Expanded View Figures

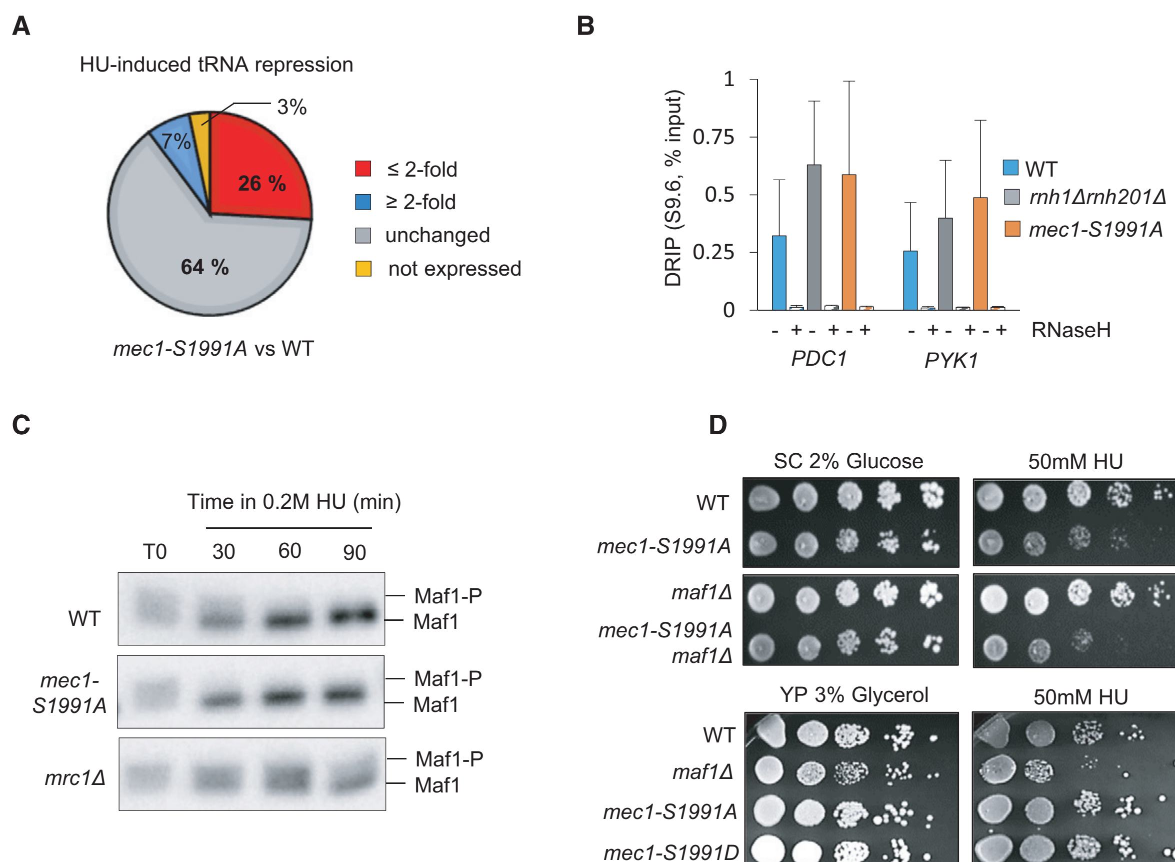

Figure EV1. Mec1-S1991 phosphorylation limits RNAPIII transcription during HU-induced replication stress.

A RNA-seq signals obtained from asynchronous culture and after 90 min of HU treatment allowed to determine the fold change of HU-induced tRNA repression (HU/as) in wild-type and *mec1-S1991A* cells. Pie charts illustrate the proportion of tRNA genes that are either not expressed (yellow), unchanged in steady-state level +/− HU treatment (unchanged, gray) and differentially repressed upon HU treatment in the *mec1-S1991A* mutant compared with wild-type cells (decreased repression in the mutant over wild-type ≤ 2-fold in red, increased ≥ 2-fold in blue).

B DNA–RNA hybrid level measured by DRIP-qPCR at *PDC1* and *PYK1* in asynchronous cells of the indicated genotype. Where indicated, samples were treated with RNase H as a technical control. SEM ($n = 3$ biological replicates) is indicated.

