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Supplemental Information

Genome-wide Map of R-Loop-Induced Damage Reveals How a Subset of R-Loops Contributes to Genomic Instability

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Supplementary Materials for

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Fig. S1

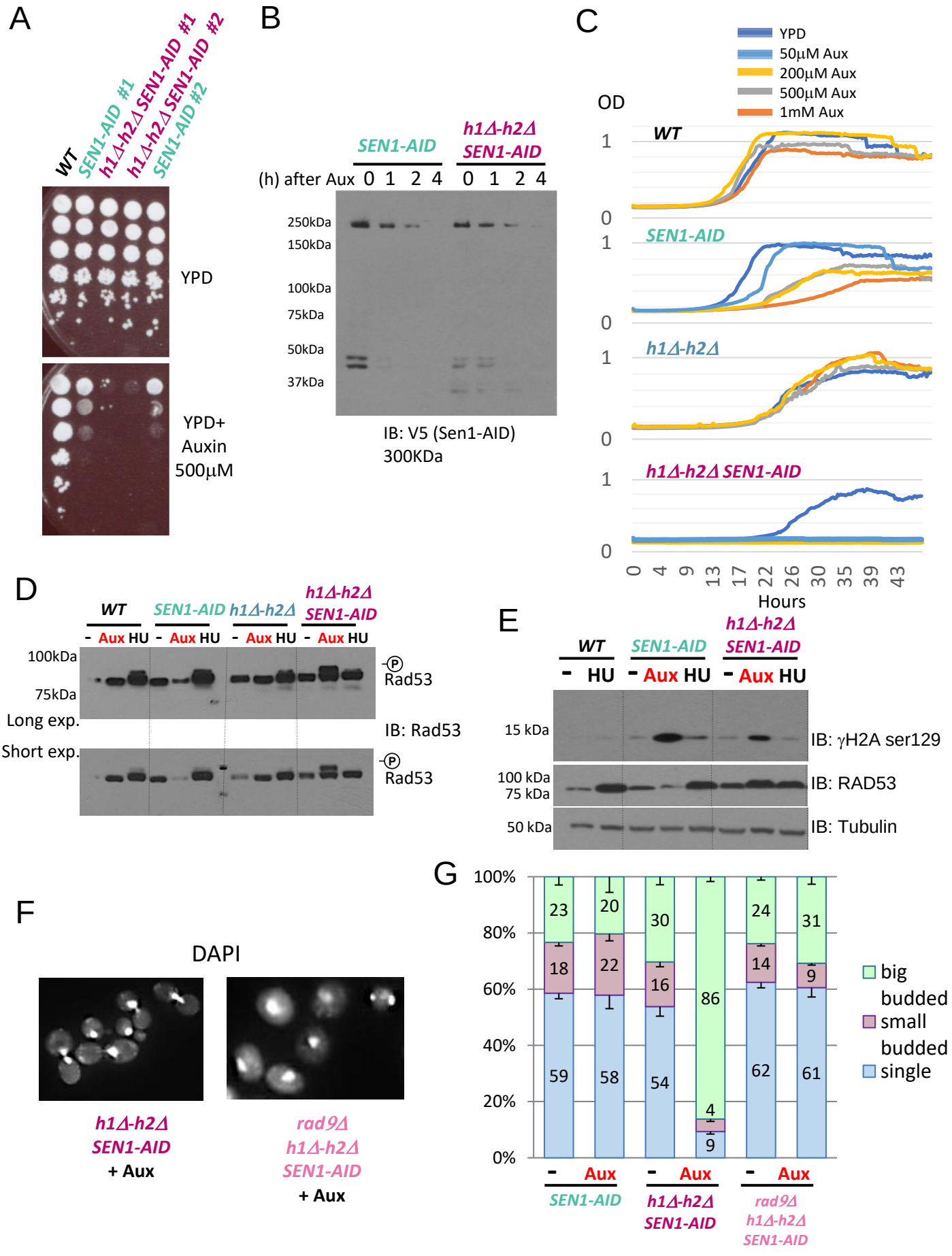

A
WT
SEN1-AID #1
h1Δ-h2Δ SEN1-AID #1
h1Δ-h2Δ SEN1-AID #2
SEN1-AID #2
YPD
YPD+ Auxin 500µM
B
SEN1-AID
h1Δ-h2Δ SEN1-AID
(h) after Aux 0 1 2 4 0 1 2 4
250kDa
150kDa
100kDa
75kDa
50kDa
37kDa
IB: V5 (Sen1-AID) 300KDa
C
YPD
50µM Aux
200µM Aux
500µM Aux
1mM Aux
OD
WT
SEN1-AID
h1Δ-h2Δ
h1Δ-h2Δ SEN1-AID
0 4 9 13 17 22 26 30 35 39 43
Hours
D
WT
SEN1-AID
h1Δ-h2Δ
h1Δ-h2Δ SEN1-AID
- Aux HU - Aux HU - Aux HU - Aux HU
100kDa
75kDa
Long exp.
Short exp.
Rad53
IB: Rad53
E
WT
SEN1-AID
h1Δ-h2Δ SEN1-AID
- HU - Aux HU - Aux HU
15 kDa
IB: γH2A ser129
100 kDa
75 kDa
IB: RAD53
50 kDa
IB: Tubulin
F
DAPI
h1Δ-h2Δ SEN1-AID + Aux
rad9Δ h1Δ-h2Δ SEN1-AID + Aux
G
100%
80%
60%
40%
20%
0%
23 18 59
20 22 58
30 16 54
86 4 9
24 14 62
31 9 61
big budded
small budded
single
- Aux
SEN1-AID
h1Δ-h2Δ SEN1-AID
rad9Δ h1Δ-h2Δ SEN1-AID

Figure S1. Related to Figure 1. SEN1-AID depletion in *h1*Δ-*h2*Δ accumulates DNA damage, activates DNA damage checkpoint and cell cycle arrest
(A) Dilution plating assays of asynchronous cultures of wild-type (*WT*), two different isolates of Sen1 with auxin-inducible degron (*SEN1-AID*) and two different isolates of Sen1 with auxin-inducible degron with deletion of the two RNases-H, RNH1 and RNH201 (*h1*Δ-*h2*Δ *SEN1-AID*). Ten-fold serial dilutions of cells were plated on YPD and YPD with 500mM auxin. Depletion of the essential Sen1 alone slows down growth on auxin, while Sen1 depletion in an RNases-H deletion background completely abolishes growth.

