellow boxes. The positions of Sc D412, Hs V411 and Sc S430 are indicated. α-helices and β-sheets are depicted in red and blue, respectively.

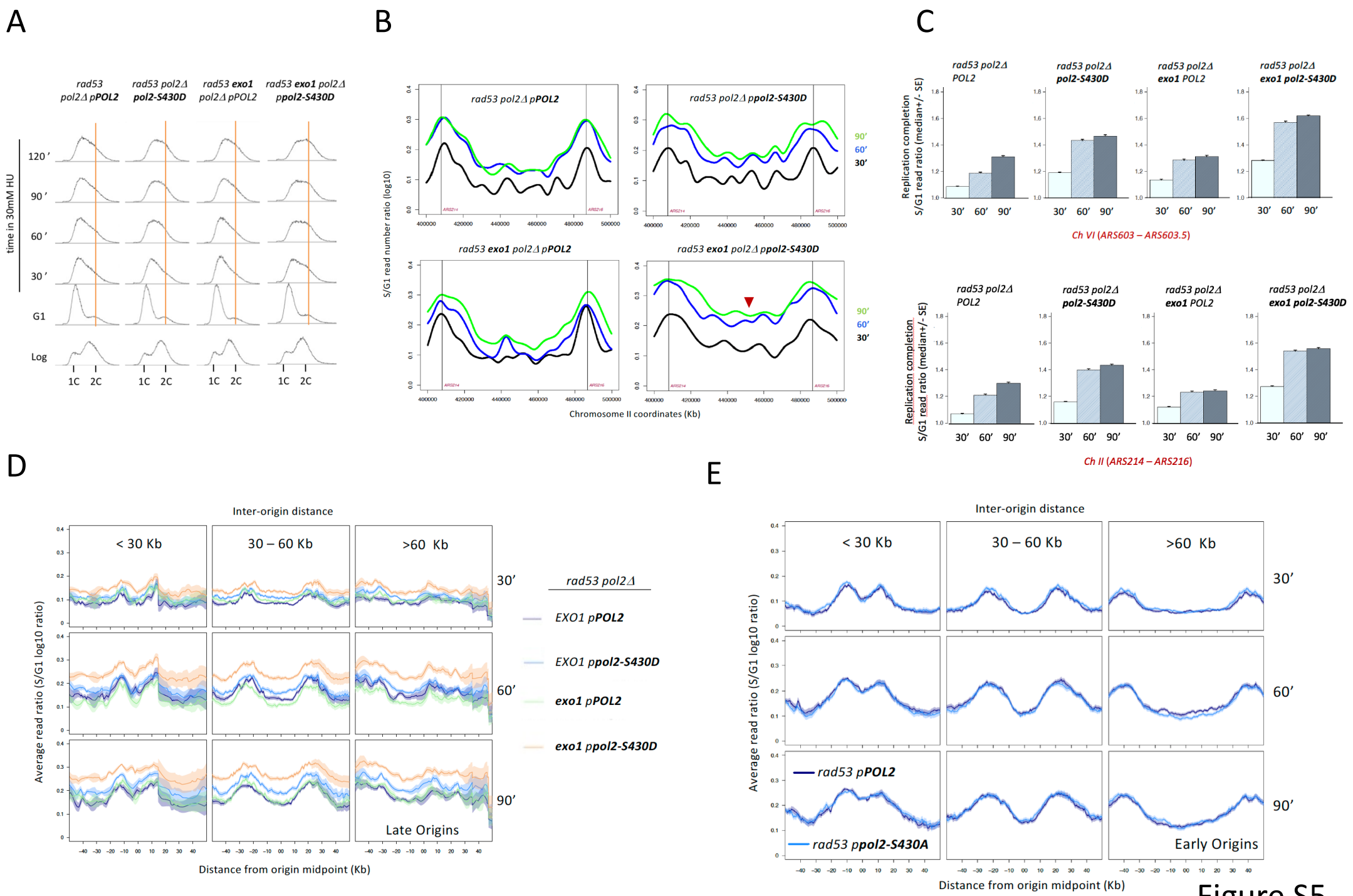
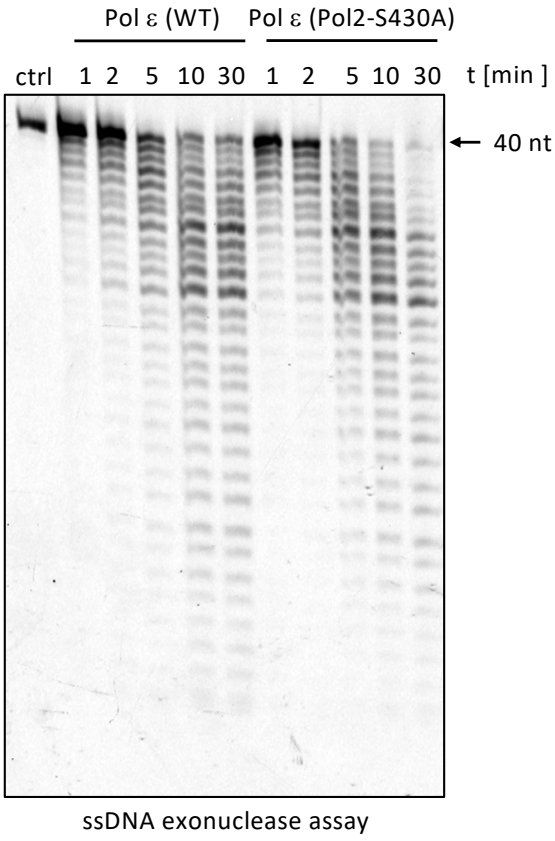