C Asynchronous cells of the indicated genotype (+Maf1-3PK) were treated with 0.2 M HU for 30, 60, and 90 min. Total protein extracts were subjected to SDS–PAGE with a low cross-linked gel for sufficient separation. Differentially phosphorylated forms of Maf1 were detected with a PK antibody.

D Drop assay showing a 10-fold dilution series of cells from exponential SC cultures of the indicated strains that were spotted on SC supplemented either with 2% glucose or 3% glycerol +/− 50 mM HU.

Source data are available online for this figure.

© 2021 The Authors

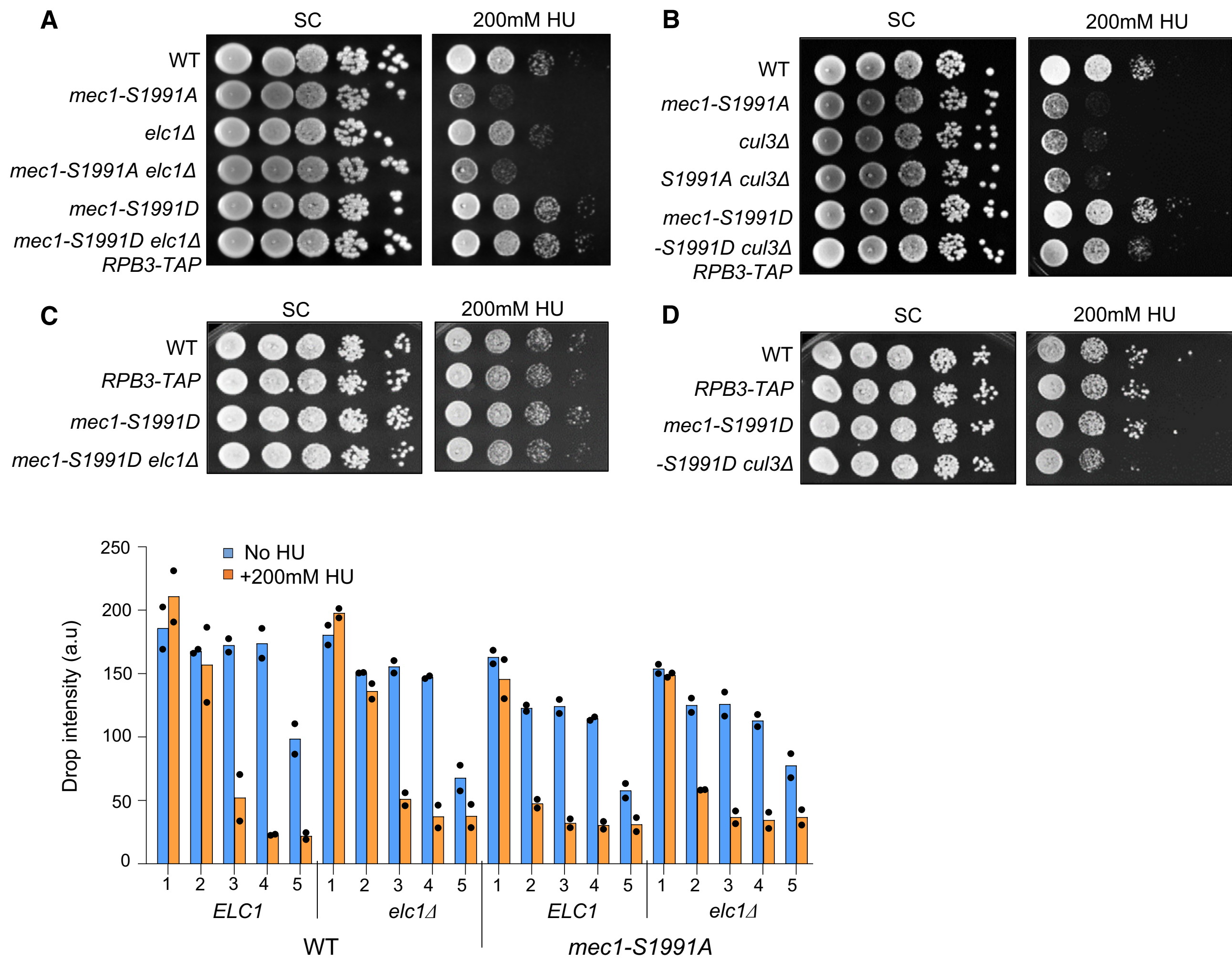

Figure EV2. Comparison of *mec1-S1991A* and *elc1Δ-cul3Δ* growth defects on HU.