(**B)** Asynchronous cultures of *SEN1-AID* and *h1*Δ-*h2*Δ *SEN1-AID* were treated for the indicated time with 500mM auxin (Aux). Western-blot was performed using a anti-V5 antibody for Sen1-AID tagged with V5. Sen1-AID was not detectable after 4h auxin treatment.

(C) Asynchronous cultures of wild-type *WT*, *SEN1-AID*, RNases-H deletion, RNH1 and RNH201 (*h1*Δ-*h2*Δ*),* and *h1*Δ-*h2*Δ *SEN1-AID* were seeded on YPD media with the indicated auxin concentration and growth at 23ºC. Growth was followed measuring the OD=600 every 10' in TECAN Spark microplate reader. Sen1-AID grows slower at high concentration of auxin, while *h1*Δ-*h2*Δ *SEN1-AID* growth is completely arrested even at the lowest auxin concentration.

(D) Asynchronous culture *WT*, *SEN1-AID*, *h1*Δ-*h2*Δ*,* and *h1*Δ-*h2*Δ *SEN1-AID* were left untreated (-), or treated for 4h with 500mM auxin (Aux), or 25mM hydroxyurea (HU) for 2h. Western-blot was performed using an anti-Rad53 antibody. Low doses of HU triggers a small amount of phosphorylated-Rad53 (upper band) in every strain, while auxin triggers a robust Rad53-phosphorylation only in *h1*Δ-*h2*Δ *SEN1-AID*.

(E) Asynchronous culture of *WT*, *SEN1-AID*, and *h1*Δ-*h2*Δ *SEN1-AID* were left untreated (-), or treated for 4h with 500mM auxin (Aux), or 25mM hydroxyurea (HU) for 2h. Western-blot was performed using an antibody for phosphorylated Ser129 H2A, anti-Rad53, tubulin for loading control. Sen1-AID depletion triggers phosphorylation of H2A.

(F) Asynchronous cultures of *h1*Δ-*h2*Δ *SEN1-AID* and *Rad9*Δ *h1*Δ-*h2*Δ *SEN1-AID* were treated for 4h with 500mM auxin (Aux). Cells were fixed, DNA was stained with DAPI and pictures were acquired with a microscope. A G2/M arrest is observed as large-budded cells with DNA in the neck of the dividing cells in *h1*Δ-*h2*Δ *SEN1-AID.* The arrest is abolished when Rad9 is deleted in *Rad9*Δ *h1*Δ-*h2*Δ *SEN1-AID.*

(G) Cell morphology of 100 cells for was quantified. *SEN1-AID, h1*Δ-*h2*Δ *SEN1-AID* and Sen1 with auxin-inducible degron with deletion of the two RNases-H, RNH1 and RNH201 in Rad9 deletion strain (*Rad9*Δ *h1*Δ-*h2*Δ *SEN1-AID*). The G2/M arrest of *h1*Δ-*h2*Δ *SEN1-AID* strain treated with auxin is abolished when Rad9 is deleted in *Rad9*Δ *h1*Δ-*h2*Δ *SEN1-AID.*

Fig. S2

A

Survival rate after transient Sen1 depletion

Auxin	0h	5h	8h
WT	100%	98%	97%
SEN1-AID	95%	89%	87%
h1Δ-h2Δ	95%	90%	89%
h1Δ-h2Δ SEN1-AID	93%	13%	7%
rad9Δ h1Δ-h2Δ SEN1-AID	92%	9%	4%

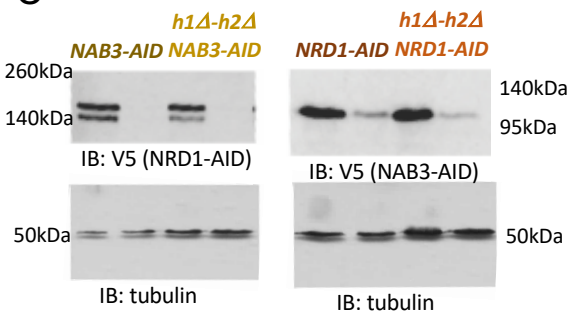
B

Colony formation rates on Auxin plates

		YPD	YPD + Auxin
Day 3	*WT*	100%	98%
	SEN1-AID	94%	0%
	h1Δ-h2Δ	97%	93%
	h1Δ-h2Δ SEN1-AID	92%	0%
	rad9Δ h1Δ-h2Δ SEN1-AID	86%	0%

		YPD	YPD + Auxin
Day 10	*WT*	100%	98%
	SEN1-AID	94%	92%
	h1Δ-h2Δ	97%	93%
	h1Δ-h2Δ SEN1-AID	92%	0%
	rad9Δ h1Δ-h2Δ SEN1-AID	86%	0%

C



D

Survival rate after transient NRD1 and NAB3 depletion

Auxin	0h	5h	8h
WT	100%	98%	97%
NAB3-AID	96%	95%	94%
h1Δ-h2Δ NAB3-AID	94%	91%	90%
NRD1-AID	95%	93%	92%
h1Δ-h2Δ NRD1-AID	93%	91%	89%

