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Supplemental Information

RNase H and Multiple RNA Biogenesis Factors Cooperate to Prevent RNA:DNA Hybrids from Generating Genome Instability

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A

	GCR Event:	YAC terminal deletions ($\times 10^{-3}$)	YAC loss ($\times 10^{-3}$)	+RNase H over-expression: YAC terminal deletions ($\times 10^{-3}$)	+RNase H over-expression: YAC loss ($\times 10^{-3}$)
	wildtype	0.23	0.41	0.23	0.41
Initiation	bur2Δ	1.04	1.36	0.19	0.62
Elongation	cdc73Δ	2.20	2.19	0.30	0.48
	leo1Δ	0.30	6.35	0.04	0.39
	spt2Δ	2.13	2.09	0.31	1.42
Repression	sin3Δ	4.68	2.83	0.54	0.17
	sds3Δ	1.76	2.16	0.25	0.50
	rpd3Δ	1.06	3.50	0.92	5.04
	not5Δ	1.50	2.08	0.31	0.62
	stb3Δ	3.00	3.90	0.13	0.20
Mediator complex: repressor module	med13Δ	2.31	2.90	0.28	0.56
	cdk8Δ	3.46	1.13	0.30	0.83
	med12Δ	2.70	6.94	0.03	0.22
	cycCΔ	3.29	2.04	0.25	0.65
Mediator complex: middle/tail module	med1Δ	0.78	2.45	0.14	1.06
	med5Δ	0.33	10.27	0.18	4.30
	med16Δ	1.02	2.05	0.81	1.23
RNA degradation	kem1Δ	2.28	2.47	0.11	0.01
	air1Δ	3.75	3.90	0.45	0.55
	rrp6Δ	1.52	2.29	0.30	0.21
	trf4Δ	1.51	2.20	0.10	0.14
RNA transport	npl3Δ	1.15	2.82	0.25	0.29

B

	GCR Event:	YAC terminal deletions ($\times 10^{-3}$)	YAC loss ($\times 10^{-3}$)
	wildtype	0.23	0.41
Activation	snf2Δ	0.78	0.49
Activation	hpa2Δ	0.50	0.74
Activation	elp3Δ	0.22	0.42
Activation	sas2Δ	0.28	0.42
Mediator complex: activator module	med2Δ	0.05	0.01
Mediator complex: activator module	med3Δ	0.28	0.30
Mediator complex: activator module	med31Δ	0.20	0.38

C

GCR Event:	YAC terminal deletions ($\times 10^{-3}$)	YAC loss ($\times 10^{-3}$)
cdk8Δ	1.42	2.09
med2Δ	0.05	0.01
cdk8Δmed2Δ	0.02	0.02

Figure S1, Related to Figures 2A and 4A. Rates of YAC Instability in Transcription Mutants

(A) Rates of YAC loss and terminal deletions in RNA biogenesis mutants, with and without the RNase H plasmid.

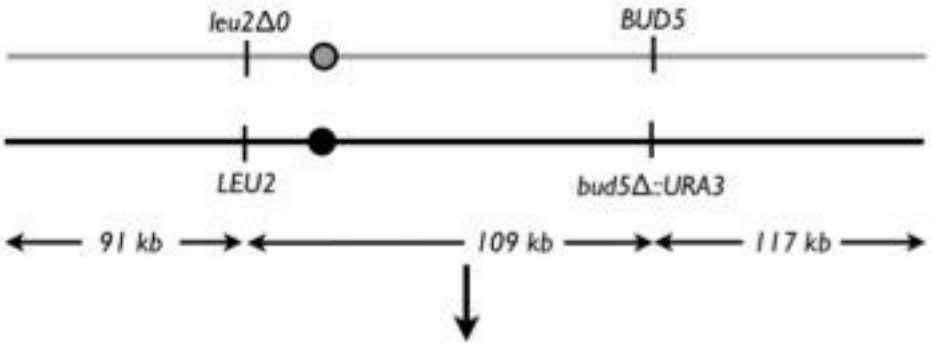
(B) Rates of YAC loss and terminal deletions in select RNA transcriptional activators. Perturbing transcriptional activation does not cause YAC instability.

(C) Rates of YAC loss and terminal deletions in mediator mutants. The deletion of an activator module subunit, *MED2*, in a *cdk8*Δ suppresses its observed YAC instability.

	GCR Event:	YAC terminal deletions ($\times 10^{-3}$)	YAC loss ($\times 10^{-3}$)	+RNase H over-expression: YAC terminal deletions ($\times 10^{-3}$)	+RNase H over-expression: YAC loss ($\times 10^{-3}$)
	wildtype	0.23	0.41	0.23	0.41
DNA damage checkpoint	ddc1Δ	2.26	2.87	2.13	2.45
DNA damage checkpoint	rad24Δ	3.00	3.09	3.10	3.11
Nucleotide excision repair	rad34Δ	1.70	10.3	1.15	6.25
Nucleotide excision repair	rad28Δ	1.64	0.98	1.97	1.00
Double-strand break repair	rad52Δ	0.34	5.84	0.25	6.40
Double-strand break repair	mre11Δ	1.75	3.37	1.90	3.10

Figure S2, Related to Figures 2B and 4B.
Rates of YAC loss and terminal deletions in DNA damage repair and checkpoint mutants, with and without the RNase H plasmid.

A



Instability Event	Rate of Instability (% of total)
Terminal Marker Loss (Leu^+,Ura^-)	$0.16*10^{-3}$ (44%)
Chromosome Loss (Leu^-,Ura^-)	$0.20*10^{-3}$ (56%)
	Total: $0.36*10^{-3}$ (100%)

B

	Rate of chr. III instability ($\times10^{-3}$) *(fold over wt)*	+RNase H overexpression ($\times10^{-3}$) *(fold over wt)*
wildtype	0.36 *(1.0)*	0.36 *(1.0)*
bur2Δ	1.60 *(4.4)*	0.39 *(1.1)*
med13Δ	2.91 *(8.0)*	0.35 *(1.0)*
sin3Δ	4.94 *(13.6)*	0.44 *(1.2)*