A–D Drop assays showing a 10-fold dilution series of cells from exponential SC cultures of the indicated strains that were spotted on SC +/− 200 mM HU. Histogram presents quantification of two independent HU sensitivity assays with mean and individual data point values indicated for each yeast dilution.

Figure EV3. Rescue of *mec1-S1991A* growth defect on HU by RBP3-TAP requires the PAF1 complex subunit Rtf1.

A Analysis of inter-origin distances at the single-molecule level by DNA combing in the indicated strains. Graph depicting the distribution of inter-origin distances determined by DNA combing after 90 min in S phase + 0.2 M HU. Box, 25–75 percentile range. Whiskers, 10–90 percentiles range. Median is indicated in kb. ns (*P*-value > 0.01), by Mann–Whitney rank-sum test. WT (n = 173), *RPB3-TAP* (n = 89), and *mec1-S1991A RPB3-TAP* (n = 118). The WT data set is from Fig 3 and is included for comparison.

B–F Drop assay showing a 10-fold dilution series of cells from exponential SC cultures of the indicated strains that were spotted on SC +/− the indicated drug. *RTF1* encodes an essential subunit of the PAF1 complex. (E) In addition to rescuing *mec1-S1991A* phenotype, expression of Rpb3-TAP slightly improves WT resistance to high doses of HU on YPD.

Source data are available online for this figure.