E

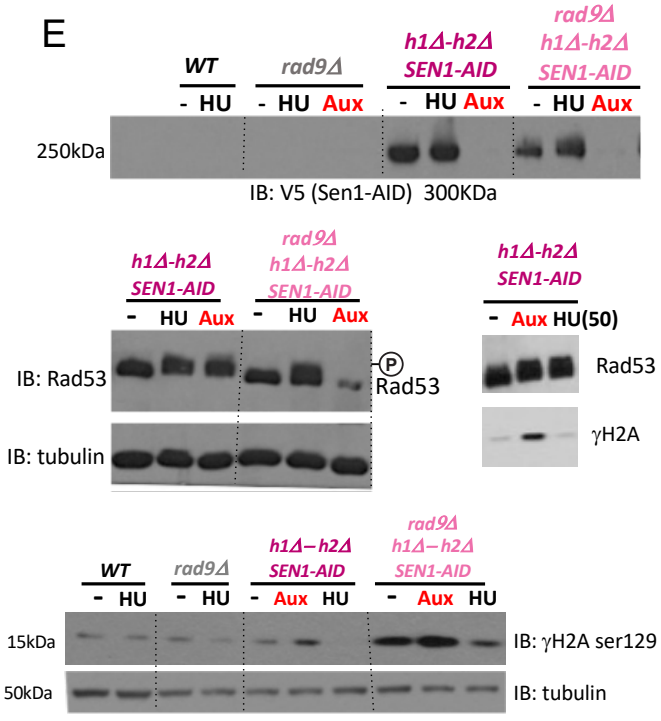


Figure S2. Related to Figure 2. Auxin treated *h1*Δ-*h2*Δ *SEN1-AID* cells accumulate irreparable damage leading to cell death.
(A) Asynchronous culture of WT, Sen1 with auxin-inducible degron (*SEN1-AID*), deletion of the two RNases-H, RNH1 and RNH201 (*h1*Δ-*h2*Δ), Sen1 with auxin-inducible degron with deletion of the two RNases-H, RNH1 and RNH201 (h1Δ-h2Δ SEN1-AID), and Sen1 with auxin-inducible degron with deletion of the two RNases-H, RNH1 and RNH201 in Rad9 deletion strain (*Rad9*Δ *h1*Δ-*h2*Δ *SEN1-AID*) were treated for the indicated time with 500μM auxin (Aux). Cells were counted and plated on YPD plates without auxin. Survival rates after transient Sen1-Aid depletion was assessed by scoring colony-forming units. *h1*Δ-*h2*Δ *SEN1-AID* and *Rad9*Δ *h1*Δ-*h2*Δ *SEN1-AID* viability was affected by Sen1-AID depletion.

(B) Asynchronous strains in (A) were left untreated, counted and plated directly on YPD plate or YPD plate with 500μM auxin. *SEN1-AID* strain did not form detectable colonies by day 3, but formed colonies by day 10, due to slow growth of this strain in auxin. No colonies were observed in *h1*Δ-*h2*Δ *SEN1-AID* nor in *Rad9*Δ *h1*Δ-*h2*Δ *SEN1-AID* strains.

(C) Asynchronous cultures of Nab3 with auxin-inducible degron (*NAB3-AID*), Nab3 with auxin-inducible degron with deletion of the two RNases-H, RNH1 and RNH201 (*h1*Δ-*h2*Δ *NAB3-AID*), Nrd1 with auxin- inducible degron (NRD1-AID) and Nrd1 with auxin-inducible degron with deletion of the two RNases-H, RNH1 and RNH201 (*h1*Δ-*h2*Δ *NRD1-AID*) were treated for 4h with 500μM auxin. Western-blot was performed using an anti-V5 antibody for Nab1-AID tagged with V5 (left panels) or Nab1-AID tagged with V5 (right panels). Tubulin was used as loading control. Nab3-AID was not detectable, while Nrd1-AID was heavily reduced after 4h auxin treatment.

(D) Asynchronous culture as in (A) were treated for the indicated time with 500μM auxin (Aux). Cells were counted and plated on YPD plates without auxin. Survival rate was scored as colony-forming units and was not affected in any strain. Differently from transient depletion of Sen1, transient depletion of Nab3 and Nrd1 in combination with deletion of the RNases-H is not lethal.

(E) Asynchronous culture of *WT, Rad9*Δ*, h1*Δ-*h2*Δ *SEN1-AID*, and *Rad9*Δ *h1*Δ-*h2*Δ *SEN1-AID* were left untreated (-), or treated for 4h with 500μM auxin (Aux), or 25mM hydroxyurea (HU) for 2h. Western-blot was performed using an anti-V5 antibody to detect Sen1-AID-V5 levels (first panel). Auxin depletes Sen1-AID in every strain. Western-blot was performed in using anti-Rad53 antibody and tubulin was used as loading control (second panel). Rad53 phosphorylation in *h1*Δ-*h2*Δ *SEN1-AID* after auxin is not present in *Rad9*Δ *h1*Δ -*h2*Δ *SEN1-AID* suggesting that Rad9 activated the DNA damage checkpoint after auxin addition resulting in Rad53 phosphorylation. Asynchronous culture of *h1*Δ-*h2*Δ *SEN1-AID* left untreated (-), or treated for 4h with 500mM auxin (Aux), or 50mM hydroxyurea (HU) for 2h. Western-blot was performed in using anti-Rad53 and phosphorylated Ser129 H2A antibody (third panel). Western-blot was performed using an antibody for phosphorylated Ser129 H2A, and tubulin as a loading control (forth panel). *Rad9*Δ *h1*Δ-*h2*Δ *SEN1-AID* has higher levels of

gH2A ser129 than *h1*Δ -*h2*Δ *SEN1-AID*, suggesting that Rad9 is not required for H2A phosphorylation after auxin treatment.