C

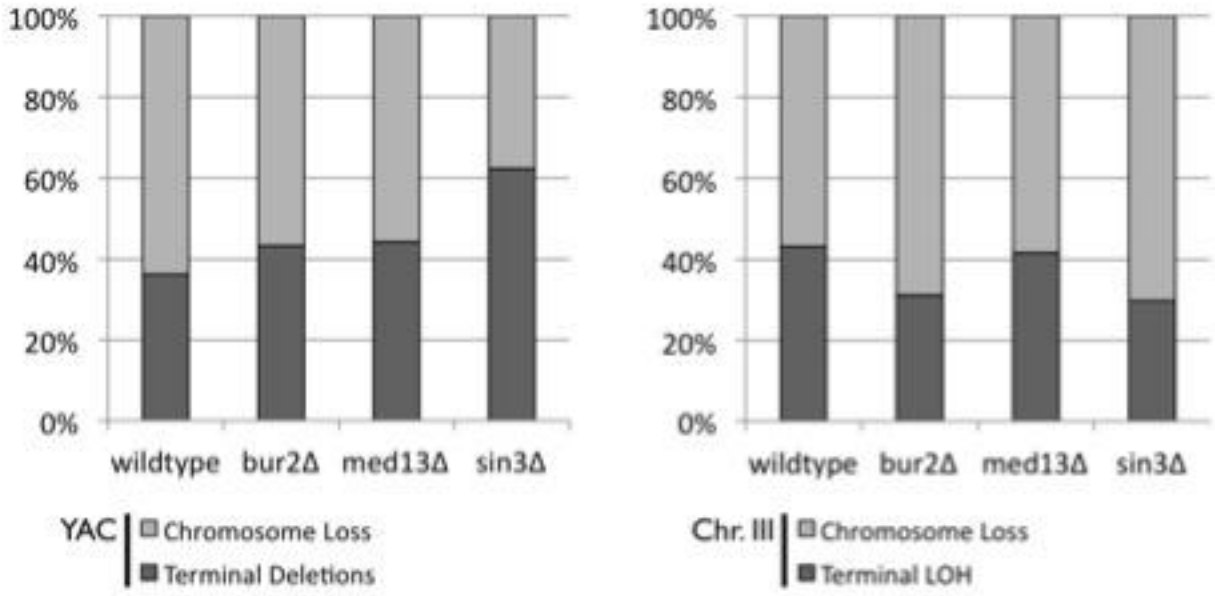


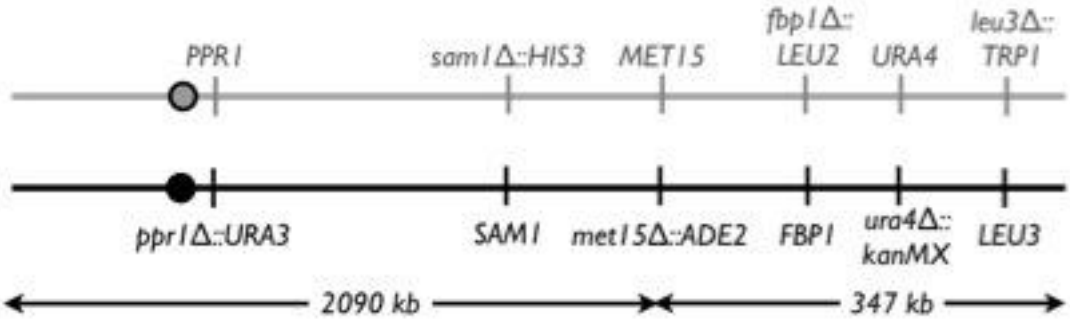
Figure S3, Related to Figure 3A. Chromosome III Instability in Diploid Transcription Mutants

(A) Assay for chromosome III instability in diploids. Rates are determined by plating 10^5 cells on 5-FOA plates and determining the number of cells that retained one marker (Leu+) but lost the other marker (Ura-), or lost the chromosome completely (Leu- and Ura-).

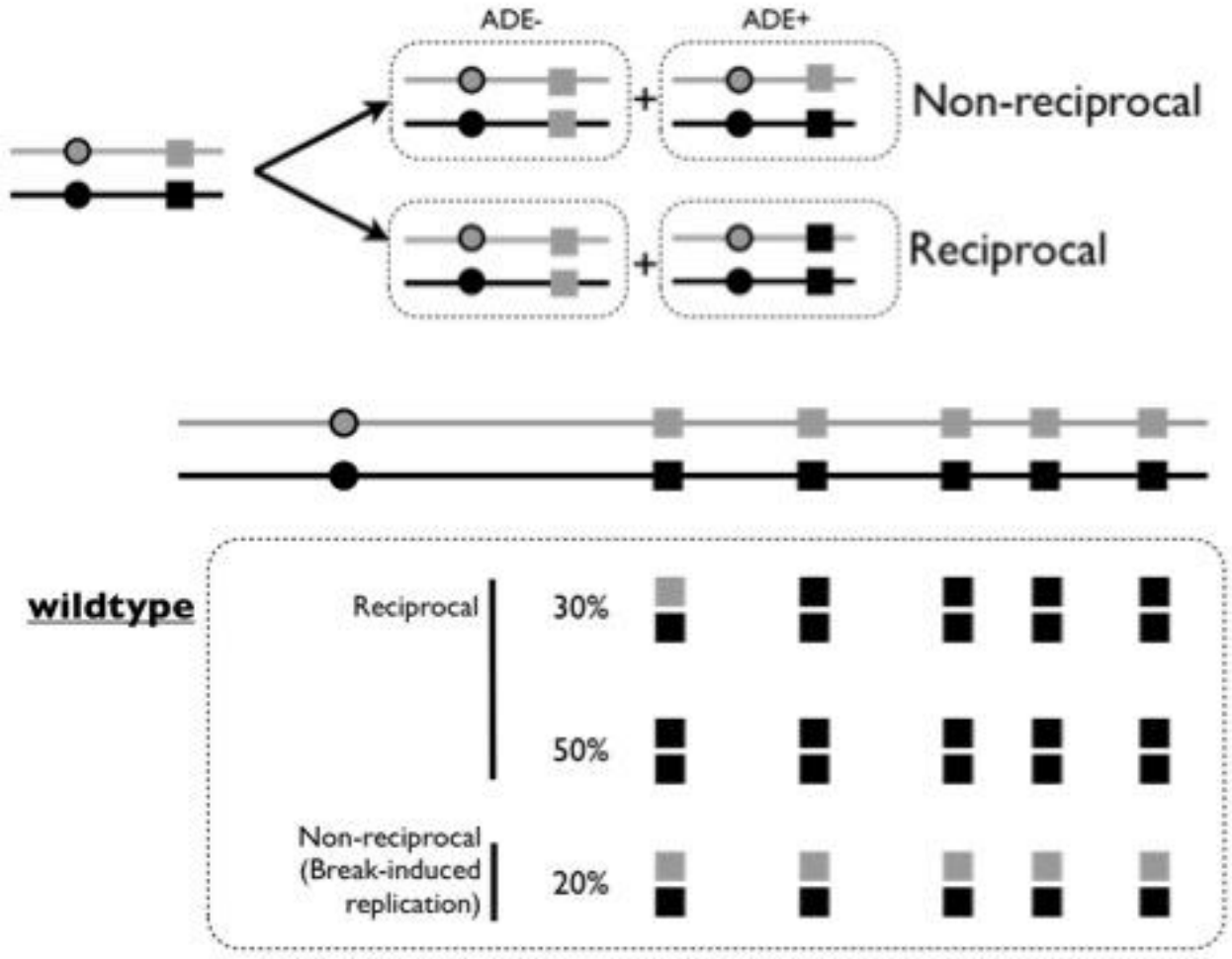
(B) Rates of chromosome III instability in select RNA biogenesis mutants.