▶

 © 2021 The Authors

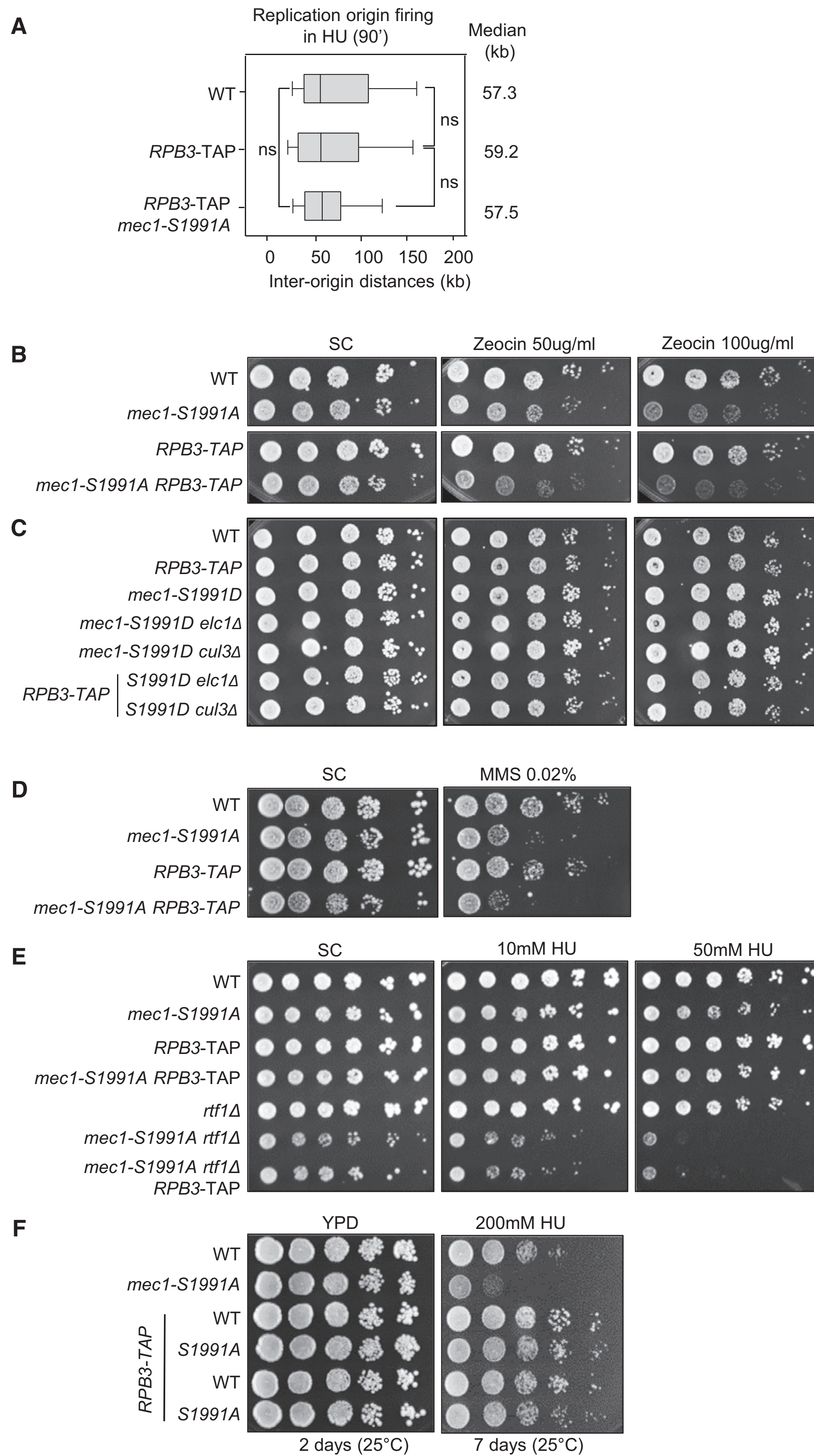

Figure EV3.

© 2021 The Authors

Figure EV4. The *mec1-S1991A* allele alters the kinome response to HU-induced replication stress.

- A Phosphopeptide abundances ($\log_2$ ratio [WT/*mec1-S1991A*]) in asynchronous cells. Colored dots are factors with significantly different phosphopeptide scores (WT/*mec1-S1991A*) > 4 (blue) or < 4 (red) and *P*-value < 0.05 (Student's paired *t*-test, biological replicates *n* = 3; significant phosphopeptides *n* = 97 and *n* = 26, respectively). Full list in Dataset EV4.
- B Venn diagram showing the overlap of *mec1-S1991A*-dependent phosphotargets between asynchronous (*n* = 75) and HU-treated (*n* = 90) cells.
- C Mec1-S1991-dependent phosphotargets in asynchronous, HU-treated cells or common between both conditions.
- D, E Drop assay showing a 10-fold dilution series of cells from exponential SC cultures of the indicated strains that were spotted on SC +/− 50 mM HU.
- F *mec1-S1991A*-dependent phosphotargets (*n* = 90) Gene Ontology on HU-treated cells.
- G LacO repeats were inserted upstream of *GAL1* in order to visualize the locus in the presence of LacI-GFP. Three zones of equal volume were defined based on erosion from the nuclear pore-tagged ring. Zone 1 corresponds to the nuclear periphery and zone 3 to the center of the nucleus.
- H Drop assay showing a 10-fold dilution series of cells from exponential SC cultures of the indicated strains that were spotted on SC +/− 0.2 M HU. *MLP1* and *MLP2* encode subunits of the inner nuclear pore basket that are implicated in the binding of highly transcribed genes.

Source data are available online for this figure.

