

SUPPLEMENTAL INFORMATIONS

Elongating RNA polymerase II and RNA:DNA hybrids hinder fork progression and gene expression at sites of head-on replication-transcription collisions.

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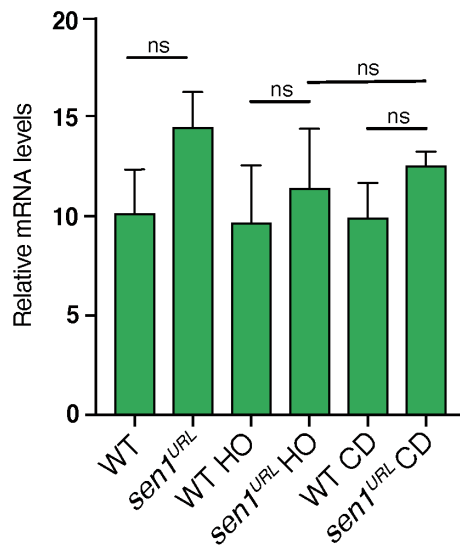
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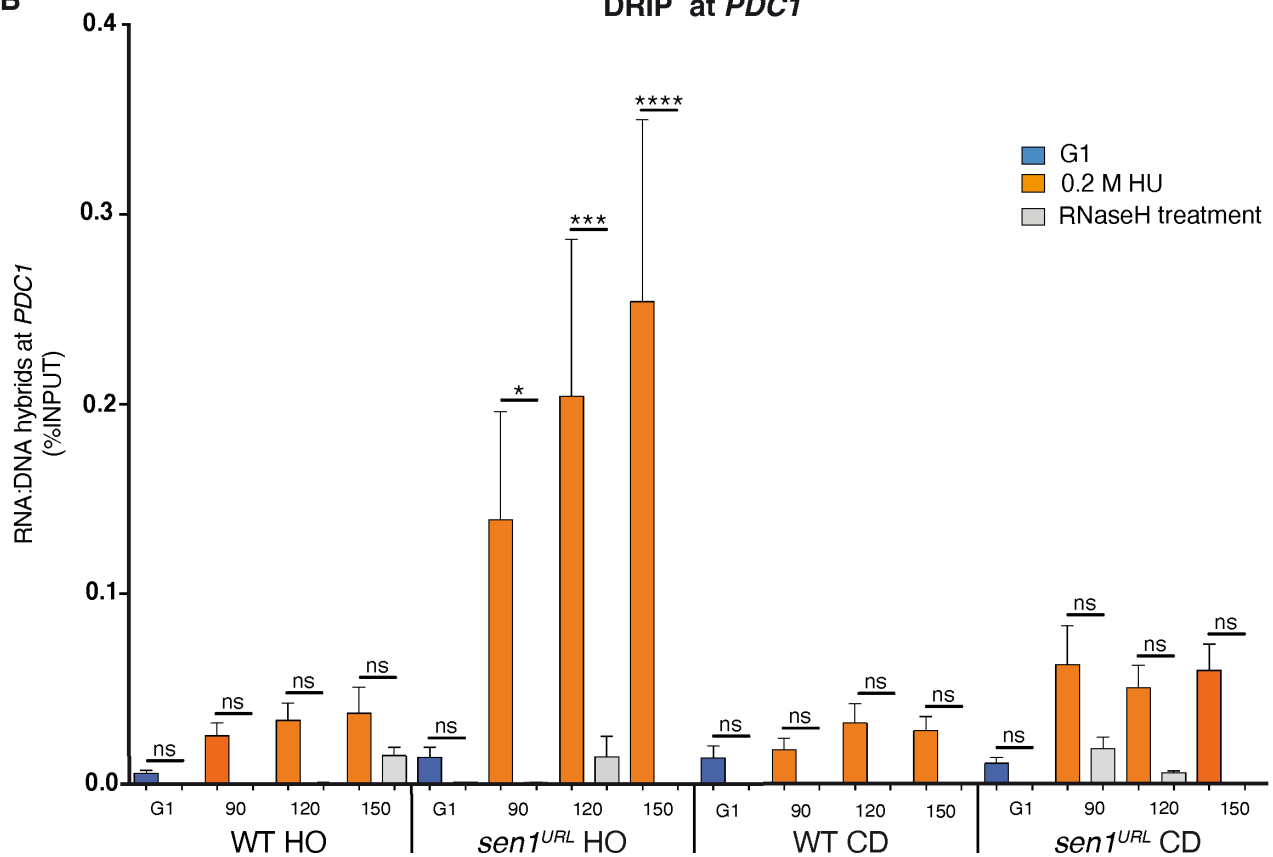
Supplementary Table S1