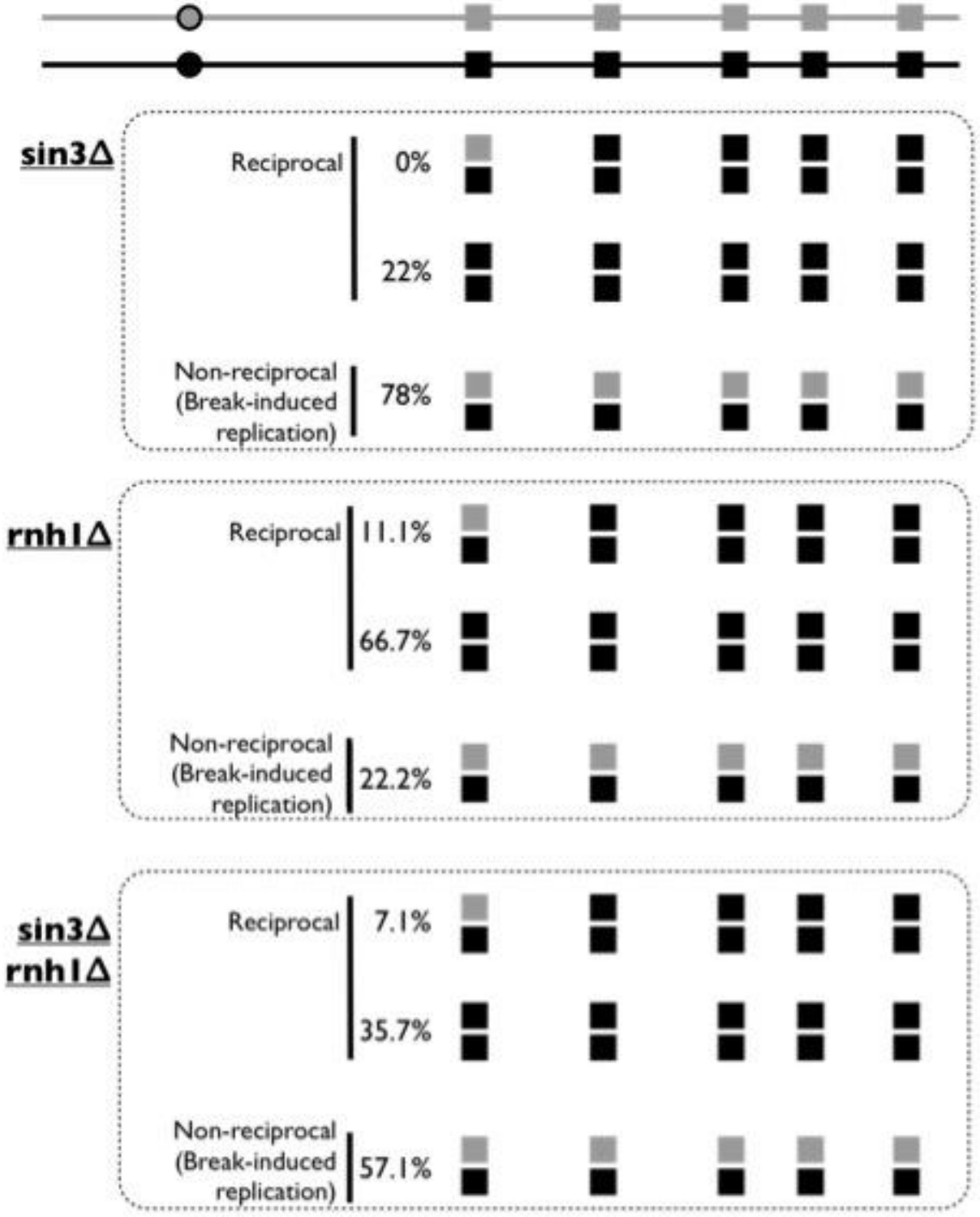
(C) The distribution of instability events on the YAC and chromosome III. On the left, are YAC loss and terminal deletion events, while on the right are chromosome III LOH and loss events in wild-type and select transcription mutants.

A


PPR1
sam1Δ::HIS3
MET15
fbp1Δ::
LEU2
URA4
leu3Δ::
TRP1
ppr1Δ::URA3
SAM1
met15Δ::ADE2
FBP1
ura4Δ::
kanMX
LEU3
2090 kb
347 kb

B


ADE-
ADE+
Non-reciprocal
Reciprocal
wildtype
Reciprocal
30%
50%
Non-reciprocal
(Break-induced
replication)
20%


sin3Δ
Reciprocal
0%
22%
Non-reciprocal (Break-induced replication)
78%
rnh1Δ
Reciprocal
11.1%
66.7%
Non-reciprocal (Break-induced replication)
22.2%
sin3Δ rnh1Δ
Reciprocal
7.1%
35.7%
Non-reciprocal (Break-induced replication)
57.1%