A
rad53 pol2Δ pPOL2
rad53 pol2Δ pol2-S430D
rad53 exo1 pol2Δ pPOL2
rad53 exo1 ppol2-S430D
time in 30mM HU
120 '
90 '
60 '
30 '
G1
Log
1C 2C
B
rad53 pol2Δ pPOL2
rad53 pol2Δ ppol2-S430D
rad53 exo1 pol2Δ pPOL2
rad53 exo1 pol2Δ ppol2-S430D
S/G1 read number ratio (log10)
90'
60'
30'
Chromosome II coordinates (Kb)
C
rad53 pol2Δ POL2
rad53 pol2Δ pol2-S430D
rad53 pol2Δ exo1 POL2
rad53 pol2Δ exo1 pol2-S430D
Replication completion
S/G1 read ratio (median+/- SE)
30' 60' 90'
Ch VI (ARS603 – ARS603.5)
Ch II (ARS214 – ARS216)
D
Inter-origin distance
< 30 Kb
30 – 60 Kb
>60 Kb
Average read ratio (S/G1 log10 ratio)
Late Origins
Distance from origin midpoint (Kb)
rad53 pol2Δ
EXO1 pPOL2
EXO1 ppol2-S430D
exo1 pPOL2
exo1 ppol2-S430D
E
Inter-origin distance
rad53 pPOL2
rad53 ppol2-S430A
Early Origins
Distance from origin midpoint (Kb)

Figure S5

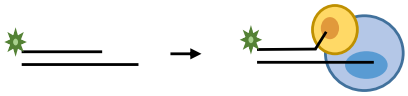
Figure S5. Pol2 serine 430 phosphomimetic substitution influences bulk genome replication under stress conditions, Related to Figure 5. (A) Flow cytometry analysis of logarithmically growing (Log) *rad53-K227A pol2Δ pRS415-POL2, rad53-K227A pol2Δ pRS415-pol2-S430D*, *rad53-K227A exo1Δ pol2Δ pRS415-POL2* and *rad53-K227A exo1Δ pol2Δ pRS415-pol2-S430D* cells blocked in G1 by alpha-factor treatment (G1) and released into S-phase in the presence of 30 mM HU. Vertical orange bars mark 2C DNA contents. (B) CGS analysis of fork progression as in the experiment shown in Figure 5C. A 120-Kb genomic region on chromosome II containing *ARS214* and *ARS216* early replication origins (marked by vertical black lines) is shown. A red arrowhead evidences increased replication completion in *rad53-K227A exo1Δ pol2Δ pRS415-pol2-S430D* cells. (C) Histogram plots showing replication completion (overall S/G1 read ratios between flanking origins) of the chromosomal regions between *ARS603*/*ARS603.5* and *ARS214*/*ARS216* shown in the CGS experiment on Figure 5C and S5B. (D) Average read ratios across genomic regions categorized by inter-origin distance between dormant and late-firing origins corresponding to the CGS experiment shown in Figure 5C. (E) Average read ratios across genomic regions categorized by inter-origin distance between early origins in *rad53-K227A Δ pol2Δ pRS415-POL2 and rad53-K227A Δ pol2Δ pRS415-pol2-S430A* cells blocked in G1 by alpha-factor treatment (G1) and released into S-phase in the presence of 25 mM HU for the indicated times.

