Figure S1:

(A) Analysis of replication of DNA in WT (VG3349-1B) and *wpl1*Δ (VG3360-3D) cells treated with 20 µg/ml CPT from time course described in Figure 2. Cells were processed for flow cytometry (Materials and Methods). Haploid αfactor arrested-cells (0 min) have a single 1C peak. DNA content increases during replication and cells reach 2C peak in G2/M. When cells exit mitosis they enter the subsequent G1 and arrest with 1C DNA content. From left to right: *WT* cells treated with DMSO or 20 µg/mL CPT then *wpl1*Δ cells treated with DMSO or 20 µg/mL CPT **(B)** Analysis of replication of DNA in WT (VG3349-1B) and *wpl1*Δ (VG3360-3D) cells treated with 0.01% MMS from time course described in Figure 2. Cells were analyzed as described in (A). From left to right: *WT* cells treated with DMSO or 0.01% MMS then *wpl1*Δ cells treated with DMSO or 0.01% MMS.

Figure S2:

(A) Schematic of time-course and analysis of cells arrested in G2/M using nocodazole then treated with CPT and released into G1 arrest. **(B) & (C)** Camptothecin treatment of wild-type (VG3349-1B) and *wpl1*Δ (VG3360-3D) cells arrested in mid-M phase has no effect on progression through mitosis. Haploid *WT* (VG3349-1B), and *wpl1*Δ (VG3360-3D) cells were synchronously arrested in mid-M phase by treatment with nocodazole (Materials and Methods). Cells were washed free of nocodazole and resuspended in YPD media buffered with 25 mM pH 7.4 HEPES and then cultures were split into two aliquots. 20 µg/mL CPT was added to one aliquot and DMSO to the other. Alpha factor was added to both aliquots and cells incubated at 23°C to allow cells to progress through and exit mitosis then arrest in G1. Aliquots were taken every 30 minutes fixed and processed for scoring DNA masses using DAPI or for DNA content by FACS. (B) Graphs show the percentage of large budded cells with a single DAPI mass (G2/M) in nocodazole arrest (T= 0 min) and at each time-point after release as described in Figure 2B. WT cells (gray) or *wpl1*Δ cells (black)**. (C)** Analysis of DNA content by FACS as described in Figure S1A. Nocodazole-arrested cells (time 0 min) have 2C DNA content, which drops to 1C when cells exit mitosis and enter G1. Left to right: *WT* cells treated with DMSO or 20 µg/mL CPT then *wpl1*Δ cells treated with DMSO or 20 µg/mL CPT.

Figure S3:

(A) Schematic of time-course and analysis of cells arrested in G2/M using nocodazole then treated with MMS and released into G1 arrest. **(B) & (C)** MMS treatment of haploid wild-type (VG3349-1B) and *wpl1*Δ (VG3360-3D) cells arrested in mid-M phase has no effect on progression through mitosis. Time course was performed as described in Figure S2B except cells were washed free of nocodazole and resuspended in YPD + alpha factor. Cultures were then split and either DMSO or 0.01% MMS was added instead of CPT. **(B)** Graphs show the percentage of large budded cells with a single DAPI mass in nocodazole arrest (T= 0 min) and at each time-point after release as described in Figure 2B. *WT* cells (gray) or *wpl1*Δ cells (black). **(C)** Analysis of DNA content by FACS as described in Figure S2C. Left to right: *WT* cells treated with DMSO or 0.01% MMS then *wpl1*Δ cells treated with DMSO or 0.01% MMS.

Figure S4:

