Expanded View Figures

Figure EV1. RNase H enzymes are required for optimal growth and cell cycle progression in the presence of HU.

A Growth of wild type and RNase H-deficient cells on synthetic complete (SC) medium ± 50 mM HU. Spots correspond to 1:10 serial dilutions. Data for the SC + 50 mM HU used here is identical to the one in Fig 2A (WT, *rnh1Δ*, *rnh201Δ*, *rnh1Δ rnh201Δ*).

B Flow-cytometry analysis of DNA content in wild-type and *rnh1Δ rnh201Δ* cells synchronized in G_1 with α-factor and released into S phase in the presence of 25 mM HU. Arrows indicate cells accumulating in G_2/M.

C ChIP-qPCR analysis of RPA enrichment at HU-arrested forks in wild-type, *rnh1Δ*, *rnh201Δ* and *rnh1Δ rnh201Δ* cells released synchronously into S phase for 60 min in the presence of 200 mM HU. Primer pairs correspond to regions located 1, 4, and 6 kb upstream of *ARS306* and 1, 3 and 6 kb downstream of *ARS607*. RPA enrichment was normalized to four unreplicated regions. Mean and SEM are indicated ($n = 3$ biological replicates). Two-way ANOVA was applied. *$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$.

D ChIP-qPCR analysis of relative RPA levels at *ARS306* and *ARS607* in cells exposed to HU or MMS. Data from Figs 1E and EV2C are expressed as relative levels in mutants and wild type cells. SEM are indicated ($n = 3$ biological replicates).

E, F ChIP-qPCR analysis of Rnh1-PK_6 and Rnh201-PK_6 enrichment near *ARS306* and *ARS607* in wild type cells. Cells were released from G_1 into S phase in medium containing 200 mM HU and were collected at the indicated timepoints. Mean and SEM are indicated ($n = 3$ biological replicates). Two-way ANOVA was applied. *$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$.

G ChIP-qPCR analysis of RPA enrichment at HU-arrested forks in the indicated cells released synchronously into S phase for 60 min in the presence of 200 mM HU. Experiments were performed as indicated in panel (C) ($n = 2$).

H ChIP-qPCR analysis of relative RPA levels at *ARS306* and *ARS607* in cells exposed to HU. Data from Fig 2B are expressed as relative levels in mutants and wild type cells. SEM are indicated ($n = 3$ biological replicates).