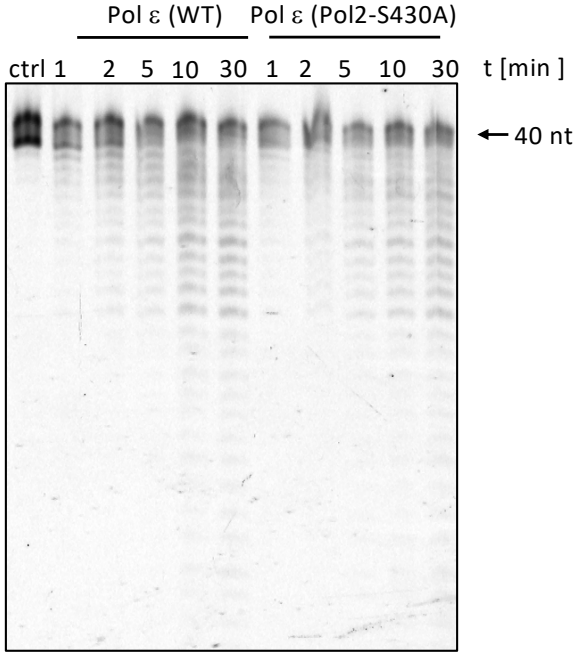
A



ssDNA exonuclease assay

B





dsDNA exonuclease assay

C

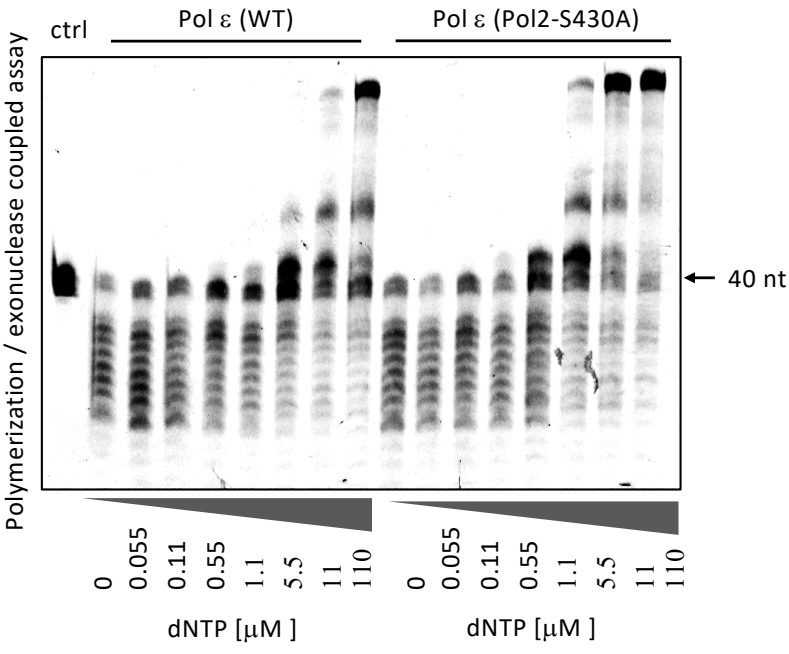


Figure S6

Figure S6. Non-phosphorylatable Pol2-S430A *in vitro* exonuclease activity, Related to Figure 6. (A-B) Single strand (A) and double strand (B) exonuclease assays performed with WT and S430A Polε variants. (C) Polymerase/exonuclease coupled assays performed with WT and S430A Polε.