Fig. S3

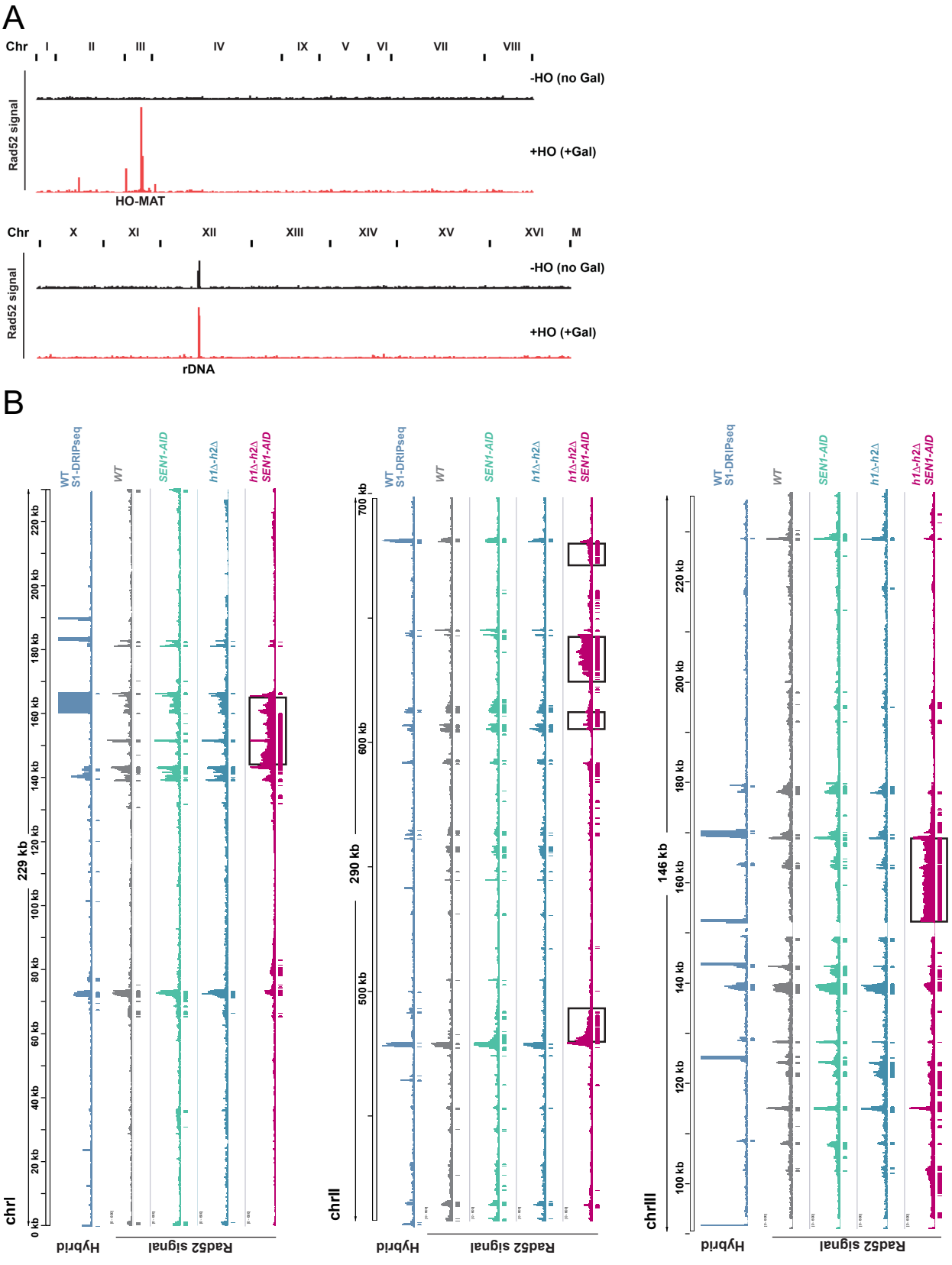

A
Chr I II III IV IX V VI VII VIII
-HO (no Gal)
Rad52 signal
+HO (+Gal)
HO-MAT
Chr X XI XII XIII XIV XV XVI M
-HO (no Gal)
Rad52 signal
+HO (+Gal)
rDNA
B
chrI
chrII
chrIII
WT S1-DRIPseq
WT
SEN1-AID
h1Δ-h2Δ
h1Δ-h2Δ SEN1-AID
Hybrid
Rad52 signal

Figure S3. Related to Figure 3. Rad52ChIP-seq signal after induction of an irreparable DSB and in strains defective for hybrid-removal machinery.
(A) In Gal-HO strain, the expression of HO is induced with 2% galactose for 1.5h. The endonuclease-HO makes a single DSB at the mating-type (HO-MAT) locus. The DSB is not repaired by recombination since the homologous sequences (HML and HMR) have been deleted. The expression of HO is induced with 2% galactose for 1.5h. Cells untreated or treated with galactose were fixed and processed for Rad52-ChIPseq. Rad52 signal from chromosomes 1-9 (top panel) and 10-16 (bottom panel) is indicated with a bar-chart in black for cells not treated (-HO, no DSB), and in red for cells induced with galactose (+HO, one DSB). A major peak signal coming from the HO-MAT is present just in induced cells (red) on chromosome III. A peak from rDNA is present in both not-induced and induced samples, since the rDNA signal is around 75 folds higher that the rest of the genome (the annotated genome used for aligning the sequencing reads has just 2 repeats of rDNA while the genome has around 150 copies).

(B) Sequencing signals from chromosome I, II and III are presented with bar-charts, and identified regions of enrichment are shown with underlining boxes. Signal coming from hybrids using published S1- DRIPseq data in a wild type (6) (WT S1-DRIPseq) is in blue (first lane). Signal coming from Rad52 (Rad52-ChIPseq) from wild type (*WT*, grey), Sen1 with auxin-inducible degron (*SEN1-AID*, green), deletion of the two RNases-H, RNH1 and RNH201 (*h1*Δ-*h2*Δ, dark blue), and Sen1 with auxin-inducible degron with deletion of the two RNases-H, RNH1 and RNH201 (*h1*Δ-*h2*Δ *SEN1-AID*, purple). All strains were treated with auxin for 4h, to deplete Sen1-AID or as a control in *WT*. In *WT*, *SEN1-AID* and *h1*Δ-*h2*Δ strains, the majority of loci bound by Rad52 co-localized with the positions of R-loops. The new Rad52 regions identified only in *h1*Δ-*h2*Δ *SEN1-AID* strain are boxed in black.