 © 2021 The Authors

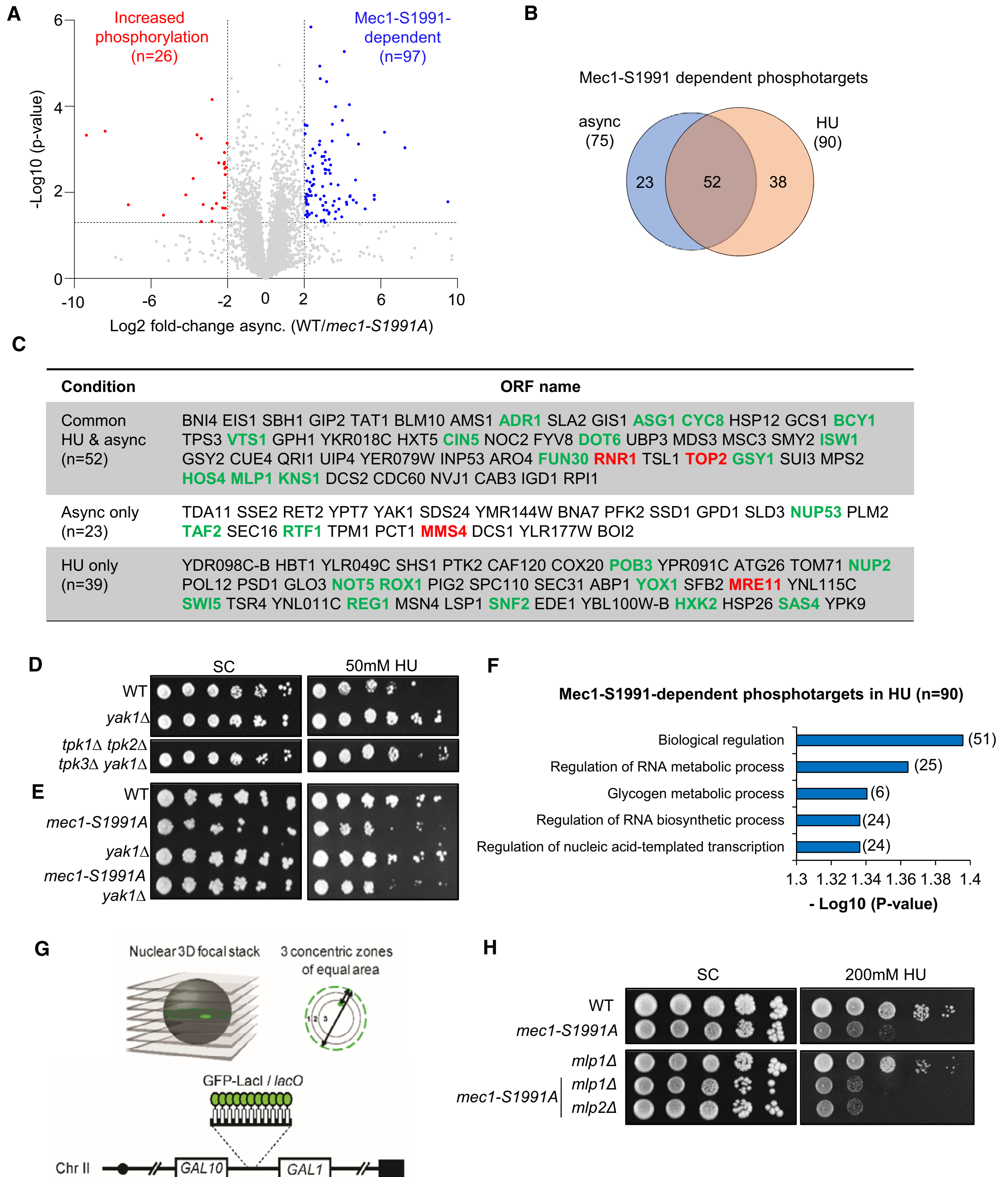

Figure EV4.

Figure EV5. Unchallenged DNA replication transiently reduces RNAPII chromatin binding in a Mec1-dependent manner.

A–C RNAPII retention on chromatin in normal S phase was assessed by ChIP-qPCR by measuring the level of Rpb1 both in G_1 and after 70 min in S phase at 16°C in wild-type and *mec1Δsml1Δ* cells. Rpb1 enrichment was quantified at loci known to generate transcription-replication conflicts: *PDC1* (A), *PYK1* (B), and *YEF3* (C). The mean percentage of Rpb1 kept on chromatin in S phase is indicated as a ratio of the S phase/G_1 level. Data are expressed as percentage of input. SEM (n = 3 biological replicates) is indicated.

D RNAPII level on chromatin was assessed by ChIP-qPCR by measuring the level of Rpb1 both in G_1 and after 70 min in S phase at 16°C in wild-type and *mec1Δsml1Δ* cells. Data are expressed as percentage of input. SEM (n = 3 biological replicates) is indicated.

E mRNA level measured by RT–qPCR in G_1 phase and after 70 min in S phase for several loci in wild-type cells. Expression levels are normalized to *ACT1*. Mean and individual data point values (n = 2 biological replicates) are indicated.