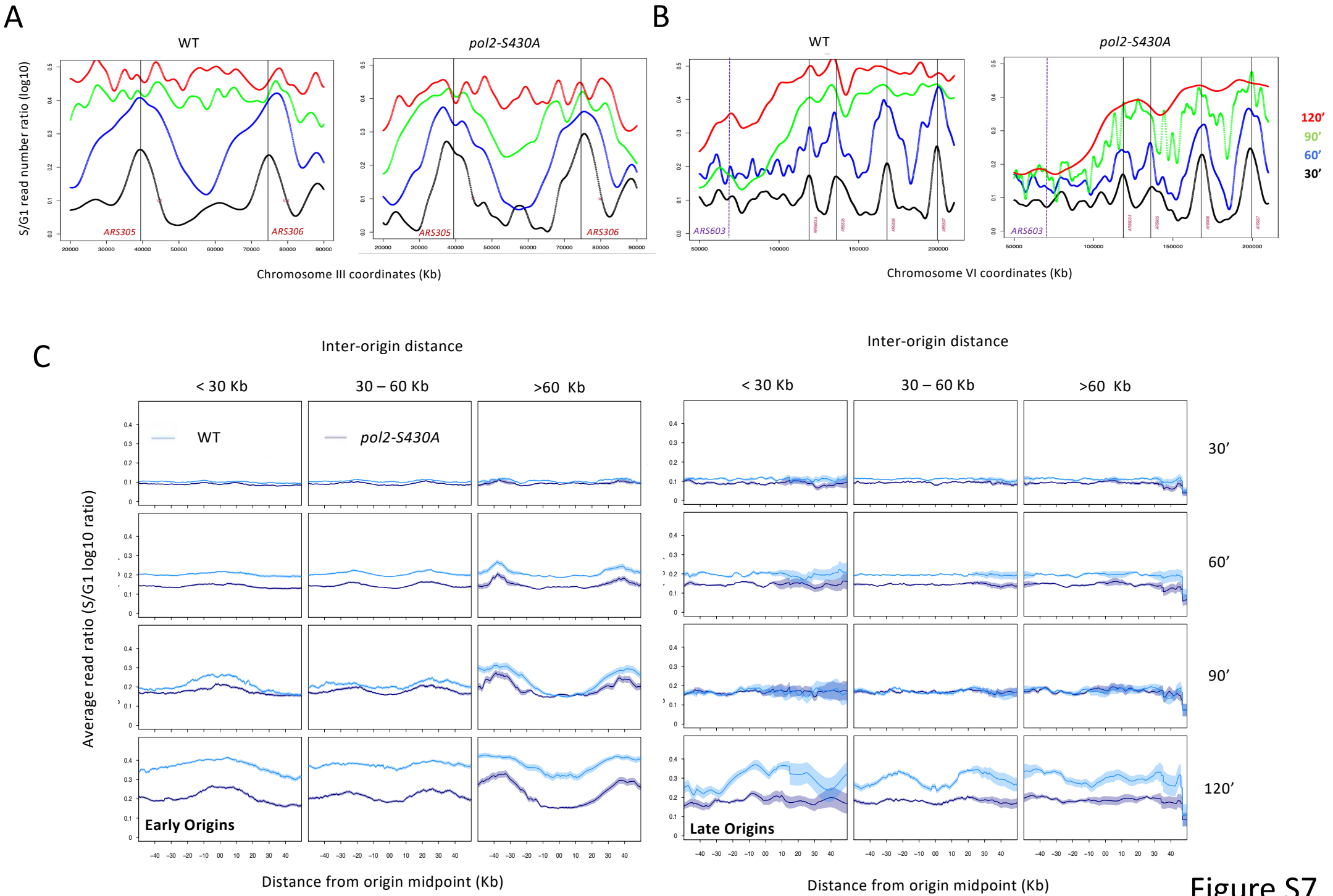

A
WT
pol2-S430A
S/G1 read number ratio (log10)
ARS305
ARS306
Chromosome III coordinates (Kb)
B
WT
pol2-S430A
ARS603
120'
90'
60'
30'
Chromosome VI coordinates (Kb)
C
Inter-origin distance
< 30 Kb
30 – 60 Kb
>60 Kb
WT
pol2-S430A
Average read ratio (S/G1 log10 ratio)
Early Origins
Late Origins
30'
60'
90'
120'
Distance from origin midpoint (Kb)

Figure S7

Figure S7. Fork progression defects in HU-challenged *pol2-S430A* cells, Related to Figure 6. (A + B) CGS analysis of fork progression as in the experiment shown in Figure 6B. A 60-Kb region genomic region on chromosome III containing the early origins *ARS305* and *ARS306* (A) and a 150-Kb genomic region on chromosome VI containing early origins *ARS603.5*, *ARS605*, *ARS606* and *ARS607* and the late origin *ARS603* (in purple color) (B) are shown. (C) Average read ratios across genomic regions categorized by inter-origin distance in early and late/dormant origin datasets of wild type and *pol2-S430A* cells along the time course experiment shown in Figure 6B.