A

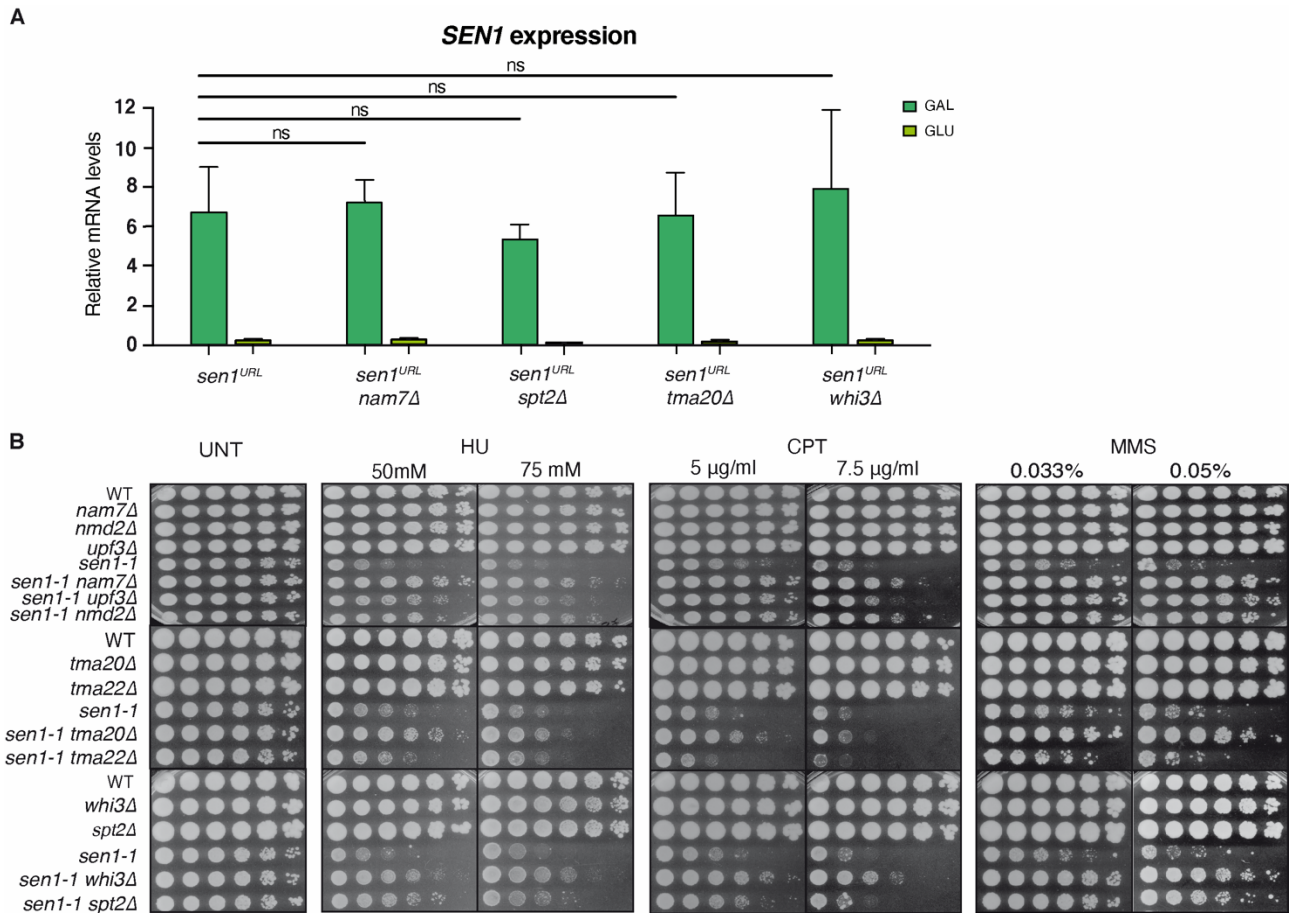
***PDC1* expression**
after 150 minutes release in 0.2 M HU