Fig. S4

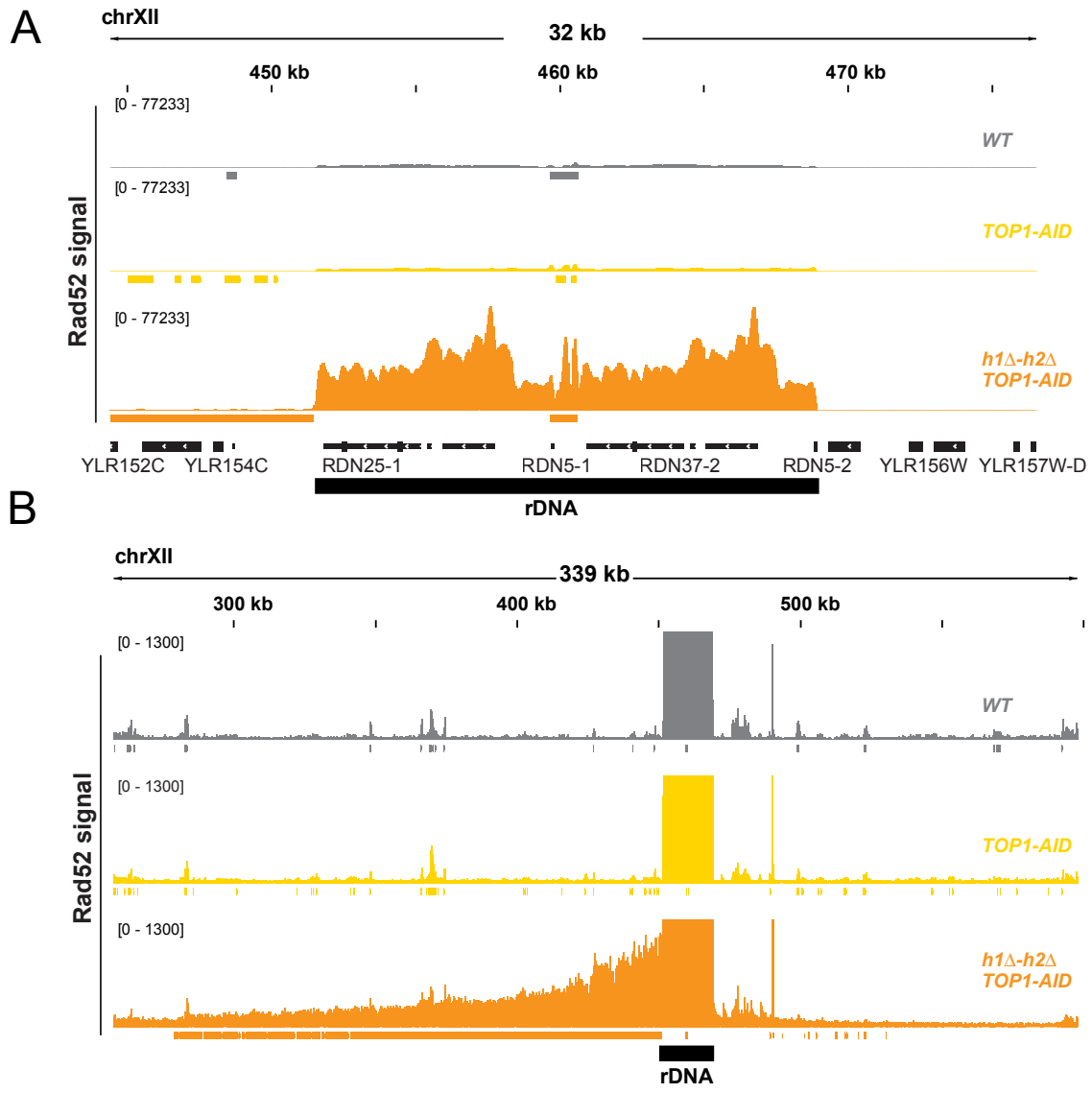


Figure S4. Related to Figure 4. Rad52ChIP-seq identifies rDNA as region of DNA damage in *h1Δ-h2Δ TOP1-AID*

(A) Rad52-ChIPseq signal from a portion of chromosome XII containing the ribosomal DNA locus (rDNA block box) is presented with bar-charts and identified regions of enrichment are shown with underlining boxes. Signal from Rad52 (Rad52-ChIPseq) from wild type (*WT*, grey), Topoisomerase-I with auxin- inducible degron (*TOP1-AID*, yellow), and Topoisomerase-I with auxin-inducible degron with deletion of the two RNases-H, RNH1 and RNH201 (*h1Δ-h2Δ TOP1-AID*, orange). All strains were treated with auxin for 4h, to deplete Sen1-AID or as a control in *WT*. Rad52-ChIPseq signal from *h1Δ-h2Δ TOP1-AID* presented a 40-fold enrichment in the rDNA locus compared to the other strains.

(B) Rad52-ChIPseq signal from a larger portion of chromosome XII containing the rDNA locus (black box). Rad52 signal from strains as in (A). The signal on the repetitive rDNA locus is around 75-folds higher than the one from unique regions; because the reference genome used in the alignment has just 2 rDNA copies while cells possess around 150 copies. So, by lowering the maximum signal by around 75 folds and showing a larger portion of the chromosome XII with the unique sequence flanking the rDNA, we detected Rad52 signal in around 150kb on the left side of the rDNA. In conclusion *h1Δ-h2Δ TOP1- AID* presents the new Rad52 signal in the rDNA and in the 150kb on the flanking left unique region.