(A) *pds5-E181K* suppresses the temperature sensitivity of an *eco1-203* mutant . Haploid *WT* (VG3349-1B), *pds5-E181K* (MSB101-3C), *eco1-203 PDS5* (VG3223-12B)*, eco1-203 pds5-E181K* (MSB189-2B) strains were serially diluted 10-fold and plated on YPD media. Plates were incubated at 23˚C and 34˚C and incubated for 3 days. **(B)** *pds5-S81R eco1*Δ and *pds5-P89L eco1*Δ double mutants have a dramatic defect in cohesion when measured at *CEN*-proximal locus. Haploid *PDS5* (MSB185-1A), *eco1*Δ *wpl1*Δ (VG3502 #A), *eco1*Δ *pds5-S81R* (MSB210-2A), *eco1*Δ *pds5-P89L* (MSB211-2J) strains were synchronously arrested in mid-M phase using nocodazole as described in Figure 3D and Material and Methods. Cells were scored for cohesion at *CEN*-proximal *TRP*1 locus by counting the percentage of cells with cohesion (one GFP focus) or that have separated sisters (two GFP foci) indicating a loss of cohesion as described in Figure 3C. The percentage of cells lacking cohesion (sister separation) is shown. **(C)** *pds5-E181K* fails to restore cohesion in *eco1-203* cells. Strains were arrested at 23°C in G1 using alpha factor. Cultures were split into two aliquots: one incubated at 23˚C or 34˚C and incubated an additional hour. Cells were released from alpha factor into YPD containing nocodazole and incubated at the same temperature as before (23°C or 34°C) until arrested in mid-M phase. Cells were scored for cohesion at CEN-distal *LYS4* locus as described in (B) and the percentage of cells with cohesion loss (Sister separation) plotted.

Figure S5:

(A) *pds5* N-terminal mutants have similar modest cohesion defect as *wpl1*Δ alone or as *pds5 wpl1*Δ double mutants. Haploid strains *PDS5* (MSB185-1A), *wpl1*Δ (VG3513-1B), *pds5-S81R* (MSB190-3E), *pds5-P89L* (MSB191-3A), *pds5-E181K* (MSB186-2E), *pds5-S81R wpl1*Δ (MSB204-1B), *pds5-P89L wpl1*Δ (MSB205-4C), *pds5-E181K wpl1*Δ (MSB206-6A) were synchronously arrested in mid-M as described in Materials and Methods and scored for cohesion at the *CEN*-proximal *TRP1* locus as described in Figure 4A. **(B)** Kinetics of cohesion loss in *pds5* N-terminal mutants. Haploid strains *PDS5* (VG3349-1B), *wpl1*Δ (*VG3360-3D*), *pds5-S81R* (MSB183-1A), *pds5-P89L* (MSB184-3A) and *pds5-E181K* (MSB101-3C) cells were assessed for sister separation. as they synchronously progress from alpha factor arrest (G1; T = 0) into mid-M phase arrest as described in Materials and Methods.

Figure S6:

Portion of clustal alignment of Pds5 homologs across species. Residues corresponding to *Saccharomyces cerevisiae* Pds5p-S81, Pds5p-P89 and, Pds5p-E181 are highlighted in black boxes.

Figure S7:

***wpl1*Δ mutants cells have decreased levels of Mcd1p**

(A) & (B) Haploid WT (VG3349-1B) and *wpl1*Δ (VG3360-3D) cells were grown in YPD at 23˚C then arrested in mid-M phase using nocodazole. Cells were processed for bulk-pelleting assay (Materials and Methods). (A) Total cellular levels of Mcd1p are decreased 2-fold in *wpl1*Δ cells. Whole cell extracts generated during bulk chromatin pelleting were analyzed by SDS-PAGE and Mcd1p levels determined by Western Blot using anti-Mcd1p (αMcd1p) antibodies. (B) Mcd1p levels are decreased in both the chromatin bound and soluble pools of Mcd1p. Bulk pelleting generates a soluble fraction (S) and a chromatin bound fraction (P) which were analyzed for Mcd1p levels as described in (A). Anti-Tubulin antibodies (αTub2p) were used as a control fractionation.