**B**

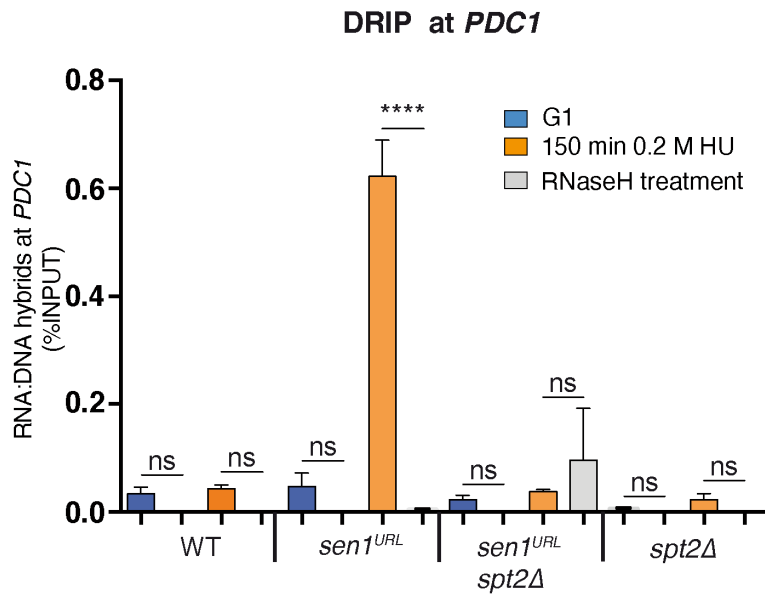
DRIP at *PDC1*



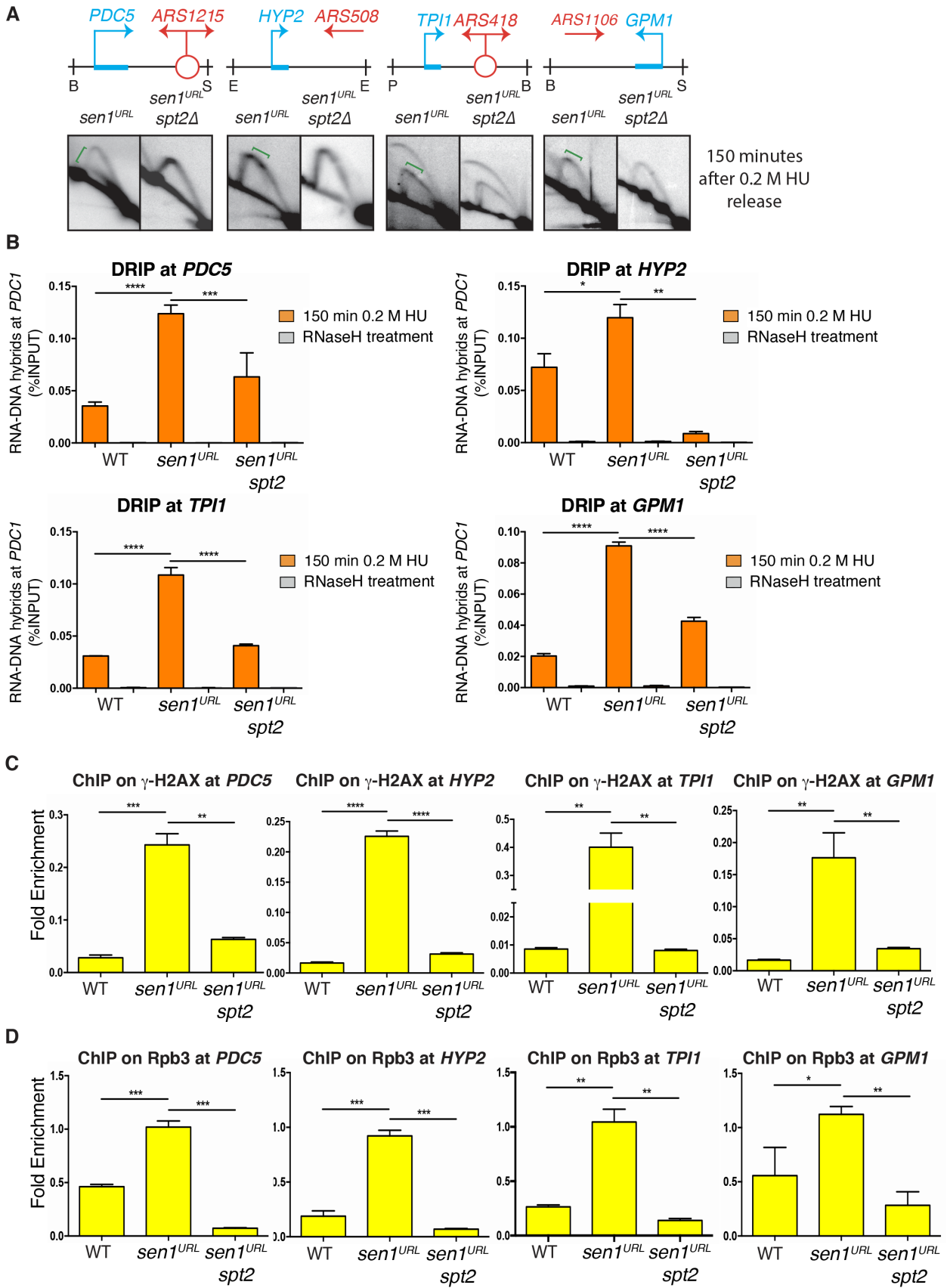
Supplementary Figure S1. Additional analysis of head-on and co-directional replication-transcription conflicts in WT and *sen1* strains. **(A)** *PDC1* mRNA levels measured by qPCR at the gene in head-on (HO) or co-directional (CD) orientation to *ARS607*-dependent replication. Data represent mean \pm SEM from three independent experiments. ns, not significant (two-way ANOVA). **(B)** DRIP-qPCR in Figure 1B shown with analysis on samples treated with RNase H. Data represent mean \pm SEM from three independent experiments. ns, not significant; * $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$ (two-way ANOVA).