Fig. S5

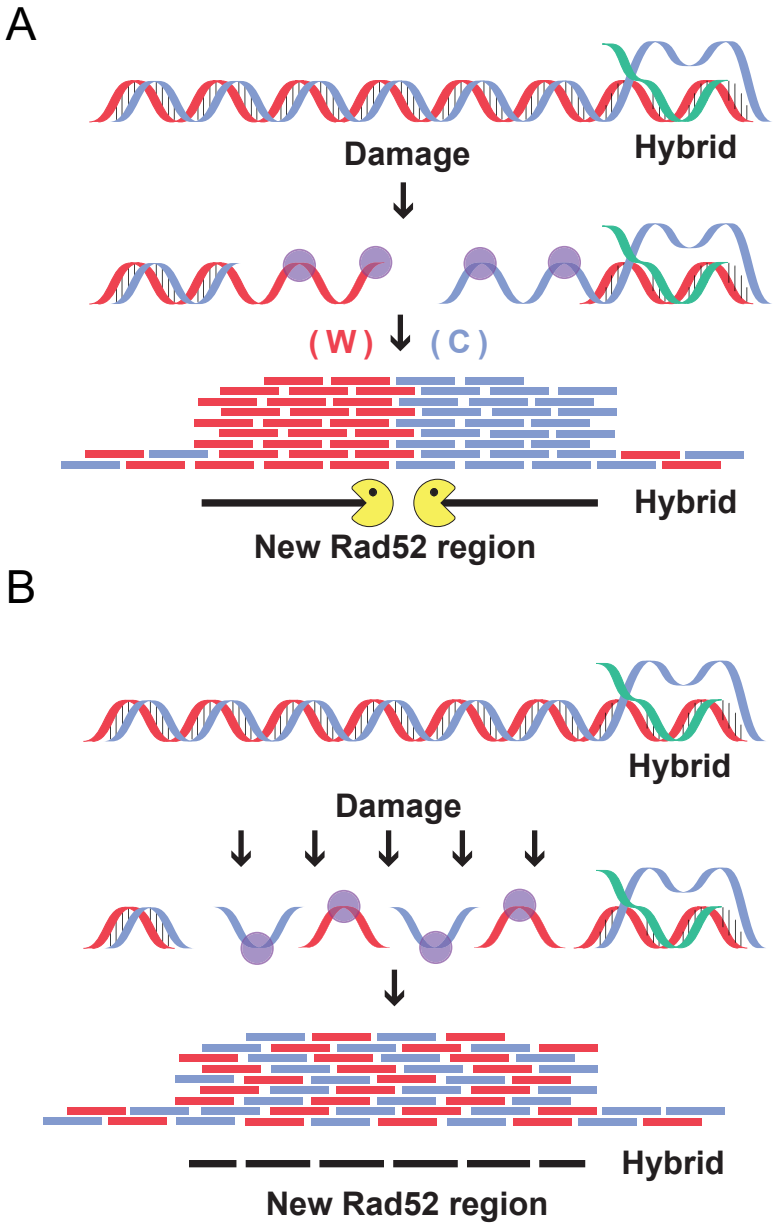


Figure S5. Related to Figure 6. Model for hybrid-induced damage originating from processing of "classical" double strand break.

(A) Expected strand specific reads for hybrid-induced damage with a single double strand break (DSB) in the same location in every cell. This DSB will be resected 5'-3' on both ends, leaving a Watson (red) ssDNA on the left side of the break and a Crick (blue) on the right side. Rad52 coats the ssDNA on both sides of the DSB, resulting in a distinct Rad52-ChIPseq signal from the Watson strand (red bricks) on one side, and from the Crick strand (blue bricks) on the other side, exactly as observed for HO DSB.

(B) Expected strand specific reads for hybrid-induced damage a single double strand break (DSB) at a different location in each cell. This DSB will be resected 5'-3'. The fact that every cell experiences the damage in a slightly different location within the new Rad52 region, will produce a mix of the Watson strand (red bricks) and the Crick strand (blue bricks) signal.