C

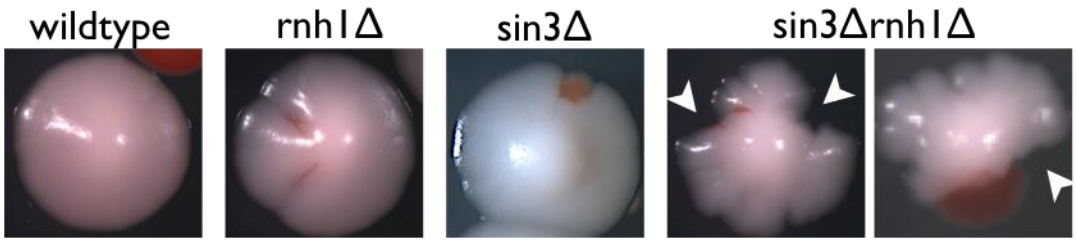
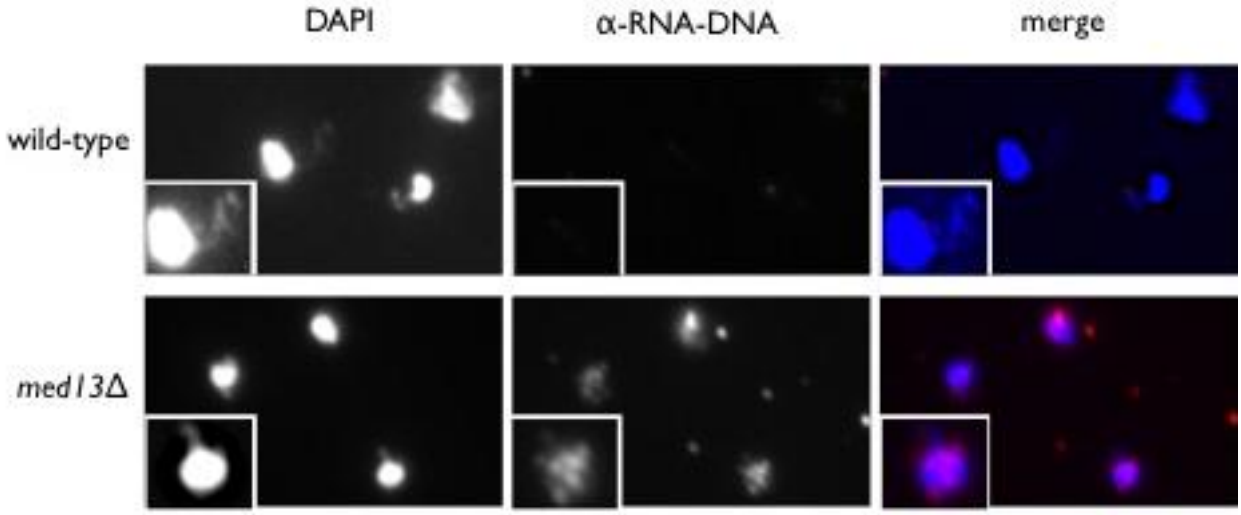
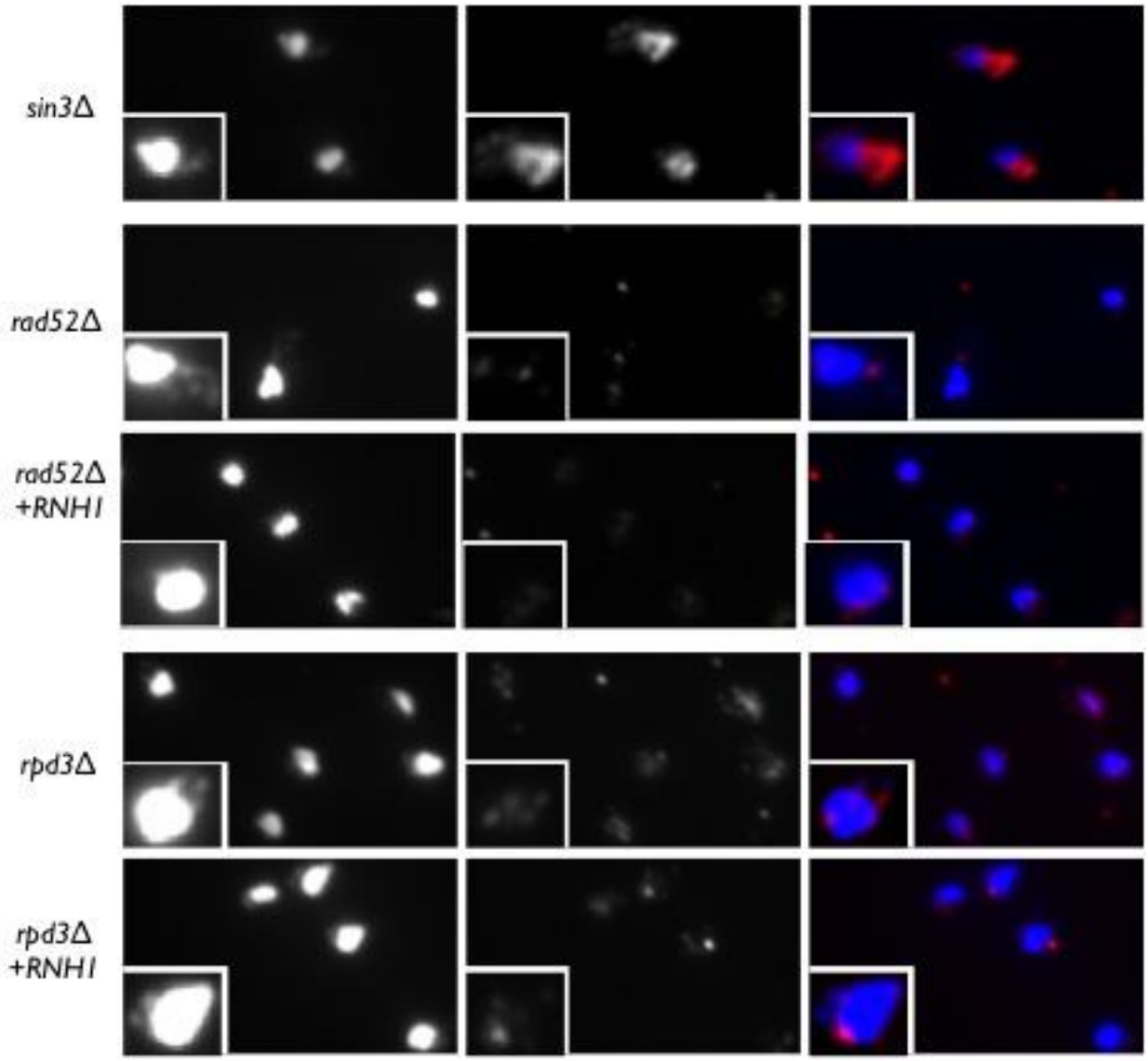


Figure S4, Related to Figure 3B. Chromosome XII Instability in Diploid *sin3Δ* Cells
(A) Schematic representation of the markers used to monitor instability on chromosome XII in diploids. For full details on the assay and distances between markers see McMurray and Gottschling, 2003.
(B) The mechanism of LOH in wildtype and *sin3Δ* cells.
Top panel- A schematic representation of the genotypes for half-sectored colonies at loci downstream of ADE2 for reciprocal and non-reciprocal events adapted from McMurray and Gottschling, 2003.
Bottom panel- The data from phenotypic analysis of half-sectored colonies, done as described in Materials and Methods. For simplicity only the genotypes for the white (ADE+) colonies is reported (n=200+ for each genotype reported).
(C) Representative images of colonies with the indicated phenotypes. Arrow heads point to possible lethal sectors.