Table S1: Strain list

Strain name	Genotype
MSB101-3C	*pds5-E181K MATa lys4:LacO(DK)-NAT* GAL+*trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB133-3C	*pds5-S81R wpl1Δ::HPH MATa lys4:LacO(DK)-NAT* GAL+*trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB134-1L	*pds5-P89L wpl1Δ::HPH MATa lys4:LacO(DK)-NAT* GAL+*trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB138-1K	*pds5-S81R eco1Δ::G418 MATa lys4:LacO(DK)-NAT* GAL+*trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB139-2J	*pds5-P89L eco1Δ::G418 MATa lys4:LacO(DK)-NAT* GAL+*trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52 pBS1030(ECO1 URA3 CEN)*
MSB147-1A	*pds5-E181K eco1Δ::G418 MATa lys4:LacO(DK)-NAT* GAL+*trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52 pBS1030(CEN URA ECO1)*
MSB183-1A	*pds5-S81R MATa lys4:LacO(DK)-NAT* GAL+*trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB184-3A	*pds5-P89L MATa lys4:LacO(DK)-NAT* GAL+*trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB185-1A	*MATa 10Kb-CEN4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB186-2E	*pds5-E181K MATa 10Kb-CEN4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB189-2B	*pds5-E181K eco1-203 MATa lys4:LacO(DK)-NAT* GAL+*trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB190-3E	*pds5-S81R MATa 10Kb-CEN4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB191-3A	*pds5-P89L MATa 10Kb-CEN4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB192-2A	*wpl1Δ::WPL1-3FLAG-KANMX MATa lys4:LacO(DK)-NAT* GAL+*trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB193-1B	*pds5-S81R wpl1Δ::WPL1-3FLAG-KANMX MATa lys4:LacO(DK)-NAT* GAL+*trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB194-1C	*pds5-P89L wpl1Δ::WPL1-3FLAG-KANMX MATa lys4:LacO(DK)-NAT* GAL+*trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB195-2D	*pds5-E181K wpl1Δ::WPL1-3FLAG-KANMX MATa lys4:LacO(DK)-NAT* GAL+*trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB204-1B	*pds5-S81R wpl1Δ::KANMX MATa 10Kb-CEN4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*

MSB205-4C	*pds5-P89L wpl1Δ::KANMX MATa 10Kb-CEN4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB206-6A	*wpl1Δ::KANMX MATa 10Kb-CEN4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52 pds5-E181K*
MSB210-2A	*pds5-S81R eco1Δ::KANMX MATa 10Kb-CEN4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52 pBS1030 (ECO1 URA3 CEN)*
MSB211-2J	*pds5-P89L eco1Δ::KANMX MATa 10Kb-CEN4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52 pBS1030 (ECO1 URA3 CEN)*
MSB249-3A	*smc3Δ::HPH mcd1Δ::SMC3-MCD1 eco1Δ::G418 MATa lys4:LacO(DK)-NAT GAL+ TIR1-CgTRP1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
MSB223-1A	*pds5-E181K wpl1Δ::HPH MATa lys4:LacO(DK)-NAT GAL+trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
VG3223-12B	*eco1-203 MATa lys4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
VG3349-1B	*MATa lys4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFPLacI-HIS3 leu2-3,112 ura3-52*
VG3360-3D	*rad61Δ::HPH MATa lys4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52*
VG3499-1B	*eco1Δ::KANMX MATa lys4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFP LacI-HIS3 leu2-3,112 ura3-52 pBS1030 (ECO1,CEN,URA3)*
VG3502 #A	*wpl1Δ::HPH eco1Δ::G418 MATa lys4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFPLacI-HIS3 leu2-3,112 ura3-52 pBS1030(ECO1,URA3,CEN)*
VG3503 #4	*wpl1Δ::HPH eco1Δ::G418 MATa 10Kb-CEN4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFPLacI-HIS3 leu2-3,112 ura3-52 pBS1030(ECO1,URA3,CEN)*
VG3513-1B	*wpl1Δ::HPH MATa 10Kb-CEN4:LacO(DK)-NAT GAL+ trp1-1 bar1 his3-11,15:pHIS3-GFPLacI-HIS3 leu2-3,112 ura3-52*
VG3620-4C	*TIR1-CaTRP1 MATa lys4:LacO(DK)-NAT GAL+ leu2-3,112 trp1-1 ura3-52 bar1 his3-11,15:pHIS3-GFPLacI-HIS3*
VG3633-2D	*eco1Δ::ECO1-3V5-AID2-KANMX MATa lys4:LacO(DK)-NAT GAL+ trp1-1 leu2-3,112 ura3-52 bar1 his3-11,15:pHIS3-GFPLacI-HIS3 TIR1-CaTRP1*
VG3940-2D	*smc3Δ::HPH mcd1Δ::SMC3-MCD1 MATa lys4:LacO(DK)-NAT GAL+ TIR1-CgTRP1 bar1 his3-11,15:pHIS3-GFPLacI-HIS3 leu2-3,112 ura3-52*
VG3957-1C	*wpl1Δ::G418 smc3Δ::HPH mcd1Δ::SMC3-MCD1 MATa lys4:LacO(DK)-NAT GAL+ TIR1-CgTRP1 bar1 his3-11,15:pHIS3-GFPLacI-HIS3 leu2-3,112 ura3-52*