Strain	Number	Genotype	Reference
WT	RB 718	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1*	Lab collection
Pol2-PK	RB 1618	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL2-6PK-HIS3MX6*	This study
Dpb2-Myc	RB 1955	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, DPB2-9MYC-HIS3MX6*	This study
Dpb3-myc	RB 1958	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, DPB3-9MYC-TRP1*	This study
Dpb4-Flag	RB 1985	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, DPB4-FLAG-KANMX6*	This study
sml1 Δ Pol2-PK	RB 1743	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL2-6PK-HIS3MX6, sml1::TRP1*	This study
sml1 Δ Dpb2-Myc	RB 2280	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, DPB2-9MYC-HIS3MX6, sml1::TRP1*	This study
sml1 Δ Dpb3-Myc	RB 2425	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, DPB3-9MYC-TRP1, sml1::TRP1*	This study
sml1 Δ mec1 Δ tel1 Δ Pol2-PK	RB 1749	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL2-6PK-HIS3MX6, sml1::TRP1, mec1::URA3, tel1::HPHMX6*	This study
sml1 Δ mec1 Δ tel1 Δ Dpb2-Myc	RB 2272	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, DPB2-9MYC-HIS3MX6, sml1::TRP1, mec1::URA3, tel1::HPHMX6*	This study
sml1 Δ mec1 Δ tel1 Δ Dpb3-Myc	RB 2443	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, DPB3-9MYC-TRP1, sml1::TRP1, mec1::URA3, tel1::HPHMX6*	This study
rad53-K227A Pol2-PK	RB 2035	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL2-6PK-HIS3MX6, rad53-K227A-KANMX6*	This study
Pol2-HA	RB 2841	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL2-3HA-TRP1*	This study
Dpb2-HA	RB 2682	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, DPB2-3HA-HIS3MX6*	This study
rad53-K227A Dpb2-HA	RB 2700	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, DPB2-3HA-HIS3MX6, rad53-K227A-KANMX6*	This study
dun1 Δ Pol2-PK	RB 3263	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL2-6PK-HIS3MX6, dun1::KANMX6*	This study
dun1 Δ Dpb2-HA	RB 3294	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, DPB2-3HA-HIS3MX6, dun1::KANMX6*	This study
Pol3-PK	RB 1621	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL3-9PK-TRP1*	This study
Pol31-Myc	RB 1962	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL31-9MYC-HIS3MX6*	This study
Pol32-Flag	RB 1965	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL32-FLAG-KANMX6*	This study
sml1 Δ Pol3-Myc	RB 3413	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL3-9MYC-TRP1, sml1::TRP1*	This study
sml1 Δ Pol31-Myc	RB 2292	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL31-9MYC-HIS3MX6, sml1::TRP1*	This study
sml1 Δ Pol32-Flag	RB 2187	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL32-FLAG-KANMX6, sml1::TRP1*	This study
sml1 Δ mec1 Δ tel1 Δ Pol3-Myc	RB 2250	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL3-9MYC-TRP1, sml1::TRP1, mec1::URA3, tel1::HPHMX6*	This study
sml1 Δ mec1 Δ tel1 Δ Pol31-Myc	RB 2287	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL31-9MYC-HIS3MX6, sml1::TRP1, mec1::URA3, tel1::HPHMX6*	This study
sml1 Δ mec1 Δ tel1 Δ Pol32-Flag	RB 2196	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, POL32-FLAG-KANMX6, sml1::TRP1, mec1::URA3, tel1::HPHMX6*	This study
rad53-K227A	RB 26	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad53-K227A-KANMX6*	Lab collection
rad53-K227A pol2-4	RB 1490	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad53-K227A-KANMX6, pol2-D290A-E292A*	This study
rad53-K227A exo1 Δ	RB 1568	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad53-K227A-KANMX6, exo1::HIS3MX6*	This study
rad53-K227A pol2-4 exo1 Δ	RB 1563	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad53-K227A-KANMX6, pol2-D290A-E292A, exo1::HIS3MX6*	This study