A



B



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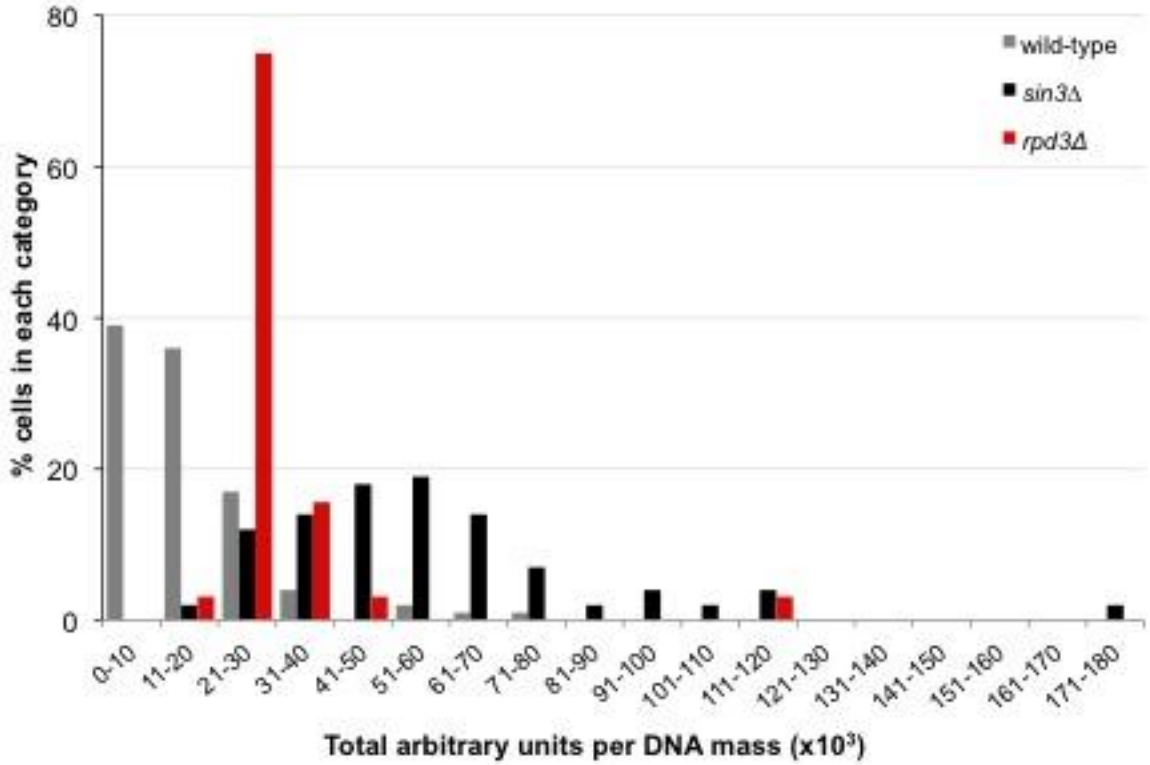


D

	Rate of rDNA instability (x10^{-4}) *(fold over wt)*	+RNase H overexpression (x10^{-4}) *(fold over wt)*
wildtype	0.28 *(1.0)*	0.51 *(1.8)*
sin3Δ	79.86 *(285.2)*	1.56 *(5.6)*
med13Δ	3.58 *(12.8)*	3.70 *(13.2)*

E

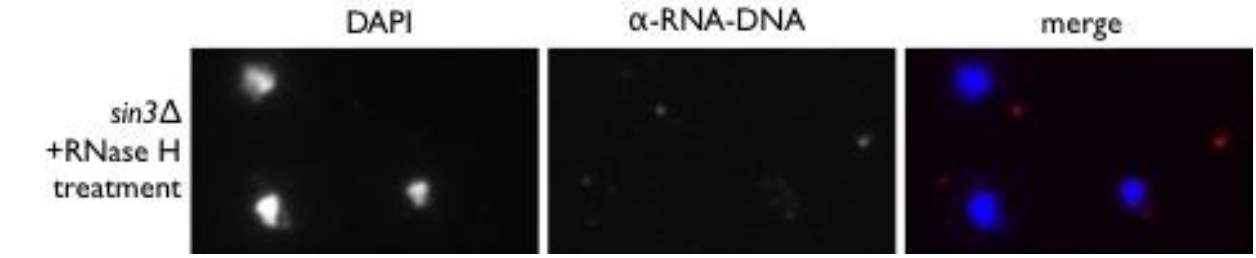


Figure S5, Related to Figures 4 and 5. RNA-DNA Hybrid Staining in Transcription Mutants

(A) Representative image of wildtype and *med13*Δ chromatin spreads stained with S9.6 antibody, recognizing RNA-DNA hybrids.