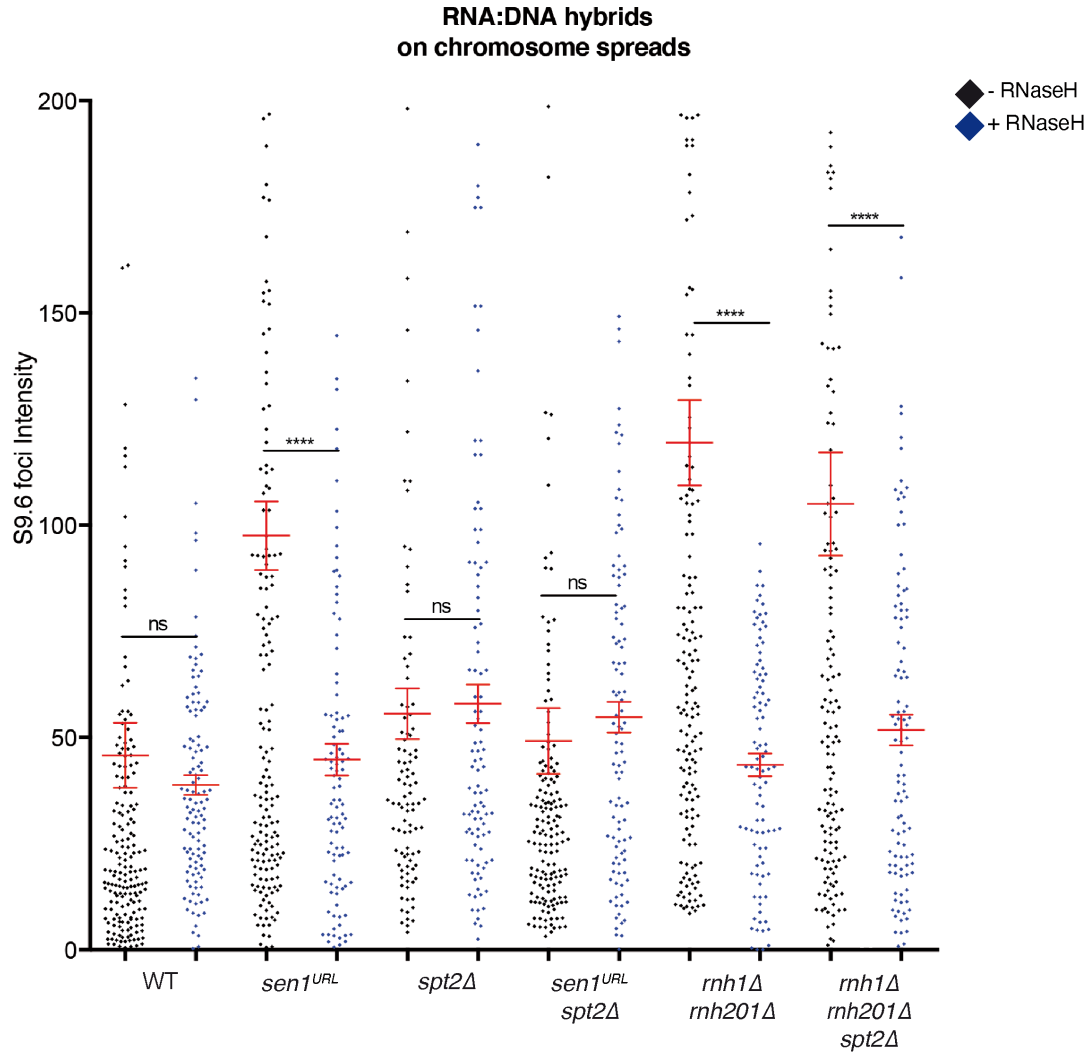
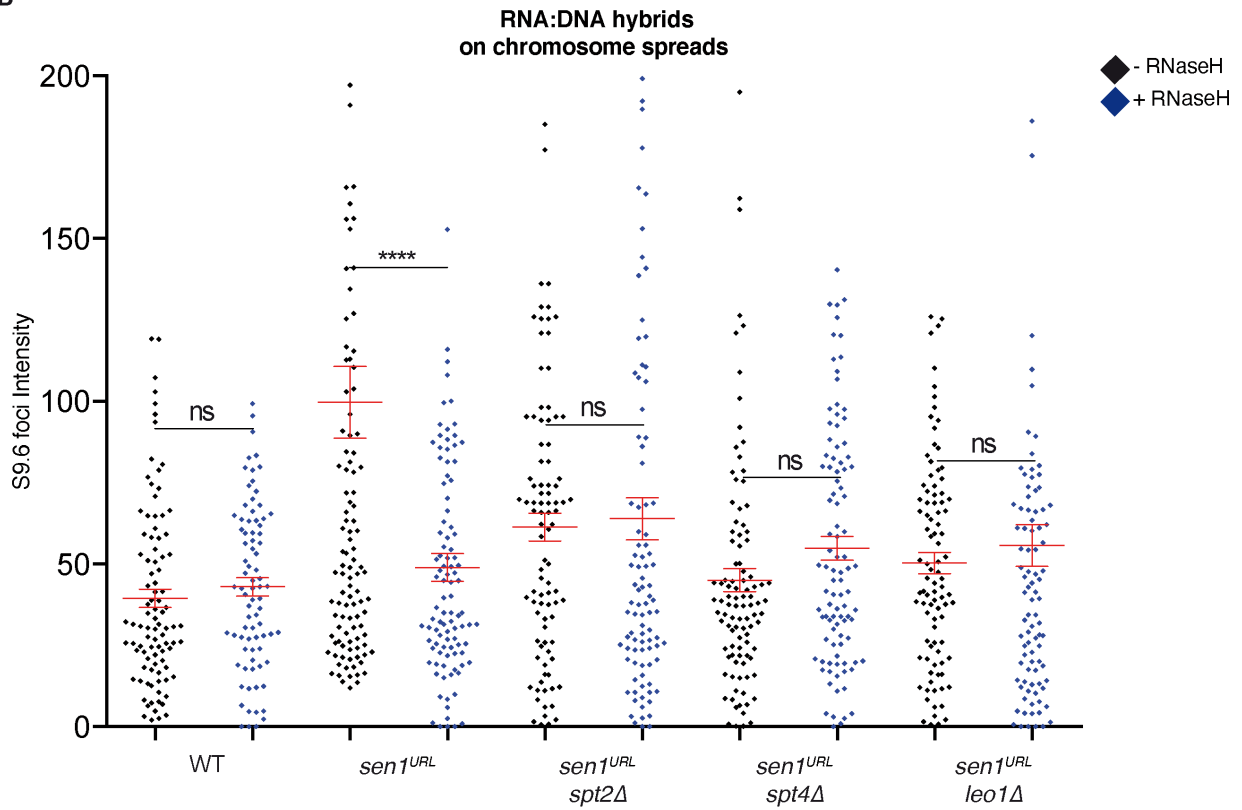
Supplementary Figure S2. *sen1^{URL}* suppressors do not regulate *SEN1* levels and rescue DNA damage sensitivity of *sen1-1* allele. (A) *SEN1* mRNA levels measured by qPCR in YPG and after 4 hours-shift in YPD. Data represent mean \pm SEM from three independent experiments. ns, not significant (two-way ANOVA). (B) Serial dilutions of the indicated strains were spotted on YPD and YPD supplemented with different concentrations of Hydroxyurea (HU), Camptothecin (CPT) and Methyl methanesulfonate (MMS) and grown at the semi-permissive temperature of 30°C for *sen1-1*.