Fig. S6

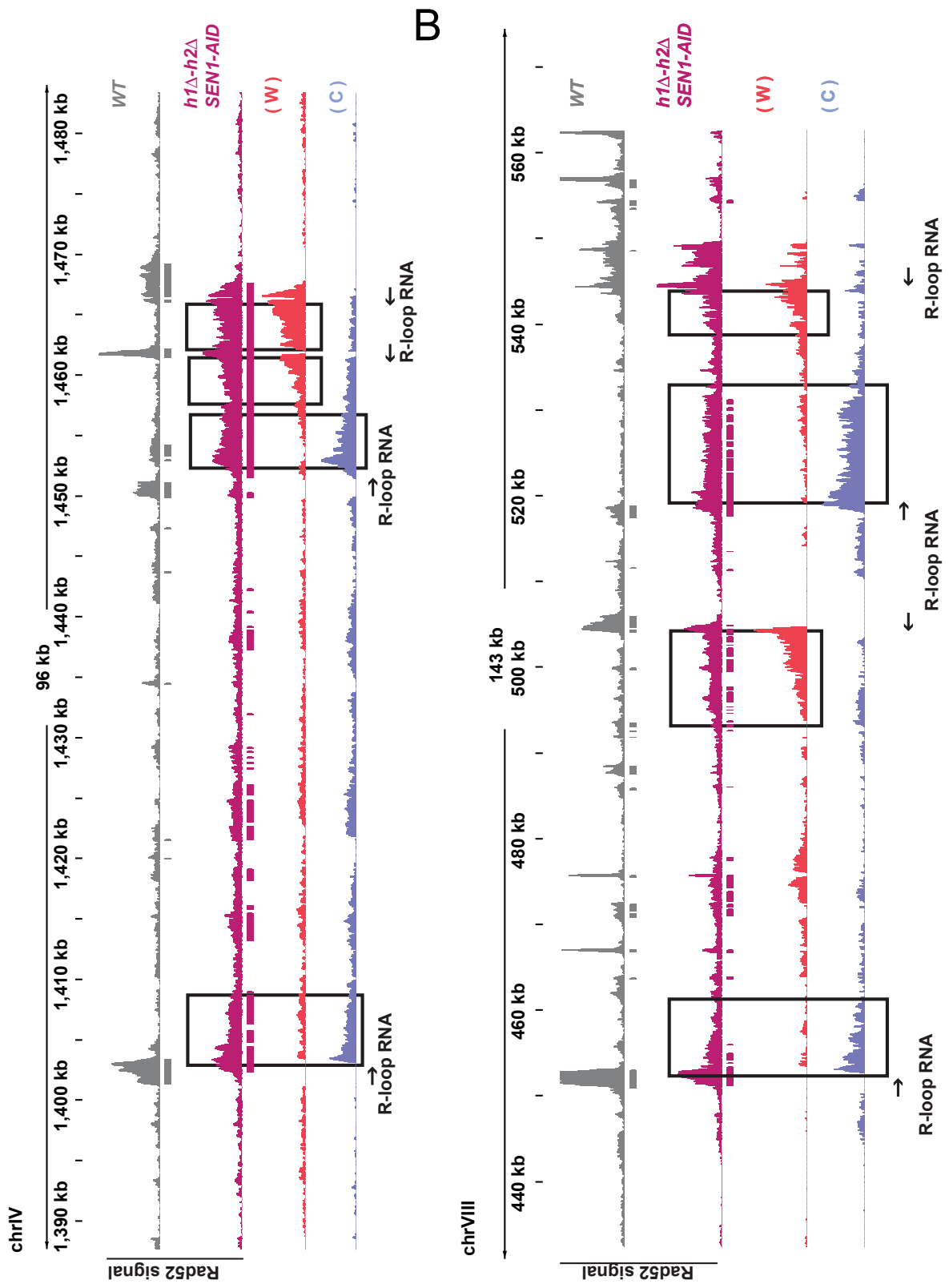

A
B
chrIV
chrVIII
96 kb
143 kb
1,390 kb
1,400 kb
1,410 kb
1,420 kb
1,430 kb
1,440 kb
1,450 kb
1,460 kb
1,470 kb
1,480 kb
440 kb
460 kb
480 kb
500 kb
520 kb
540 kb
560 kb
WT
h1Δ-h2Δ SEN1-AID
(W)
(C)
R-loop RNA
Rad52 signal

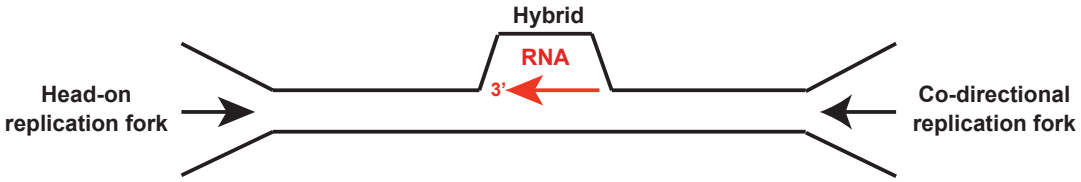
Fig. S6. Related to Figure 6. Regions of DNA damage in *h1Δ-h2Δ SEN1-AID* present a unique strand-signature of Rad52-ChIPseq signal

(A) Rad52-ChIPseq signal from a portion of chromosome IV is presented as bar-charts and identified regions of enrichment are shown with underlining boxes. Signal coming from Rad52 (Rad52-ChIPseq) from wild type (*WT*, grey), and Sen1 with auxin-inducible degron with deletion of the two RNases-H, RNH1 and RNH201 (*h1Δ-h2Δ SEN1-AID,* purple*)*. All strains were treated with auxin for 4h, to deplete Sen1-AID or as control in *WT*. The extra Rad52 signal present in *h1Δ-h2Δ SEN1-AID* versus *WT* coming from the Watson strand (W, red), and coming from the Crick strand (C, blue). The signal of the new Rad52 regions present in *h1Δ-h2Δ SEN1-AID* (boxed in black) are originated mainly from one of the two strands, and the associated R-loop is indicated with an arrow following the direction of transcription.

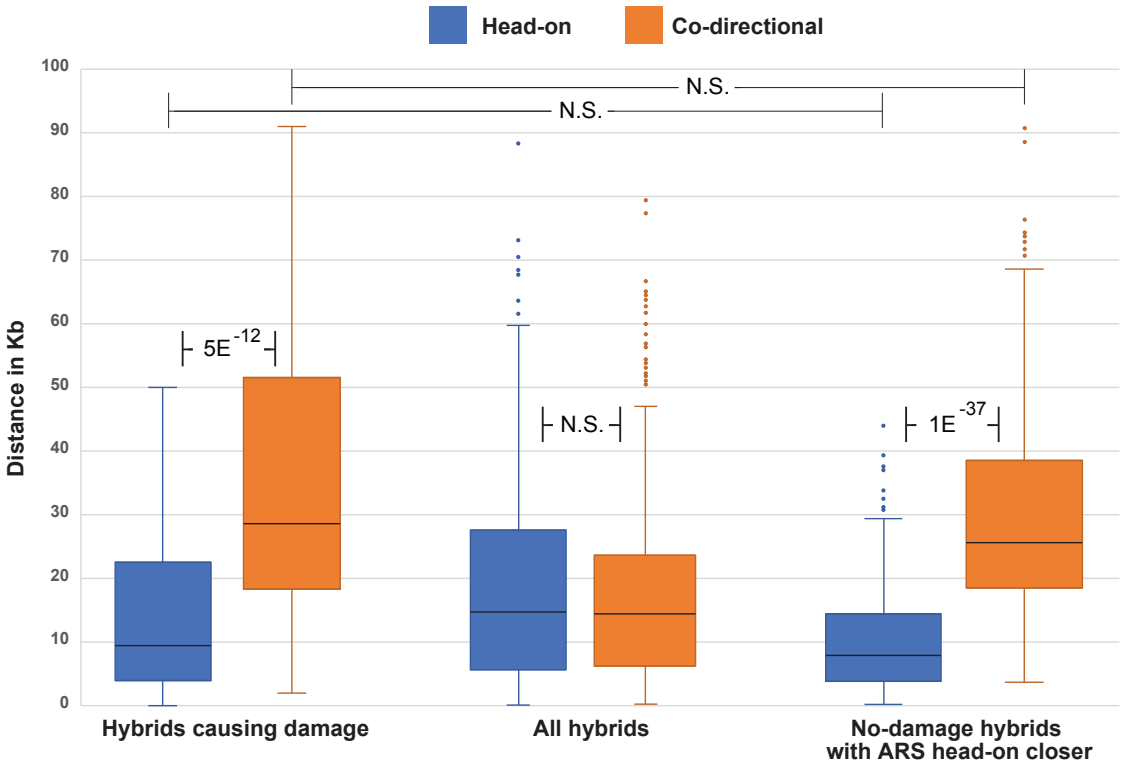
(B) Rad52-ChIPseq signal from a portion of chromosome VII with same strains as in (A)