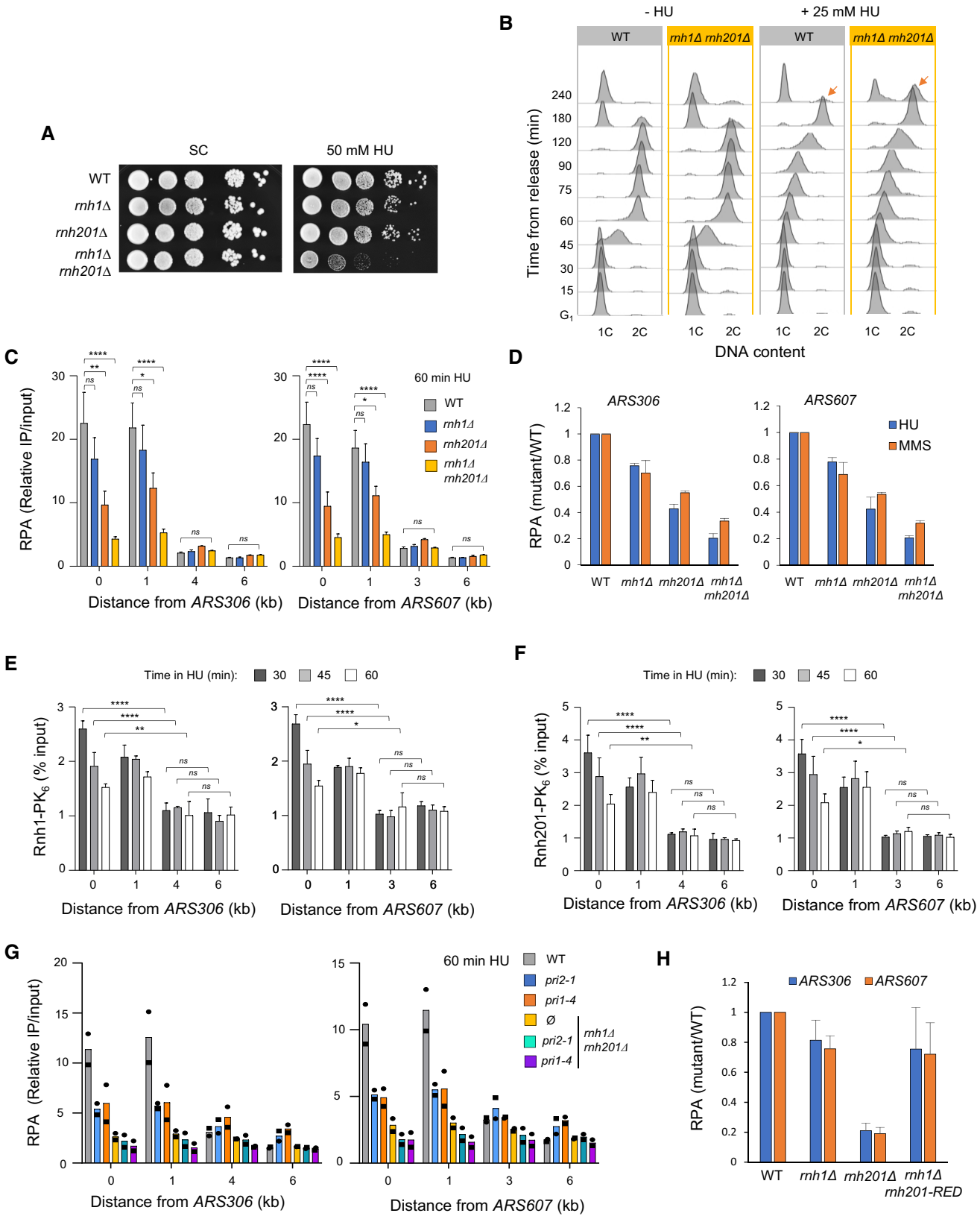
A
SC
50 mM HU
WT
rnh1Δ
rnh201Δ
rnh1Δ rnh201Δ
B
- HU
+ 25 mM HU
WT
rnh1Δ rnh201Δ
Time from release (min)
240
180
120
90
75
60
45
30
15
G1
1C
2C
DNA content
C
RPA (Relative IP/input)
60 min HU
WT
rnh1Δ
rnh201Δ
rnh1Δ rnh201Δ
Distance from ARS306 (kb)
Distance from ARS607 (kb)
D
ARS306
ARS607
RPA (mutant/WT)
HU
MMS
E
Time in HU (min): 30 45 60
Rnh1-PK6 (% input)
F
Rnh201-PK6 (% input)
G
RPA (Relative IP/input)
60 min HU
WT
pri2-1
pri1-4
Ø
rnh1Δ rnh201Δ
H
ARS306
ARS607
RPA (mutant/WT)
rnh201-RED

Figure EV1.

Figure EV2. RNase H activity is dispensable for the activation of early replication origins and the repression of late origins in the presence of HU.

A Genome-wide analysis of origin usage in wild-type and *rnh1Δ rnh201Δ* cells released synchronously into S phase for 60 min in the presence of 200 mM HU. Relative DNA copy number was determined by deep sequencing as the ratio of normalized reads in HU and G_1 cells. A representative region on chromosome IV is shown. Positions of early (black) and late (orange) origins are indicated. Arrowheads point to active origins.

B Number of active and inactive origins in the experiment shown in panel (A). The *rad53Δ sml1Δ* mutant is used as a positive control for the derepression of late origins in HU (Poli *et al*, 2012).