Figure S1

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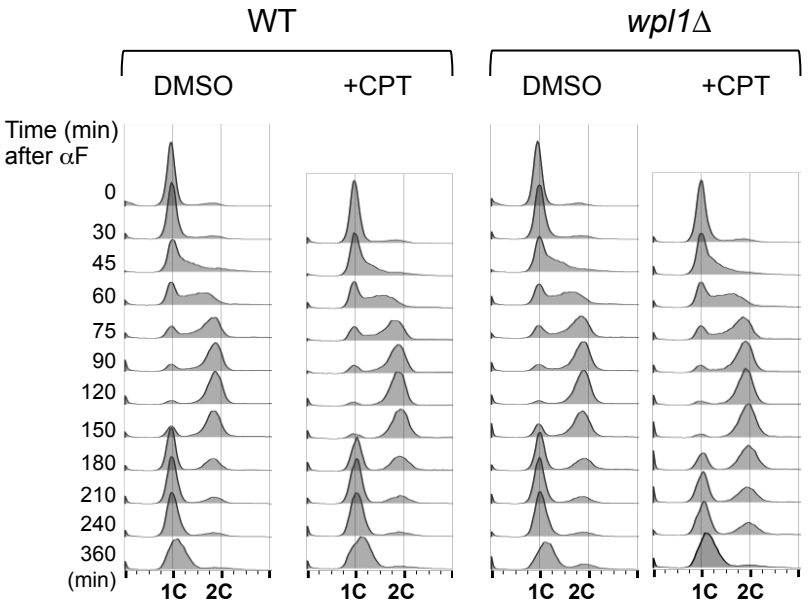
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+CPT
DMSO
+CPT
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60
75
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210
240
360
(min)
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1C 2C
1C 2C
1C 2C

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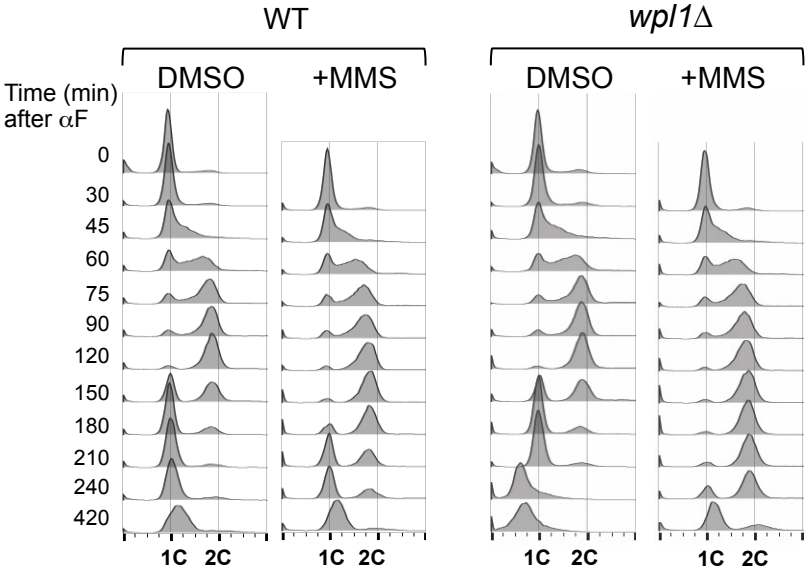
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1C 2C
1C 2C

Figure S2

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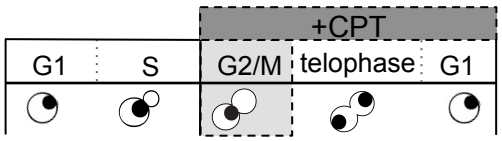