Fig.S7

A



B



	Head-on	Co-dir
Min	30	2000
Q1	4000	18700
Median	9400	28549
Q3	22000	51000
Max	50000	91000

	Head-on	Co-dir
Min	36	312
Q1	9190	10078
Median	23638	23221
Q3	44258	37821
Max	141611	127377

	Head-on	Co-dir
Min	36	5061
Q1	5171	25965
Median	11025	35791
Q3	19934	53532
Max	61569	127377

Fig. S7. Related to Figure 6. Distance between hybrid regions in *h1Δ-h2Δ SEN1-AID* and head-on and co-directional replication origins.

(A) Model for head-on and co-directional replication origins. Head-on replication origins will collide head-on with the 3' end of the RNA forming the hybrid. Co-directional replication origins will collide with the 5' of the RNA, in the same direction of the RNA forming the hybrid.

(B) The distribution of the distance between the hybrid and the corresponding head-on replication origin (blue) and co-directional origin (orange) is showed using box and whiskers graph (we used the origin of replication dataset from the database of http://www.oridb.org). The first and second bars show the distance between the hybrid originating the new Rad52 regions in *h1Δ-h2Δ SEN1-AID* strain and the head-on and co-directional replication origins. 75% of them have a head-on origin closer than the co-directional one. The third and fourth bars show the distance between every hybrid forming regions in the genome that contains a transcript, and the corresponding head-on and co-directional origin of replication. 50% of the hybrids have a head-on replication origin closer than the co-directional one. The fifth and sixth bars show the distance between the hybrids that do not cause damage and contains a transcript (and have a head-on replication origin closer than the co-directional one) with the head-on and co-directional replication origins. The corresponding values are showed below. The statistical significance was calculated using the T-test (T-test value or N.S. for not statistically significant).

Table S1.
Strains table

Strain name	strain name in paper	genotype	mating type
LC-A01	WT	leu2 his3::TIR1-HI8S3 ura3 TRP1 ade-	MATa
LC-A02	SEN1-AID	leu2 his3::TIR1-HIS3 ura3 TRP1 ade- SEN1-3xV5-AID-KanMX	MATa
LC-A03	h1Δ-h2Δ	leu2 his3::TIR1-HIS3 ura3 TRP1 ade- rnh1Δ::HYG-B rnh201Δ::CLONAT	MATa
LC-A04	h1Δ-h2Δ SEN1-AID	leu2 his3::TIR1-HIS3 ura3 TRP1 ade- rnh1Δ::HYG-B rnh201Δ::CLONAT SEN1-3xV5-AID-KanMX	MATa
LC-A05	rad9Δ	leu2 his3::TIR1-HIS3 ura3 TRP1 ade- (not red) rad9::LEU2	MATa
LC-A06	rad9Δ SEN1-AID	leu2 his3::TIR1-HIS3 ura3 TRP1 ade- (not red) SEN1-3xV5-AID1-KanMX rad9::LEU2	MATa
LC-A07	rad9Δ h1Δ-h2Δ	leu2 his3::TIR1-HIS3 ura3 TRP1 ade- rnh1Δ::HYG-B rnh201Δ::CLONAT rad9::LEU2	MATa
LC-A08	rad9Δ h1Δ-h2Δ SEN1-AID	leu2 his3::TIR1-HIS3 ura3 TRP1 ade- rnh1Δ::HYG-B rnh201Δ::CLONAT SEN1-3xV5-AID1-KanMX rad9::LEU2	MATa
LC-A09	TOP1-AID	leu2 his3::TIR1-HIS3 ura3 TRP1 ade- TOP1-3xV5-AID-KanMX	MATa
LC-A10	h1Δ-h2Δ TOP1-AID	leu2 his3::TIR1-HIS3 ura3 TRP1 ade- rnh1Δ::HYG-B rnh201Δ::CLONAT TOP1-3V5-AID1-KanMX6	MATa
Ref.(17)	Gal-HO	Δho Δhml1:ADE1 Δhmr::ADE1 ade1-110 leu2,3-112 lys5 trp1::hisG ura3-52 ade3::GAL10:HO	MATα

Table S2.
Oligos table

Ch1_A_F	ggtagaaccaccgaccaaga
Ch1_A_R	tgtcttcctccgctcaaact
Ch1_B_F	tatcgccacacgtttctttg
Ch1_B_R	agaatggctagcggactgaa
Ch1_C_F	tatcgcatggtctccagatg
Ch1_C_R	accggatcattgctgaagtc
Ch2_A_F	tgaggagactgcagcaagaa
Ch2_A_R	ttatatgtggggctggcact
Ch2_B_F	tgcaatcagtacggtgttcg
Ch2_B_R	cgggtccatttcctacttca
Ch2_C_F	ggacagattacccacctcca
Ch2_C_R	atattgcctgcaccacacaa