C Distribution of inter-origin distances determined by DNA combing after releasing cells from G_1 into S phase for 90 min in the presence of 200 mM HU. Box, 25–75 percentile range. Whiskers, 10–90 percentiles range. Median is indicated in kb. *ns*: not significant, Mann–Whitney rank-sum test. The DNA combing experiment was repeated twice ($n = 2$ biological replicates) with similar results, one representative experiment is shown.

D Growth of the indicated strains on synthetic complete (SC) ± 50 mM HU or 0.01% MMS. Spots correspond to 1:10 serial dilutions.

E ChIP-qPCR analysis of RPA enrichment around *ARS306* and *ARS607* in the indicated cells released synchronously into S phase for 60 min in the presence of 200 mM HU. RPA enrichment was normalized to four unreplicated regions. Mean and SEM are indicated ($n = 3$ biological replicates). For statistical analysis, two-way ANOVA was applied. $^{**}P < 0.01$; $^{***}P < 0.001$.

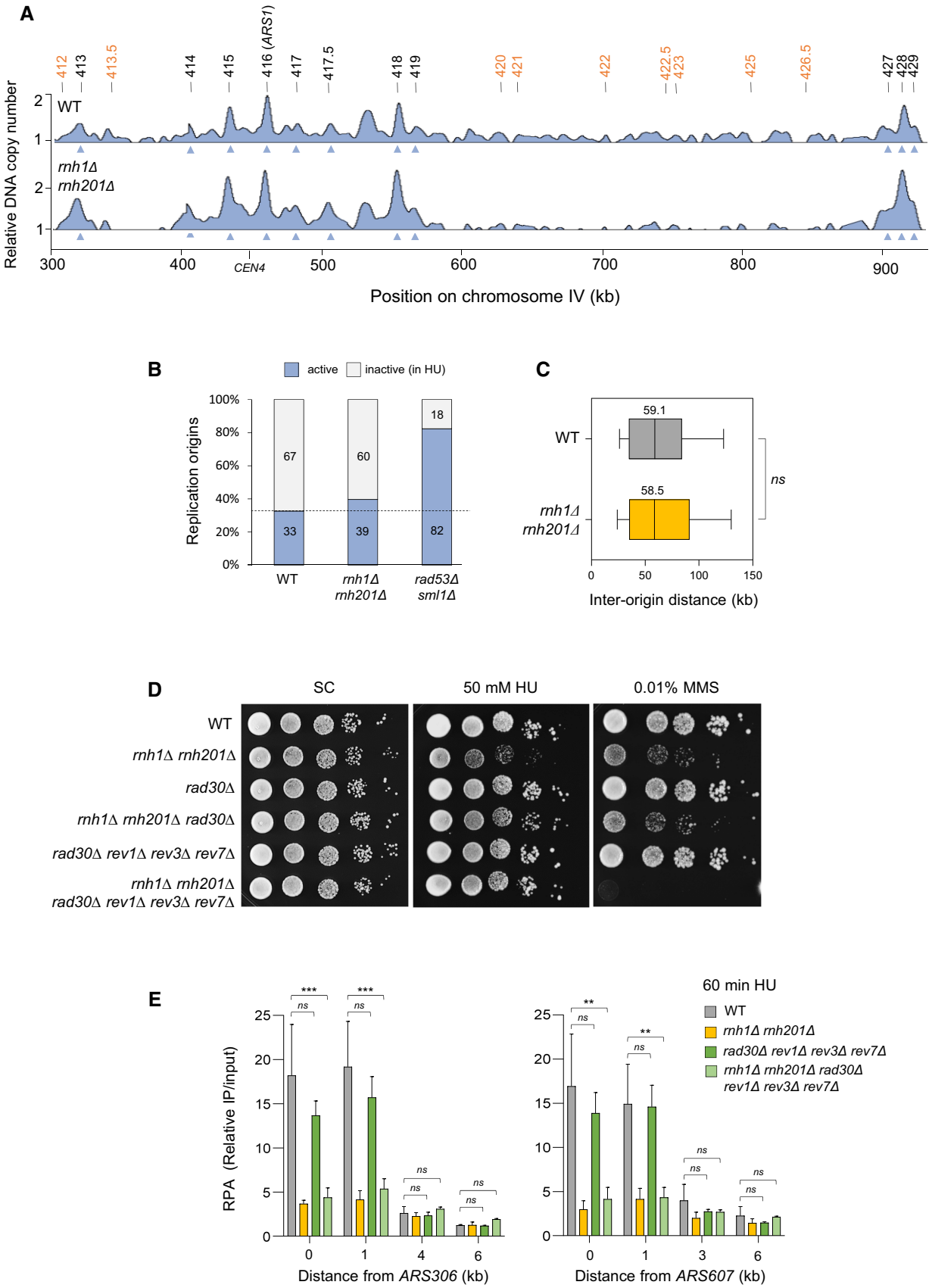
A
412
413
413.5
414
415
416 (ARS1)
417
417.5
418
419
420
421
422
422.5
423
425
426.5
427
428
429
WT
rnh1Δ
rnh201Δ
Relative DNA copy number
CEN4
Position on chromosome IV (kb)
B
active
inactive (in HU)
Replication origins
67
33
60
39
18
82
WT
rnh1Δ rnh201Δ
rad53Δ sml1Δ
C
59.1
58.5
ns
Inter-origin distance (kb)
D
SC
50 mM HU
0.01% MMS
WT
rnh1Δ rnh201Δ
rad30Δ
rnh1Δ rnh201Δ rad30Δ
rad30Δ rev1Δ rev3Δ rev7Δ
rnh1Δ rnh201Δ rad30Δ rev1Δ rev3Δ rev7Δ
E
60 min HU
WT
rnh1Δ rnh201Δ
rad30Δ rev1Δ rev3Δ rev7Δ
rnh1Δ rnh201Δ rad30Δ rev1Δ rev3Δ rev7Δ
RPA (Relative IP/input)
Distance from ARS306 (kb)
Distance from ARS607 (kb)