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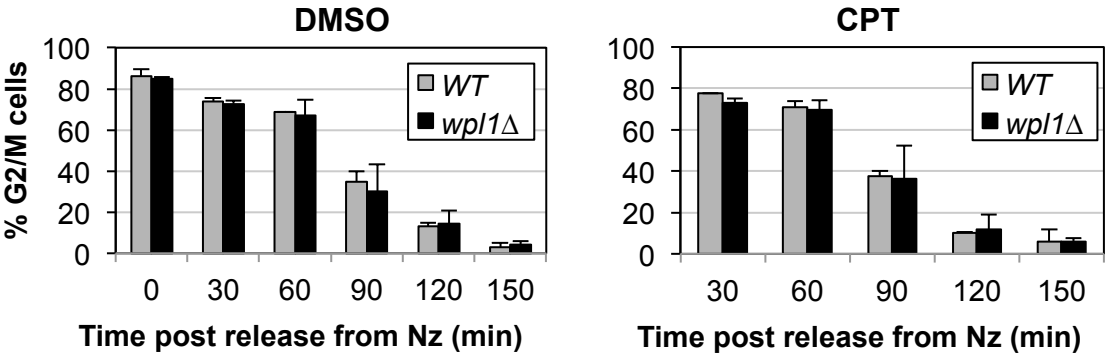

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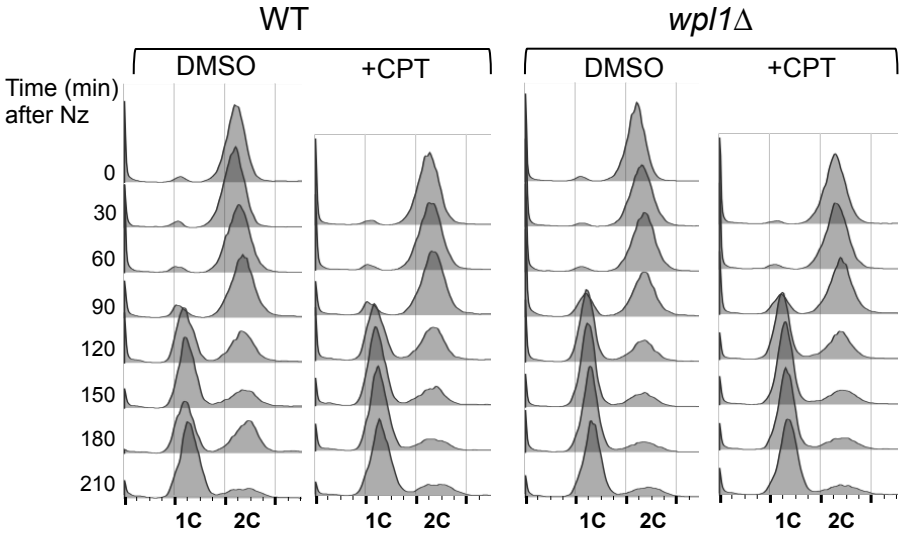

Figure S3

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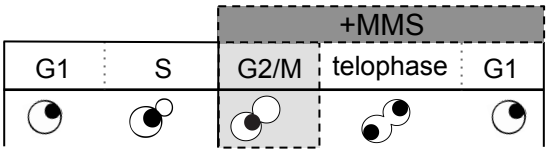

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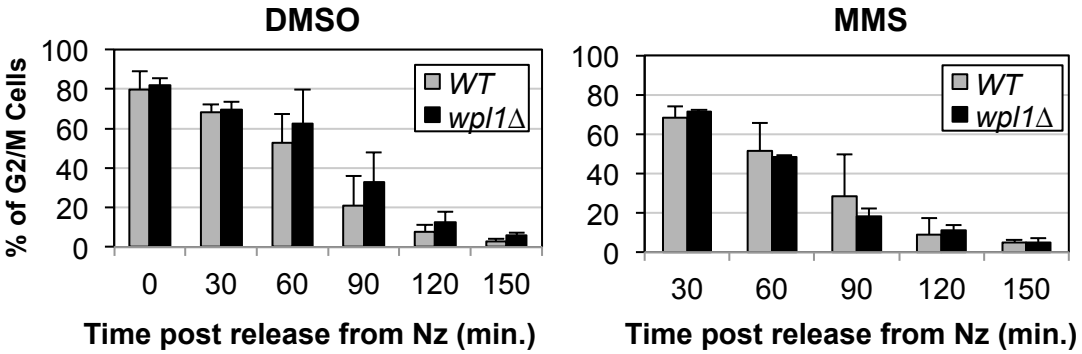