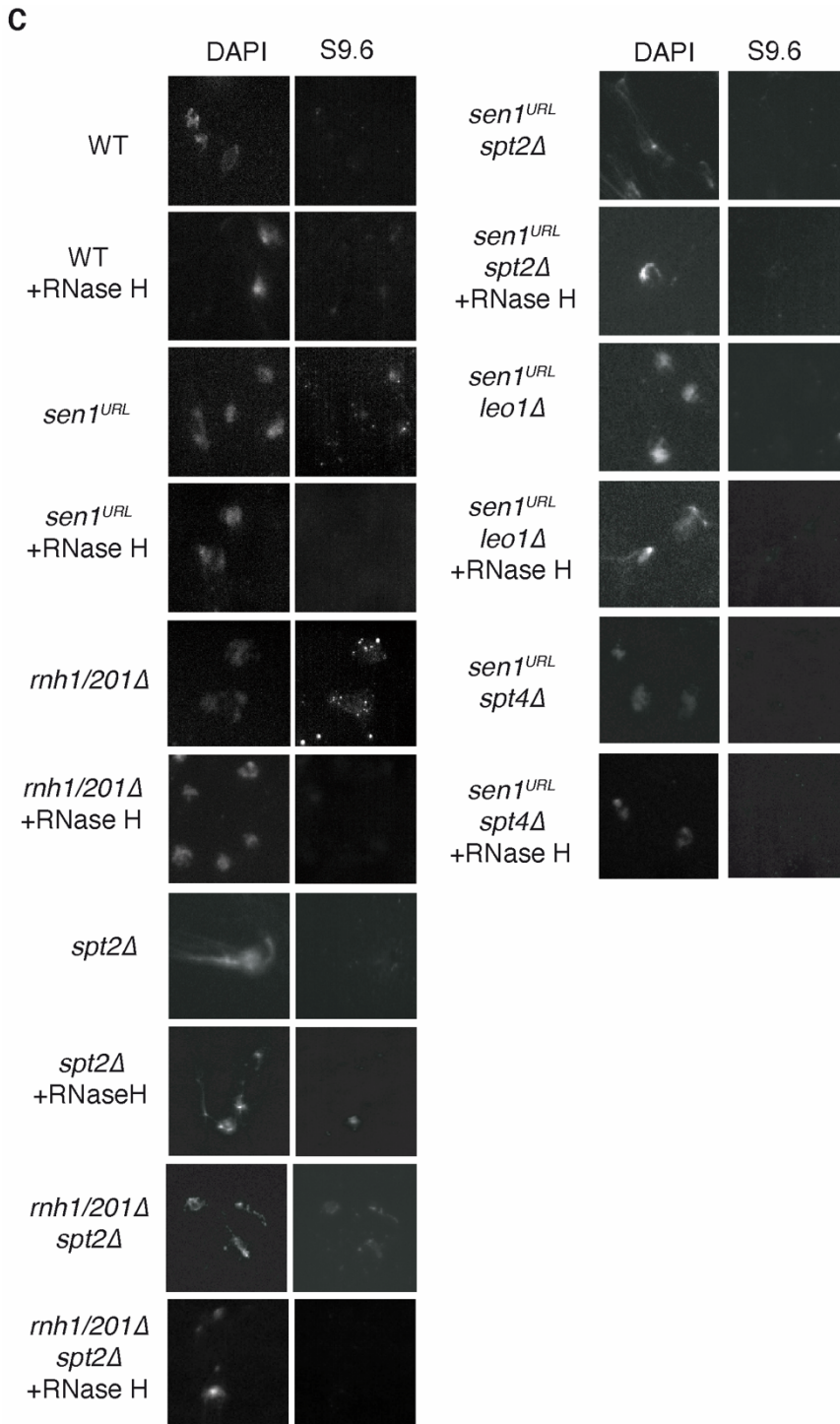


Supplementary Figure S3. *In vitro* RNase H treatment of RNA:DNA hybrids detected by DRIP-qPCR. DRIP-qPCR in Figure 3B shown with the analysis on samples treated with RNase H. Data represent mean \pm SEM from three independent experiments. ns, not significant; **** $P < 0.0001$ (two-way ANOVA).

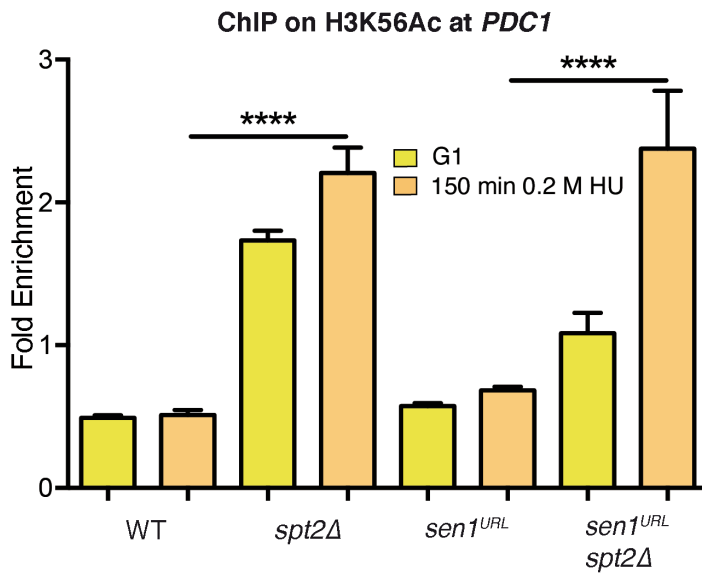


Supplementary Figure S4. Dangerous outcomes of head-on replication-transcription collisions at highly expressed genes in *sen1* mutants and suppression by Spt2 inactivation. **(A)** Yeast strains were treated in HU as in Figure 3A and replication intermediates were analyzed by 2D gel technique at the *PDC5*, *HYP2*, *TPI1* and *GPM1* loci upon digestion with BglII/SphI (B/S), EcoRI (E), PstI/BamHI (P/B) and BamHI/StuI (B/S), respectively. Green brackets indicate *sen1*-dependent replication fork defects at sites of collisions with transcription. At the same transcribed loci, the levels of RNA:DNA hybrids **(B)** were analyzed by DRIP-qPCR and the levels of γ -H2AX **(C)** or Rpb3 **(D)** by ChIP-qPCR. Data represent mean \pm SEM from three independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$ (two-way ANOVA). Analysis at *PDC5* gene were conducted in strains not expressing *PDC1*.