Figure EV2.

Figure EV3. *SEN1* overexpression alleviates the accumulation of *rnh1Δ rnh201Δ* cells in G_2/M induced by HU and MMS exposure.

A *SEN1* mRNA levels measured by RT–qPCR in asynchronous control cells in the indicated strains. Expression is normalized to *ACT1* ($n = 2$ biological replicates).

B Effect of *SEN1* overexpression on cell cycle progression in wild type and *rnh1Δ rnh201Δ* cells exposed to low doses of HU or MMS. Cells were arrested in G_1 with α-factor and were released into S phase in the presence of 25 mM HU or 0.015% MMS. The percentage of G_2 cells at 180 and 240 min after release from G_1 was calculated after flow cytometry analysis of DNA content. Mean and SEM are indicated ($n = 3$ biological replicates). *$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$, two-way ANOVA.

C, D Representative flow cytometry profiles used for calculating the percentage of G_2/M cells in panel B. Arrows indicate differences due to *SEN1* overexpression.

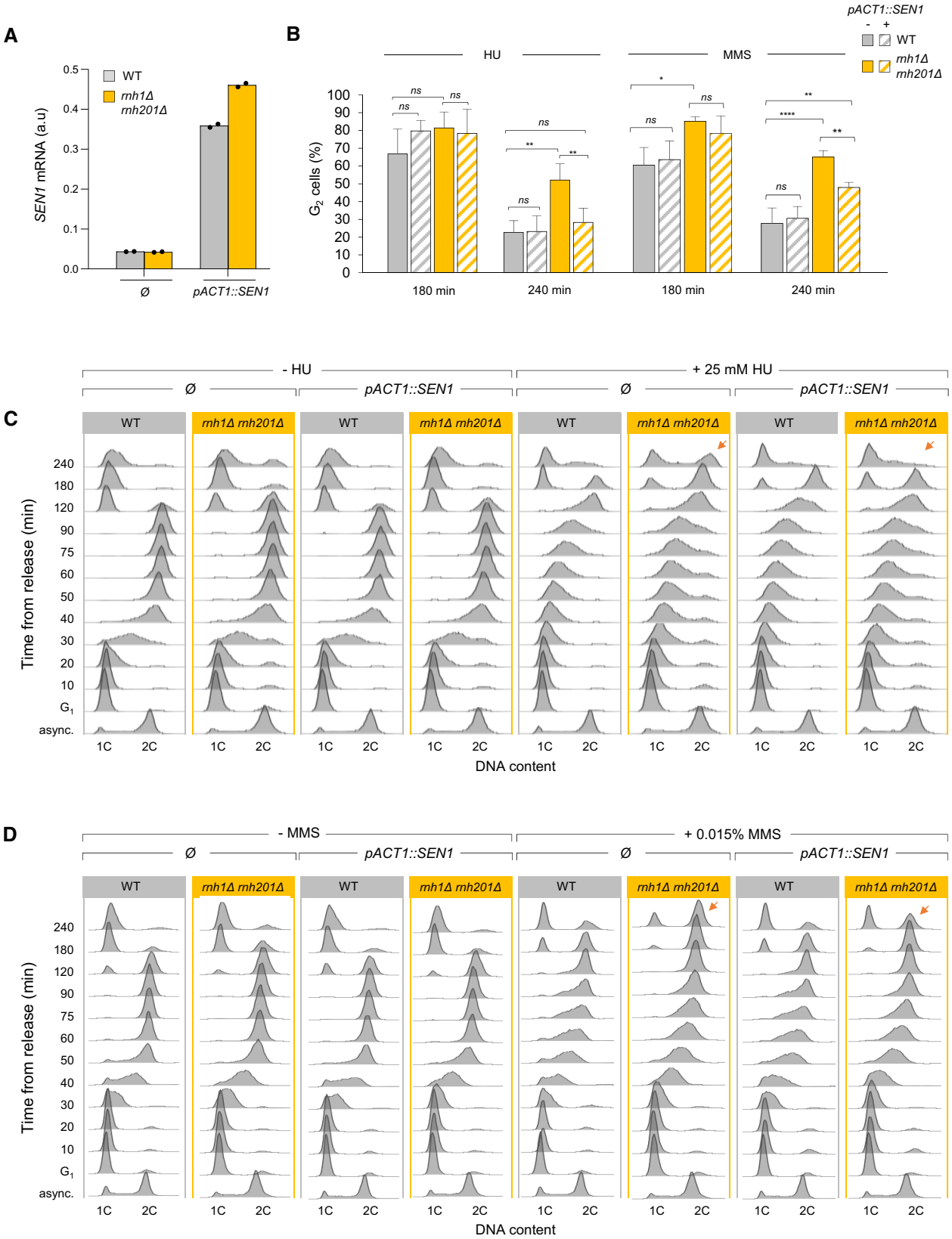
A
SEN1 mRNA (a.u)
WT
rnh1Δ rnh201Δ
Ø
pACT1::SEN1
B
pACT1::SEN1
WT
rnh1Δ rnh201Δ
HU
MMS
G2 cells (%)
180 min
240 min
C
- HU
+ 25 mM HU
pACT1::SEN1
Time from release (min)
async.
DNA content
D
- MMS
+ 0.015% MMS

Figure EV3.

Figure EV4. Analysis of the levels of chromatin-bound RNAPII around HU-arrested forks.

A Chromatin-bound proteins from cells in indicated phases of the cell cycle were prepared and subjected to SDS–PAGE and immunoblotting with the indicated antibodies in a wild-type strain that either does or does not overexpress *SEN1* (Table EV1).

B Quantification of chromatin-bound Rpb1-S2P, Rpb1-S5P and Rpb1-CTD from data shown in panel F. Mcm2 is used as a loading control. Mean and SEM are indicated ($n = 3$ biological replicates). *P*-values are indicated (paired *t*-tests).