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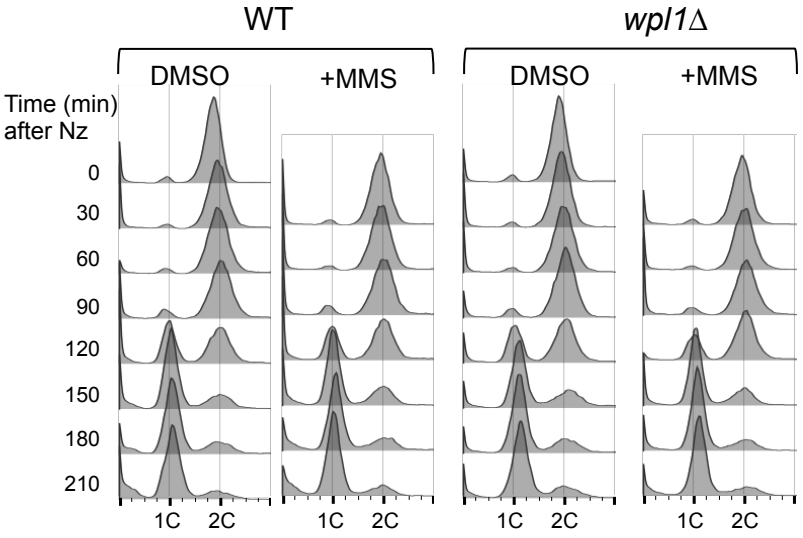
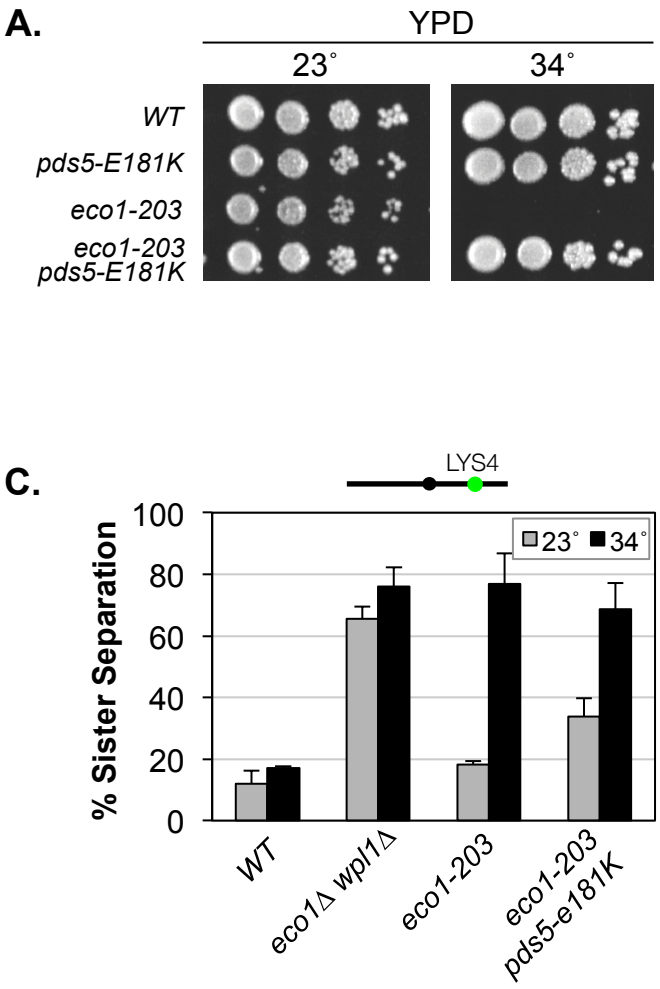