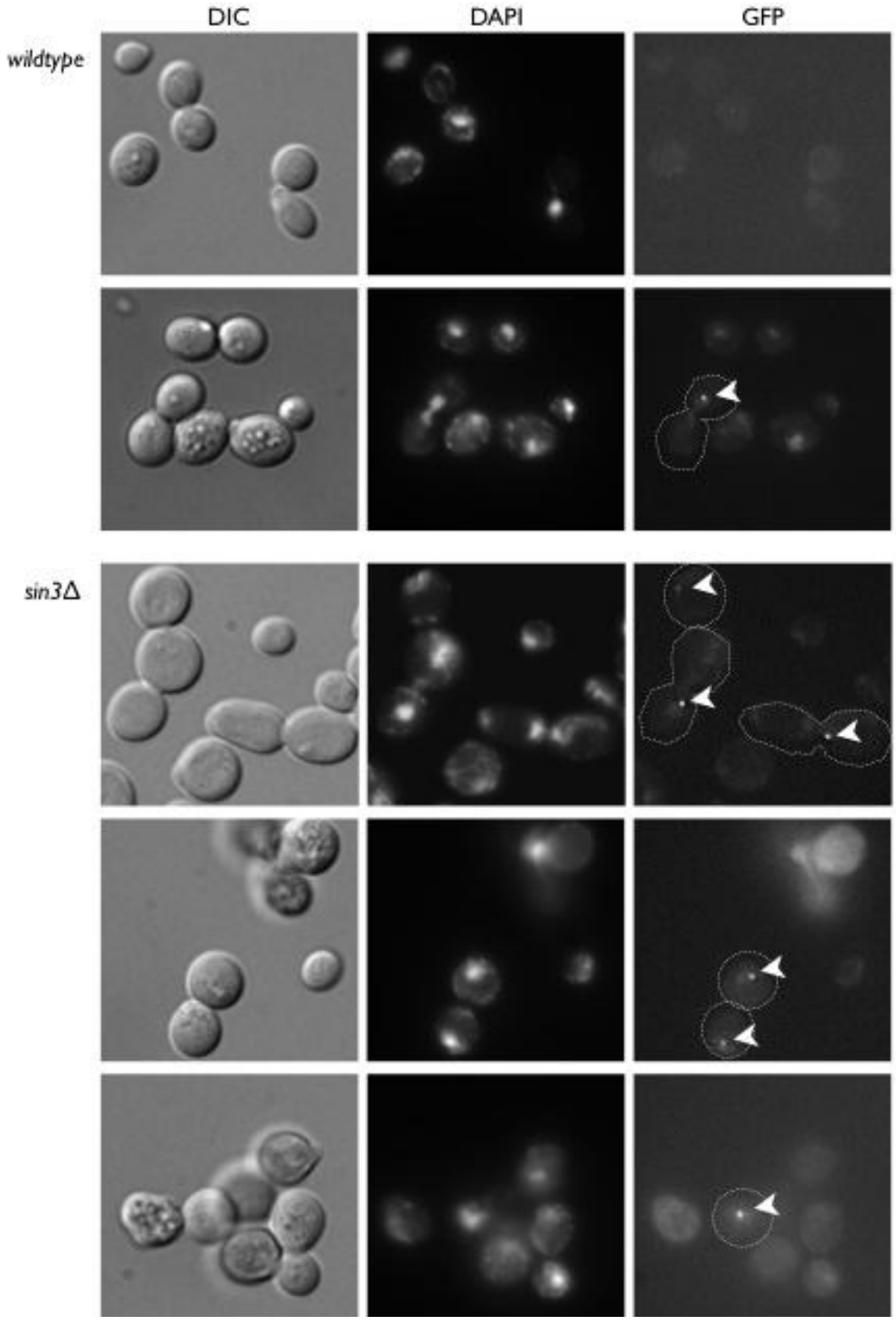
(B) Representative image of *sin3*Δ*, rad52*Δ and *rpd3*Δ chromatin spreads stained with S9.6 antibody, recognizing RNA-DNA hybrids. Strains were harboring either an empty control *2μ* plasmid or one expressing RNase H1.

(C) Measurement distribution of staining intensity in *rpd3*Δ*,* as compared to wildtype and *sin3*Δ nuclei. A total of 32 nuclei were scored for *rpd3*Δ*.*

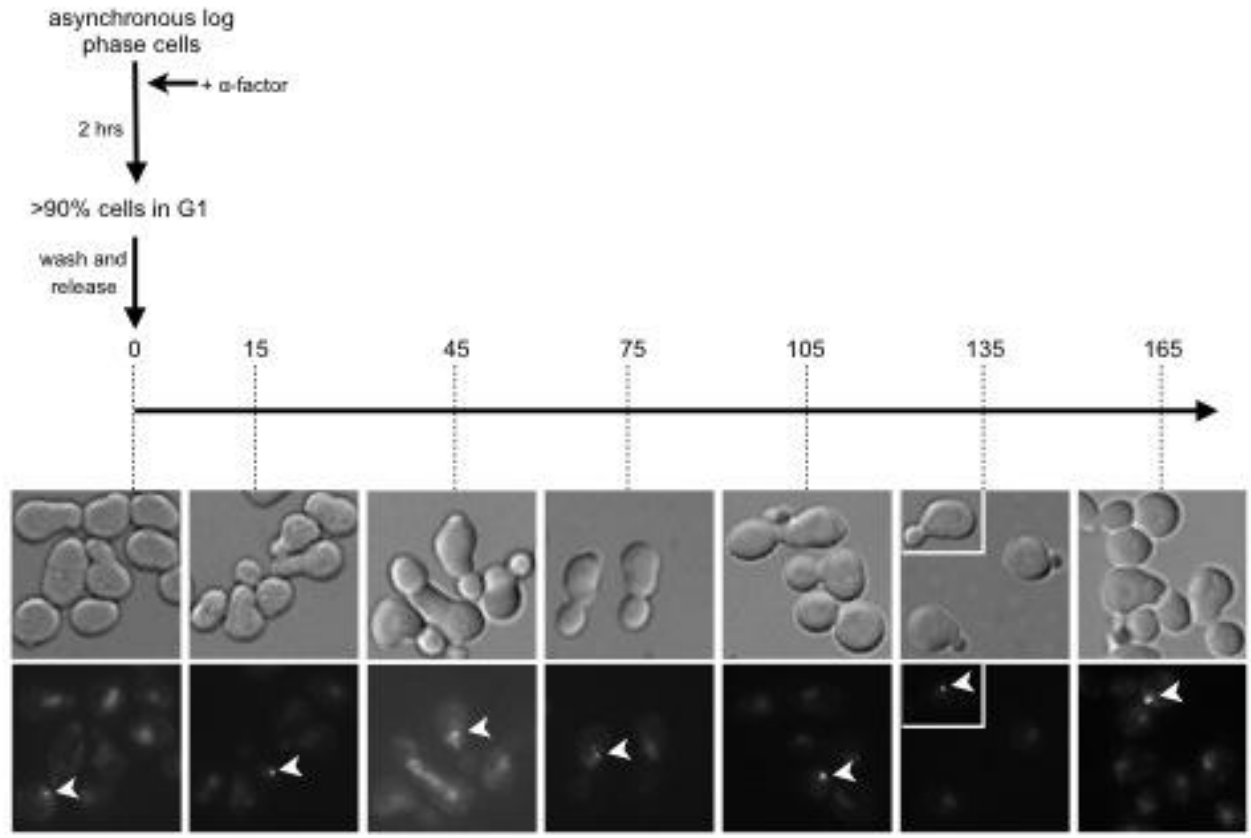
(D) Rates of rDNA instability in transcription mutants with and without the RNase H plasmid.

(E) Chromatin spreads of *sin3*Δ, where slides were pre-treated with RNase H prior to staining with the RNA-DNA hybrid antibody.