C Schematic representation of the genes exhibiting transcription-replication conflicts.

D ChIP-qPCR analysis comparing Rpb1-CTD enrichment in G_1-arrested versus HU-arrested cells (60 min, 0.2 M). Rpb1-CTD occupancy is expressed as percentage of input DNA. Data are expressed as individual data points. Mean is indicated ($n = 2$ biological replicates).

E Variation of DNA copy number around the early origin *ARS305* in wild type cells released synchronously into S phase after an α-factor arrest in medium containing 200 mM HU. Samples were collected every 3 min. DNA copy number was quantified by qPCR at *ARS305* and 4 other loci located at +1, +3, −1 and −3 kb from *ARS305*.

F ChIP-qPCR of Rpb1-CTD enrichment in cells collected in the experiment described in panel (E). Rpb1-CTD occupancy is expressed as percentage of input DNA.

G DRIP-qPCR analysis of RNA:DNA hybrid enrichment at an intergenic locus (*HO*) and two active genes (*YEF3* and *RPL15A*) in wild type and *rnh1Δ rnh201Δ* cells ± *in vitro* RNase H treatment. Data are expressed as a percentage of input. Individual points are indicated ($n = 2$ biological replicates).

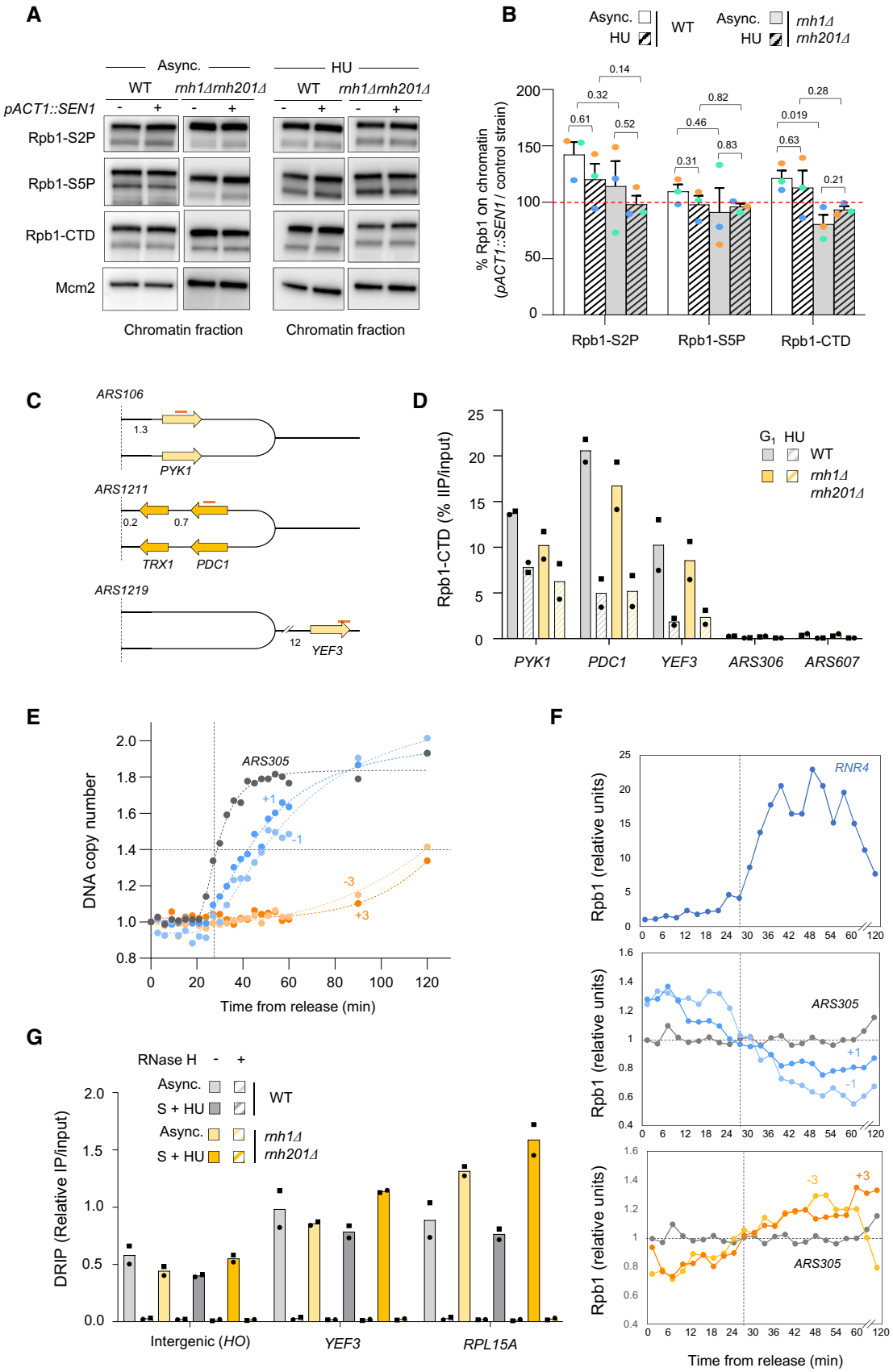
A
Async.
HU
WT
rnh1Δrnh201Δ
pACT1::SEN1
Rpb1-S2P
Rpb1-S5P
Rpb1-CTD
Mcm2
Chromatin fraction
B
WT
rnh1Δ rnh201Δ
% Rpb1 on chromatin (pACT1::SEN1 / control strain)
Rpb1-S2P
Rpb1-S5P
Rpb1-CTD
C
ARS106
PYK1
ARS1211
TRX1
PDC1
ARS1219
YEF3
D
Rpb1-CTD (% IIP/input)
G1 HU
PYK1
PDC1
YEF3
ARS306
ARS607
E
DNA copy number
ARS305
Time from release (min)
F
Rpb1 (relative units)
RNR4
ARS305
G
RNase H
S + HU
DRIP (Relative IP/input)
Intergenic (HO)
YEF3
RPL15A