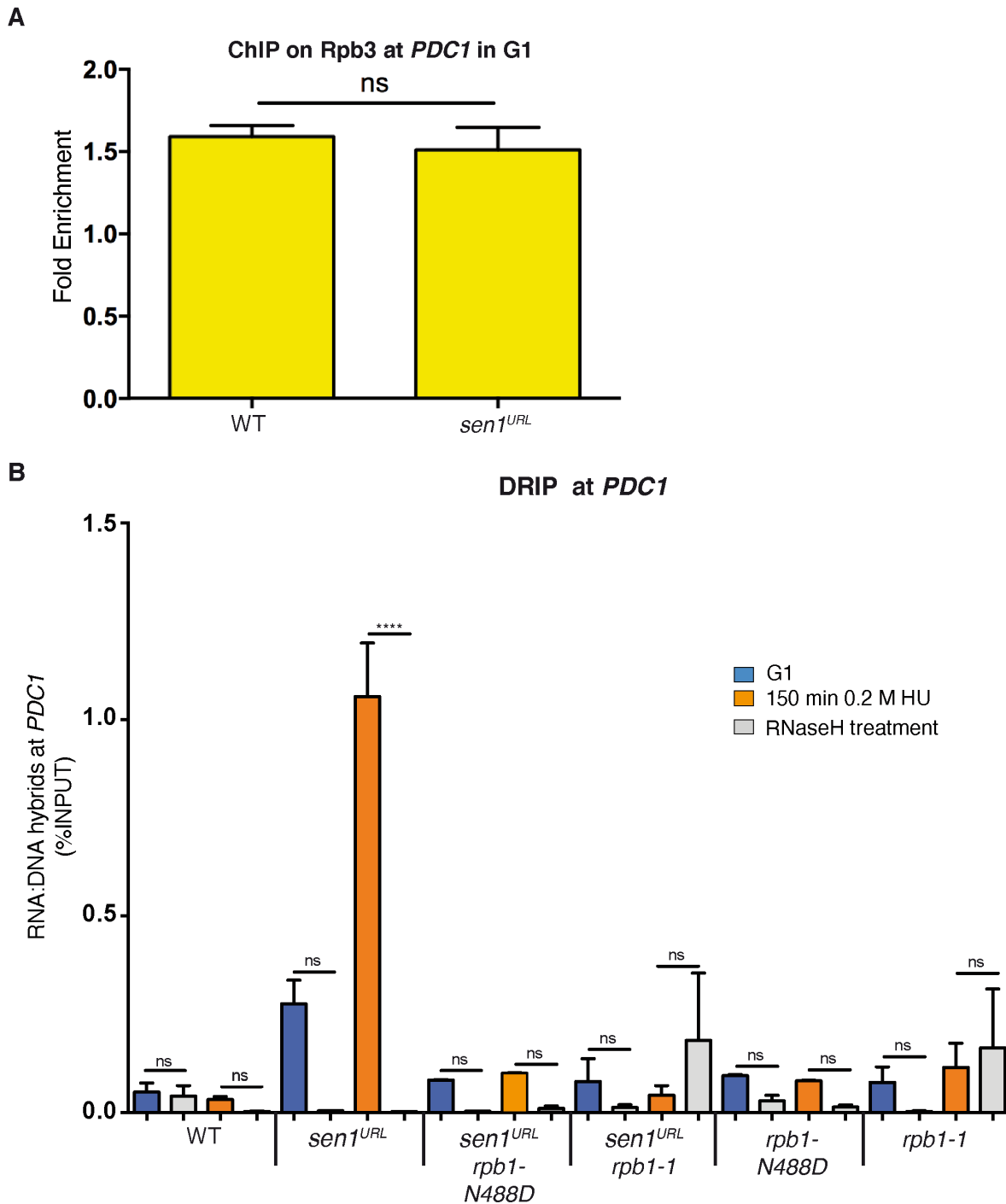
A**B**



Supplementary Figure S5. In *vitro* RNase H treatment of S9.6 foci detected by IF. S9.6 foci quantification on chromosome spreads in Figure 4A (A) or in Figure 5C (B) shown with the analysis on samples treated with RNase H enzyme. Data represent mean \pm SEM from three independent experiments. At least 100 nuclei for each experiment were analyzed. ns, not significant; ****P<0.0001 (unpaired two-tails t-test). (C) Representative images of S9.6 immunostaining on chromosome spreads treated or not with RNase H.



Supplementary Figure S6. Analysis of H3K56ac levels. ChIP-qPCR analysis at *PDC1* locus of histone H3 lysine 56-acetylated (H3K56ac). Data represent mean \pm SEM from three independent experiments. ****P<0.0001 (two-way ANOVA).



Supplementary Figure S7. Additional analysis of RNAPII accumulation at *PDC1* gene in *sen1* mutants. (A) ChIP-qPCR analysis at *PDC1* locus of the Rpb3 subunit of the RNAPII complex in cells arrested in G1-phase of the cell cycle. Data represent mean \pm SEM from three independent experiments. ns, not significant (two-way ANOVA). (B) *In vitro* RNase H treatment of RNA:DNA hybrids detected by DRIP-qPCR. DRIP-qPCR in Figure 6F shown with analysis on samples treated with RNase H. Data represent mean \pm SEM from three independent experiments. ns, not significant; **** $P < 0.0001$ (two-way ANOVA).