F, G Flow-cytometry analysis of DNA content. Asynchronous cells were synchronized in G_1 with α-factor and released into S phase at 25°C (F) or 16°C (G) in YPAD. The kinetics of G_1/S transition as well as progression through S phase are shown over time.

 © 2021 The Authors

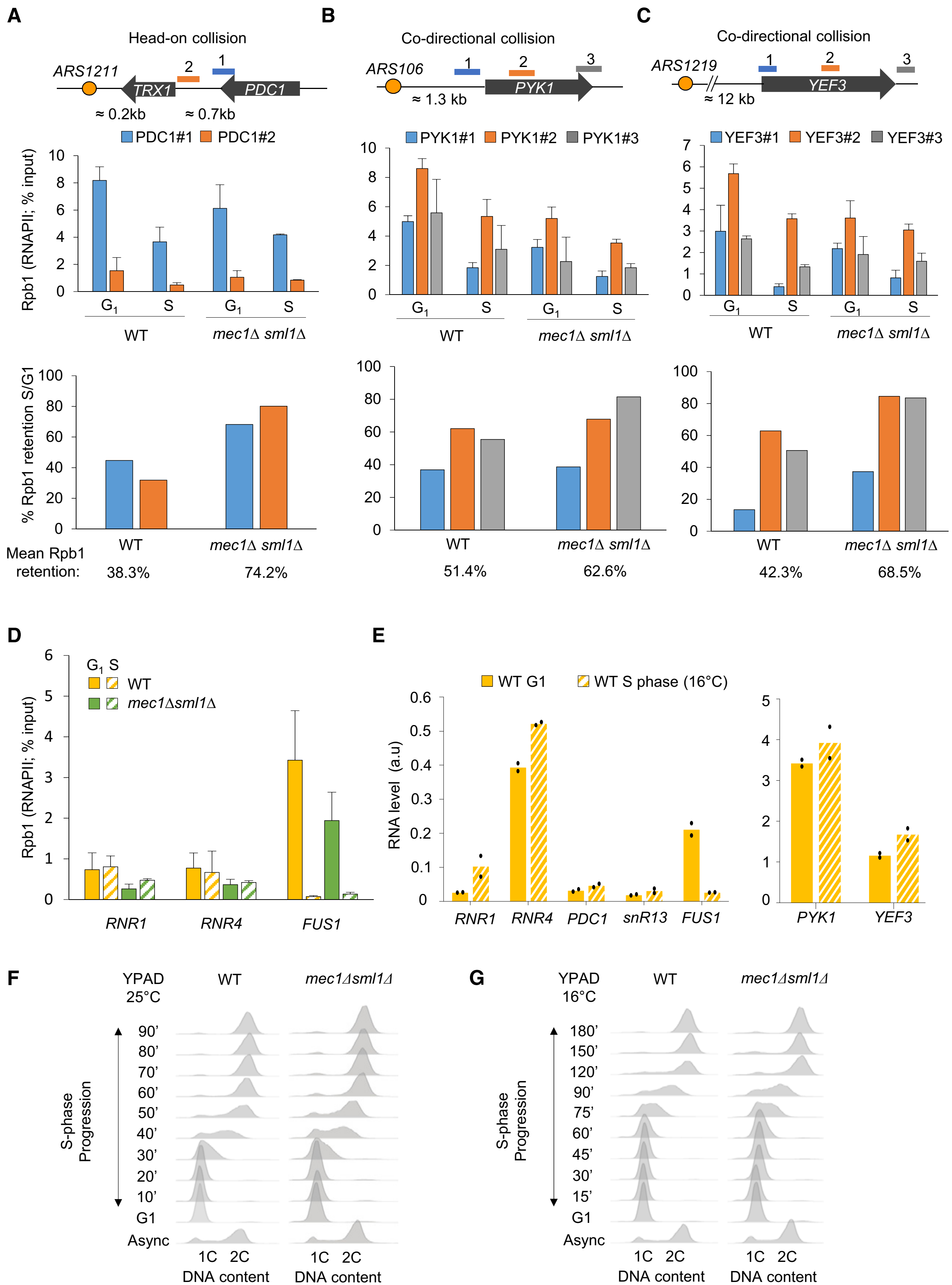

Figure EV5.