Figure EV4.

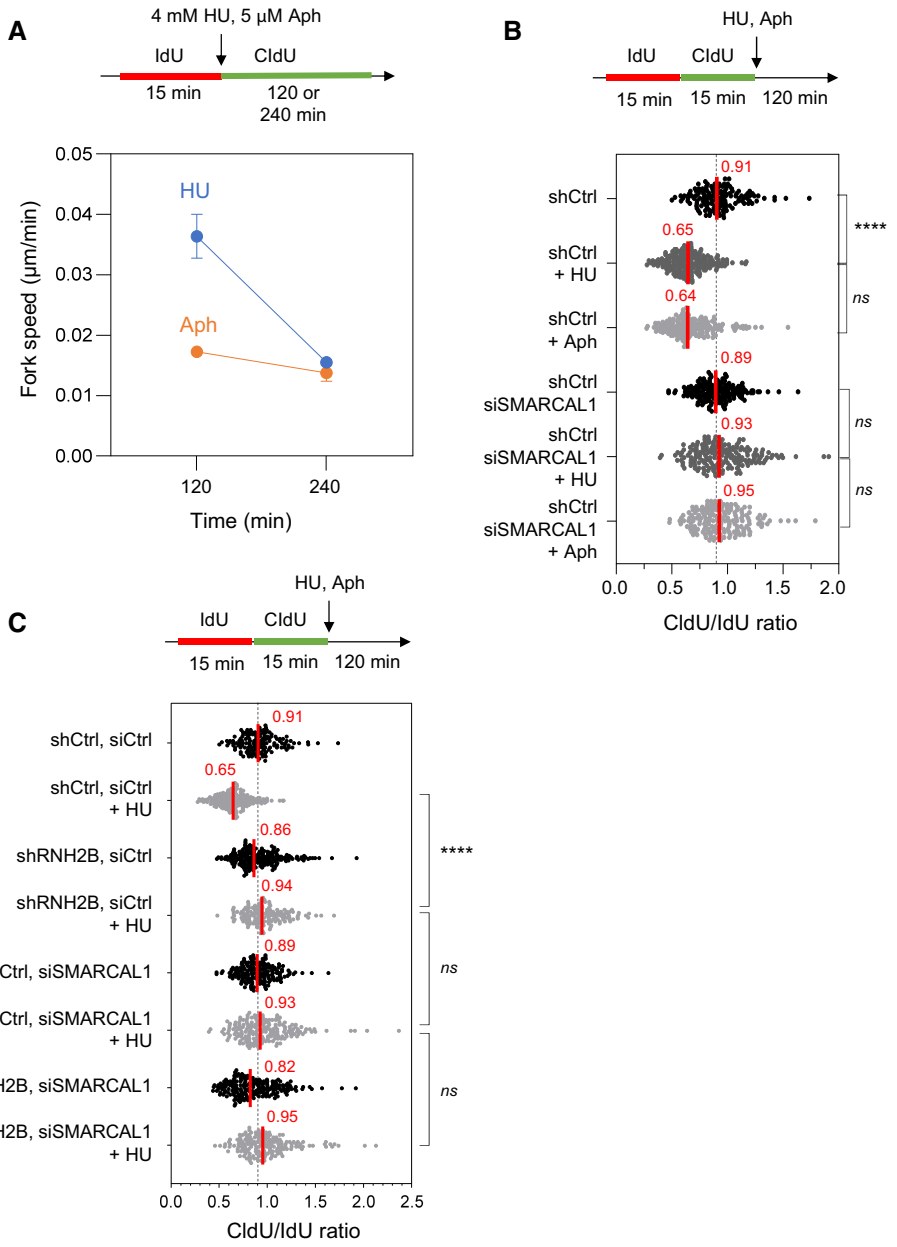

Figure EV5. Effect of aphidicolin and hydroxyurea on fork velocity and on the resection of nascent DNA.

A HeLa cells were labeled for 15 min with IdU and for 120 or 240 min with CldU in the presence of 4 mM hydroxyurea (HU) or 5 µM aphidicolin (Aph). DNA fibers were stretched on glass slides and the length of CldU tracks were measured. Fork speed was calculated during the first 120 min after HU addition and between 120 and 240 min. Mean and SEM are shown for two independent experiments.

B HeLa cells were transfected with siRNA against SMARCAL1 or a control sequence (siCtrl) for 48 h. They were sequentially labeled for 15 min with IdU and for 15 min with CldU. Then, they were treated for 2 h with 4 mM hydroxyurea (HU) or 5 µM aphidicolin (Aph) before DNA fiber analysis. The lengths of the IdU and CldU tracks were plotted as the ratio of CldU to IdU. Median CldU/IdU ratios are indicated in red. ****$P < 0.0001$; ns, non-significant, Mann–Whitney rank-sum test ($n = 2$ biological replicates).

C DNA fiber analysis of fork resection in control HeLa cells (shCtrl) and in HeLa cells depleted for RNase H2B (shRNH2B) and transfected with siRNA against SMARCAL1 or a control sequence (siCtrl), and exposed or not to 4 mM HU. Cells were treated with doxycycline (10 µg/ml) for 24 h and were then transfected with siRNAs for 48 h in the presence of doxycycline. Cells were sequentially labeled for 15 min with IdU and for 15 min with CldU. Then, they were either collected immediately or treated for 2 h with 4 mM hydroxyurea (HU) before DNA fiber analysis. The lengths of the IdU and CldU tracks were plotted as the ratio of CldU to IdU. Median CldU/IdU ratios are indicated in red. ****$P < 0.0001$; ns, non-significant, Mann–Whitney rank-sum test ($n = 2$ biological replicates).