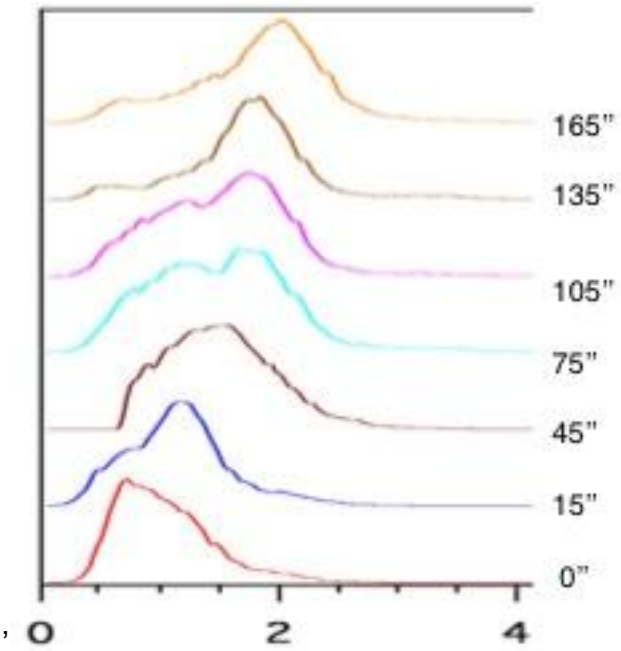
A



B


asynchronous log phase cells
+ α-factor
2 hrs
>90% cells in G1
wash and release
0
15
45
75
105
135
165

C


165"
135"
105"
75"
45"
15"
0"
0
2
4

D

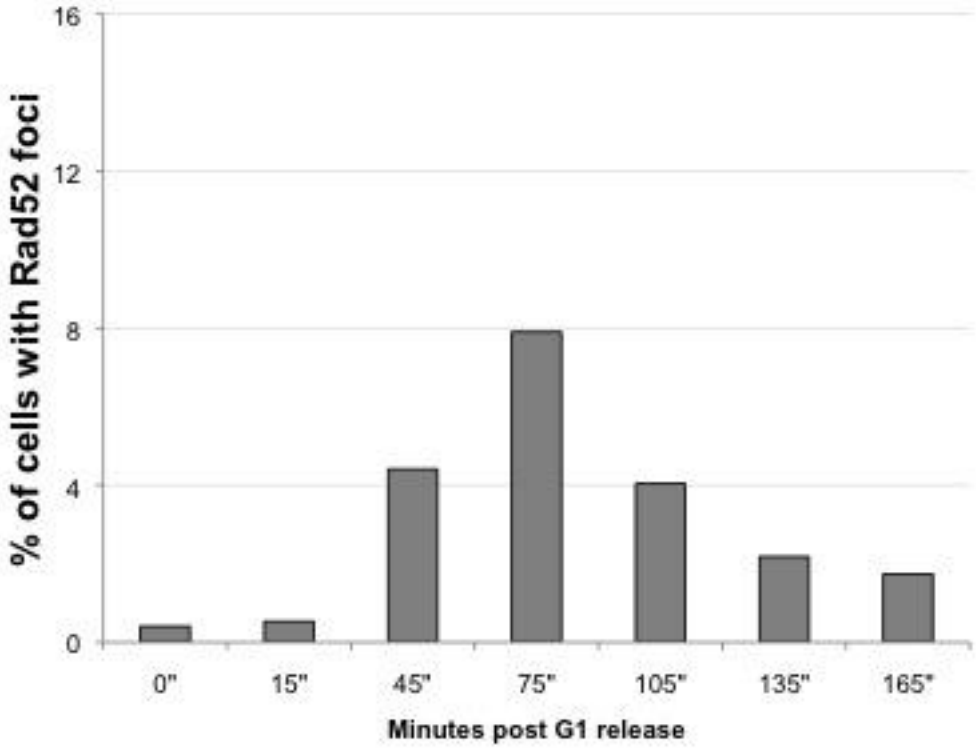


Figure S6, Related to Figure 6. Rad52-GFP Foci in *sin3*Δ Cells
(A) Representative image of asynchronous log phase wildtype and *sin3*Δ cells expressing Rad52-GFP. White arrows point to examples of scored Rad52-GFP foci.
(B) Overview of the synchronization experiment, and representative images of DIC and GFP *sin3*Δ cells at indicated time points.
(C) FACs profile of *sin3*Δ cells used in the synchronization experiment. Standard FACs techniques were used (Guthrie and Fink, 2002).
(D) The number of cells with Rad52-GFP foci in wildtype cells at indicated time points after release from G1 arrest. 500 cells were scored for each time point.

A

GCR Event:	YAC terminal deletions ($\times 10^{-3}$)	YAC loss ($\times 10^{-3}$)	+RNase H over-expression: YAC terminal deletions ($\times 10^{-3}$)	+RNase H over-expression: YAC loss ($\times 10^{-3}$)
wildtype	0.23	0.41	0.23	0.41
rnh1Δ	1.08	0.71	0.09	0.13
rnh201Δ	1.37	1.22	0.30	0.27
rnh1Δ rnh201Δ	3.82	3.70	0.45	1.26
bur2Δ rnh1Δ	1.26	13.76	0.49	1.01
med13Δ rnh1Δ	0.56	17.74	0.53	0.04
sin3Δ rnh1Δ	0.95	19.56	0.32	2.88
rad52Δrnh1Δ	0.22	4.80	0.22	4.80

B

	Rate of chr. III instability ($\times 10^{-3}$) *(fold over wt)*
wildtype	0.36 *(1.0)*
rnh1Δ	0.35 *(1.0)*
rnh201Δ	0.33 *(0.9)*
rnh1Δ rnh201Δ	3.05 *(8.5)*
bur2Δ	1.60 *(4.4)*
bur2Δ rnh1Δ	4.21 *(11.6)*
med13Δ	2.91 *(8.0)*
med13Δ rnh1Δ	6.05 *(16.7)*
sin3Δ	4.94 *(13.6)*
sin3Δ rnh1Δ	10.45 *(28.8)*

C

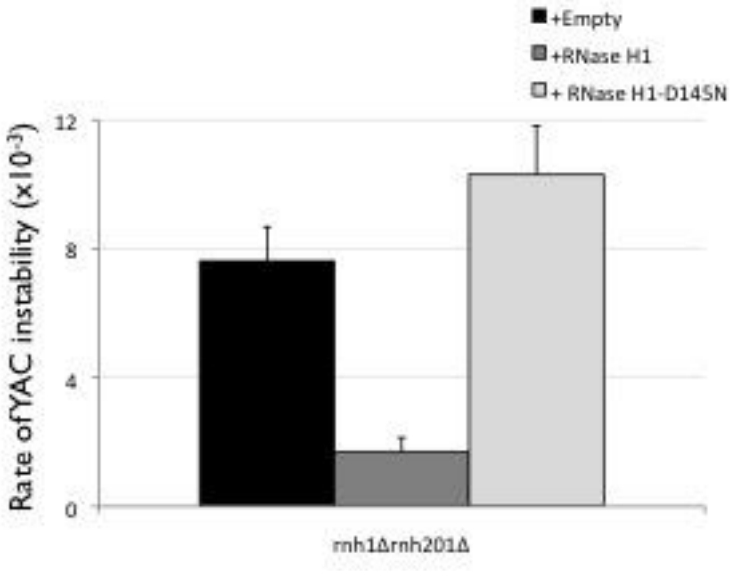


Figure S7, Related to Figure 7. Genome Instability in Transcription Mutants in an *rnh1*Δ Background

(A) Rates of YAC loss and terminal deletions in RNase H1 mutants, with and without the RNase H plasmid.