Supplementary Table S1

number	Genotype	Reference
GF 9	<i>MATa ade2-1 trp1-1 leu2-3 112 his3-11 15 ura3-1 can1-100 GALPSI⁺ RAD5⁺</i>	R.Rothstein, H.Klein
GH 788	<i>MATa, KANMX6-GAL-URL-3HA-SEN1</i>	Lab stock
GH 176	<i>MATa, sen1-1-NAT</i>	Lab stock
GH 1059	<i>MATa, PDC1 CD at 948bp from ARS607, pdc1::URA3</i>	This study
GH 1067	<i>MATa, KANMX6-GAL-URL-3HA-SEN1, PDC1 CD at 948bp from ARS607, pdc1::URA3</i>	This study
GH 1083	<i>MATa, PDC1 HO at 948bp from ARS607, pdc1::URA3</i>	This study
GH 1082	<i>MATa, KANMX6-GAL-URL-3HA-SEN1, PDC1 HO at 948bp from ARS607, pdc1::URA3</i>	This study
GH1349	<i>MATa, PDC1 CD at 948bp from ARS607, pdc1::HIS3MX6</i>	This study
GH1351	<i>MATa, sen1-1-NAT, PDC1 CD at 948bp from ARS607, pdc1::HIS3MX6</i>	This study
GH1350	<i>MATa, PDC1 HD at 948bp from ARS607, pdc1::HIS3MX6</i>	This study
GH1345	<i>MATa, sen1-1-NAT, PDC1 HD at 948bp from ARS607, pdc1::HIS3MX6</i>	This study
GH 500	<i>MATa, KANMX6-GAL-URL-3HA-SEN1, nam7::HIS3MX6</i>	This study
GH 707	<i>MATa, NAT-GAL-URL-3HA-SEN1, nmd2::HIS3MX6</i>	This study
GH 703	<i>MATa, NAT-GAL-URL-3HA-SEN1, upf3::HIS3MX6</i>	This study
GH 622	<i>MATa, NAT-GAL-URL-3HA-SEN1, tma20::HIS3MX6</i>	This study
GH 624	<i>MATa, NAT-GAL-URL-3HA-SEN1, tma22::HIS3MX6</i>	This study
GH 410	<i>MATa, KANMX6-GAL-URL-3HA-SEN1, whi3::HIS3MX6</i>	This study
GH 595	<i>MATa, KANMX6-GAL-URL-3HA-SEN1, spt2::HIS3MX6</i>	This study
GH 354	<i>MATa, nam7::HIS3MX6</i>	This study
GH 678	<i>MATa, nmd2::HIS3MX6</i>	This study
GH 676	<i>MATa, upf3::HIS3MX6</i>	This study
GH 633	<i>MATa, tma20::HIS3MX6</i>	This study
GH 635	<i>MATa, tma22::HIS3MX6</i>	This study
GH 459	<i>MATa, whi3::HIS3MX6</i>	This study
GH 593	<i>MATa, spt2::HIS3MX6</i>	This study
GH 502	<i>MATa, sen1-1-NAT, nam7::HIS3MX6</i>	This study
GH 727	<i>MATa, sen1-1-NAT, upf3::HIS3MX6</i>	This study
GH736	<i>MATa, sen1-1-NAT, nmd2::HIS3MX6</i>	This study
GH 622	<i>MATa, sen1-1-NAT, tma20::HIS3MX6</i>	This study
GH 630	<i>MATa, sen1-1-NAT, tma22::HIS3MX6</i>	This study
GH748	<i>MATa, sen1-1-NAT, whi3::HIS3MX6</i>	This study
GH616	<i>MATa, sen1-1-NAT, spt2::HIS3MX6</i>	This study
GH 616	<i>MATa, sen1-1-NAT, spt2:: HIS3MX6</i>	This study
GH 37	<i>MATa, rnh1::HIS3MX6, rnh201::NAT</i>	Lab stock
GH 1305	<i>MATa, rnh1::HIS3MX6, rnh201::NAT, spt2::URA3</i>	This study
GH 855	<i>MATalpha, mrc1::URA3</i>	Lab stock
GH 1167	<i>MATa, KANMX6-GAL-URL-3HA-SEN1, spt4::HIS3MX6</i>	This study

GH 620	<i>MATa, NAT-GAL-URL-3HA-SEN1, leo1::HIS3MX6</i>	Lab stock
GH 1256	<i>MATa, NAT-GAL-URL-3HA-SEN1, nhp6A::KANMX6, nhp6B::URA3</i>	This study
GH 1106	<i>MATa, NAT-GAL-URL-3HA-SEN1, hmo1::KANMX6</i>	This study
GH 1146	<i>MATa, KANMX6-GAL-URL-3HA-SEN1, hho1::URA3</i>	This study
GH 1176	<i>Mata, KANMX6-GAL-URL-3HA-SEN1, hht1::URA3,</i>	This study
GH 1269	<i>MATa, NAT-GAL-URL-3HA-SEN1, bre1::KANMX6</i>	This study
GH 1114	<i>MATa, NAT-GAL-URL-3HA-SEN1, set1::URA3</i>	This study
GH 374	<i>MATa, KANMX6-GAL-URL-3HA-SEN1, jhd2::HIS3MX6</i>	Lab stock
GH 444	<i>MATa, KANMX6-GAL-URL-3HA-SEN1, rtt109::TRP1</i>	Lab stock
GH 1252	<i>MATa, NAT-GAL-URL-3HA-SEN1, hst4::HIS3MX6, hst3::URA3</i>	This study
GH587	<i>MATa, Δ promoter PDC1 (deletion from coordinates 234402 to 235141 on chromosome XII)</i>	Lab Stock
GH 612	<i>MATa, Δ promoter PDC1, KANMX6-GAL-URL-3HA-SEN1</i>	This study
GH 1346	<i>MATa, Δ promoter PDC1, KANMX6-GAL-URL-3HA-SEN1, spt2::HIS3MX6</i>	This study
GF 113	<i>MATa, rpb1-1</i>	Lab Stock
GF 145	<i>MATa, rpb1-N488D</i>	This study
GH 1351	<i>MATa, KANMX6-GAL-URL-3HA-SEN1, rpb1-1</i>	This study
GH 1357	<i>MATa, KANMX6-GAL-URL-3HA-SEN1, rpb1-N488D</i>	This study