Figure S4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34˚
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eco1-203
eco1-203
pds5-E181K
B.
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% sister Separation
80
60
40
20
0
PDS5
eco1Δ wpl1Δ
eco1Δ
pds5-S81R
eco1Δ
pds5-P89L
C.
LYS4
% Sister Separation
100
80
60
40
20
0
23˚
34˚
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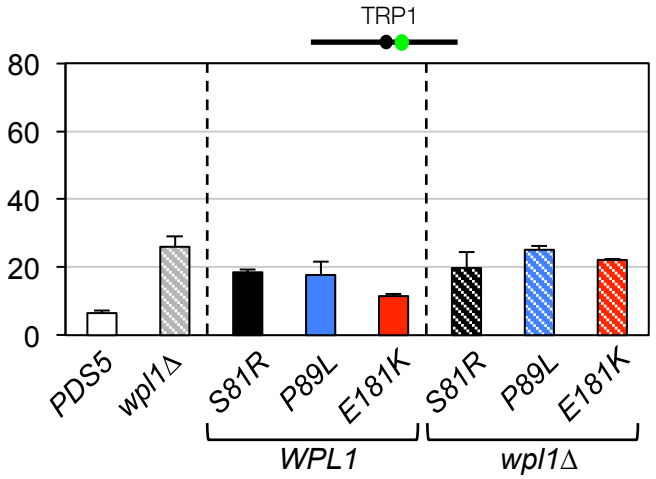
eco1Δ wpl1Δ
eco1-203
eco1-203
pds5-e181K

Figure S5

A.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P89L
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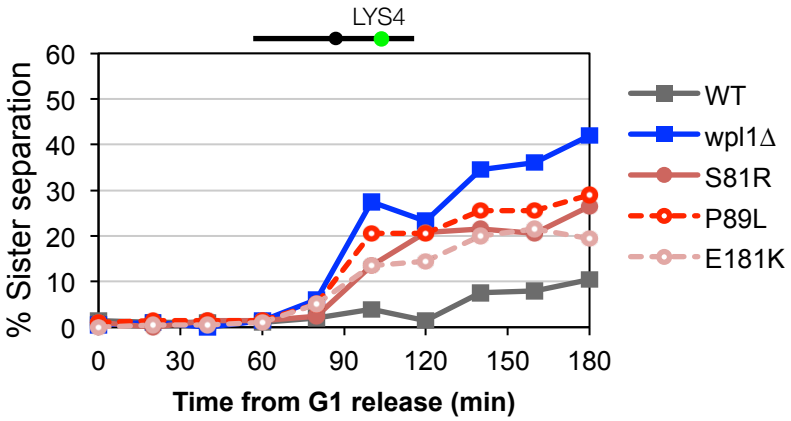
WPL1
wpl1Δ

B.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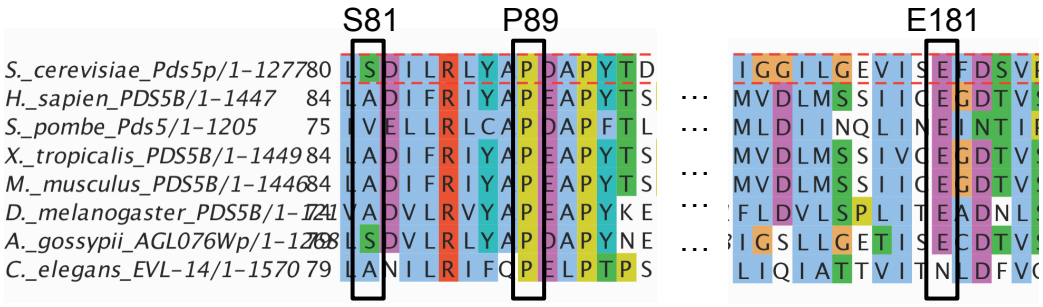
50
40
30
20
10
0
0
30
60
90
120
150
180
Time from G1 release (min)
WT
wpl1Δ
S81R
P89L
E181K

Figure S6

A.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E181
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Figure S7