rad53-K227A pol3-01	RB 1436	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad53-K227A-KANMX6, pol3-D520V*	This study
rad53-K227A pol2-4 pol3-01	RB 2788	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad53-K227A-KANMX6, ppol2-D290A-E292A, pol3-D520V*	This study
rad53-K227A GAL-exo1-AID	RB 2447	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad53-K227A-KANMX6, tir1::URA3, TRP1-GAL1-3HA-EXO1-AID-HPHMX6*	This study
rad53-K227A pol2-4 GAL-exo1-AID	RB 2936	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad53-K227A-KANMX6, tir1::URA3, TRP1-GAL1-3HA-EXO1-AID-HPHMX6, pol2-D290A-E292A*	This study
rad53-K227A pol3-D520V GAL-exo1-AID	RB 3015	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad53-K227A-KANMX6, tir1::URA3, TRP1-GAL1-3HA-EXO1-AID-HPHMX6, pol3-D520V*	This study
sml1Δ mec1Δ	RB 323	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, sml1::TRP1, mec1::URA3*	Lab collection
sml1Δ mec1Δ pol2-4	RB 2845	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, sml1::TRP1, mec1::URA3, pol2-D290A-E292A*	This study
sml1 Δ mec1 Δ exo1 Δ	RB 2838	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, sml1::TRP1, mec1::URA3, exo1::HIS3MX6*	This study
sml1 Δ mec1 Δ pol2-4 exo1 Δ	RB 2858	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, sml1::TRP1, mec1::URA3, pol2-D290A-E292A, exo1::HIS3MX6*	This study
dun1 Δ	RB 326	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, dun1::KANMX6*	This study
dun1 Δ pol2-4	RB 3374	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, dun1::KANMX6, pol2-D290A-E292A*	This study
dun1 Δ exo1 Δ	RB 3371	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, dun1::KANMX6, exo1::HIS3MX6*	This study
dun1 Δ pol2-4 exo1 Δ	RB 3394	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, dun1::KANMX6, pol2-D290A-E292A, exo1::HIS3MX6*	This study
rad53-K227A pol2Δ pPOL2	RB 2910	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad53-K227A-KANMX6, POL2::HPH, pRS415-POL2*	This study
rad53-K227A pol2 Δ ppol2-S430D	RB 3100	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad53-K227A-KANMX6, POL2::HPH, pRS415-pol2-S430D*	This study
rad53-K227A pol2Δ pPOL2 exo1Δ	RB 2918	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad53-K227A-KANMX6, POL2::HPH, pRS415-POL2, exo1::HIS3MX6*	This study
rad53-K227A pol2 Δ ppol2-S430D	RB 3103	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad53-K227A-KANMX6, POL2::HPH, pRS415-pol2-S430D, exo1::HIS3MX6*	This study
rad53-K227A pol2 Δ ppol2-4	RB 3110	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, rad53-K227A-KANMX6, POL2::HPH, pRS415-pol2-D290A-E292A, exo1::HIS3MX6*	This study
pol2-S430A	RB 3647	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, pol2-S430A*	This study
pol2-S430D	RB 3479	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, pol2-S430D*	This study
pol2-4	RB 1292	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, pol2-D290A-E292A*	This study
pol2-4-S430A	RB 3758	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, pol2-D290A-E292A-S430A*	This study
GAL-Pol ε	RB 3649	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, bar1::HYG, pep4::KANMX6, POL2-3FLAG-NAT, ura3::URA3pRS306/Dpb2, Dpb3, trp1::TRP1pRS304/POL2, Dpb4-Tev-CBP*	J. Yeeles
GAL-Pol ε-Pol2-S430D	RB 3652	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, bar1::HYG, pep4::KANMX6, POL2-3FLAG-NAT, ura3::URA3pRS306/Dpb2, Dpb3, trp1::TRP1pRS304/pol2-S430D, Dpb4-Tev-CBP*	This study
GAL-Pol ε-Pol2-S430A	yJY109	*MATa, his3-11,15, leu2-3,112, trp1-1, ura3-1, bar1::HYG, pep4::KANMX6, POL2-3FLAG-NAT, ura3::URA3pRS306/Dpb2, Dpb3, trp1::TRP1pRS304/pol2-S430A, Dpb4-Tev-CBP*	This study

Table S1. Strains used in this study.