(B) Rates of chromosome III instability in RNase H1 mutants.

(C) Rates of YAC instability with over-expression of the catalytic mutant RNase H1-D145N.

GCR Event:	YAC terminal deletions ($\times 10^{-3}$)	YAC loss ($\times 10^{-3}$)
wildtype	0.23	0.41
rnh1Δ	1.08	0.71
rnh201Δ	1.37	1.22
rnh1Δ rnh201Δ	3.82	3.70
bur2Δ rnh201Δ	0.71	4.44
med13Δ rnh201Δ	1.32	11.32
sin3Δ rnh201Δ	0.75	11.56

Figure S8, Related to Figure 7.
Rate of YAC instability in transcription mutants in an *rnh201*Δ background.

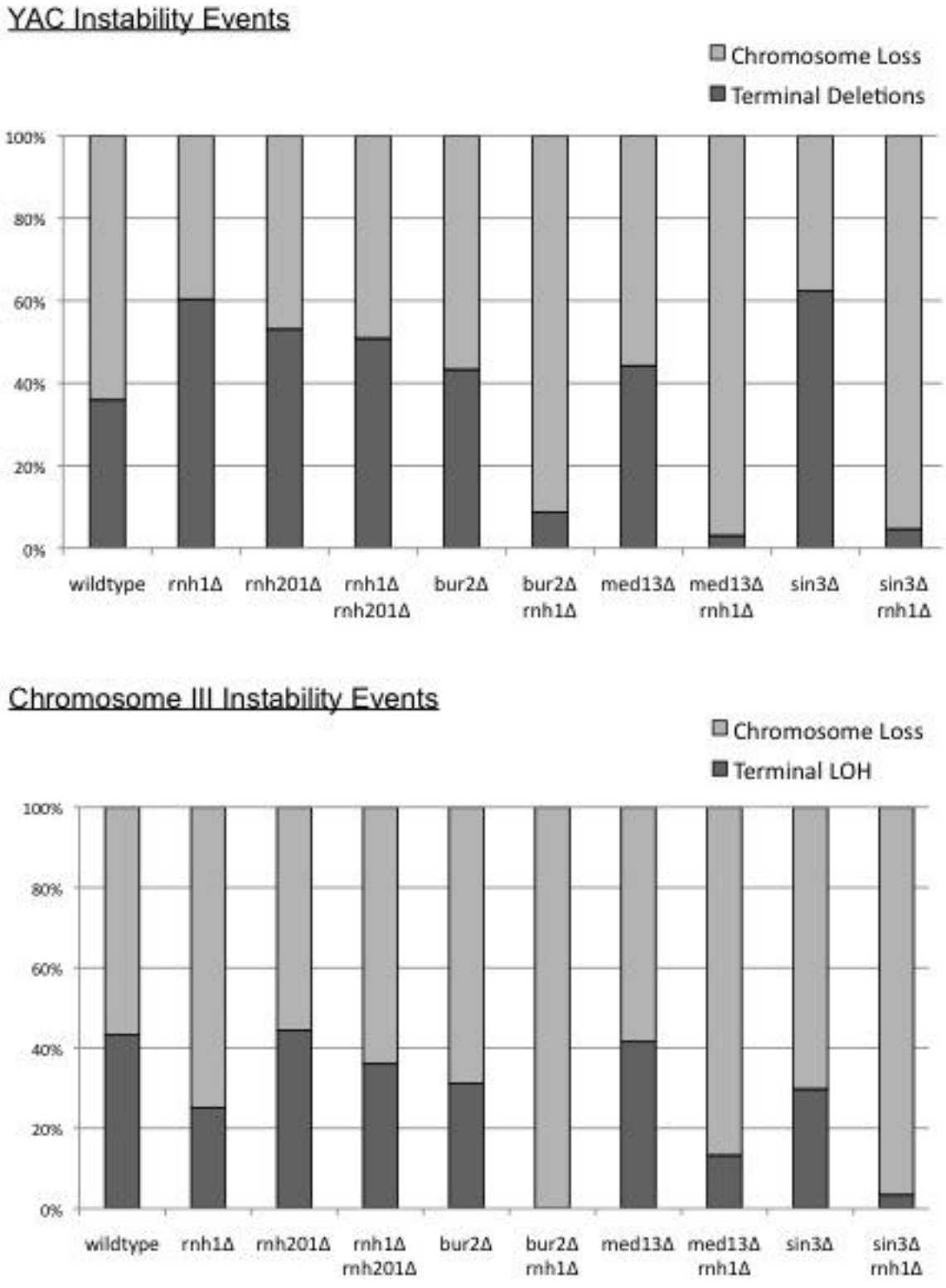


Figure S9, Related to Figure 7.
The distribution of instability events on the YAC and chromosome III in various RNase HI double mutants. Deletion of *RNH1* in a transcription mutant background leads to most events being chromosome loss events.

Homo sapiens RNase H1:
ATGagctggt ttctgttcct ggcccacaga gtcgccttgg ccgccttgcc ctgccgccgc ggctctcgca ggttcgggat gttctatgcc gtgaggaggg gccgcaagac cggggtcttt ctgacctgga atgagtgcag agcacaggtg gaccggtttc ctgctgccag atttaagaag tttgccacag aggatgaggc cagggccttt gtcaggaaat ctgcaagccc ggaagtttca gaagggcatg aaaatcaaca tggacaagaa tcggaggcga aagccagcaa gcgactccgt gagccactgg atggagatgg acatgaaagc gcagagccgt atgcaaagca catgaagccg agcgtggagc cggcgcctcc agttagcaga gacacgtttt cctacatggg agacttcgtc gtcgtctaca ctgatggctg ctgctccagt aatgggcgta gaaggccgcg agcaggaatc ggcgtttact gggggccggg ccatccttta aatgtaggca ttagacttcc tgggcggcag acaaaccaaa gagcggaaat tcatgcagcc tgcaaagcca ttgaacaagc aaagactcaa aacatcaata aactggttct gtatacagac agtatgttta cgataaatgg tataactaac tgggttcaag gttggaagaa aaatgggtgg aagacaagtg cagggaaaga ggtgatcaac aaagaggact ttgtggcact ggagaggctt acccagggga tggacattca gtggatgcat gttcctggtc attcgggatt tataggcaat gaagaagctg acagattagc cagagaagga gctaaacaat cggaagacta g

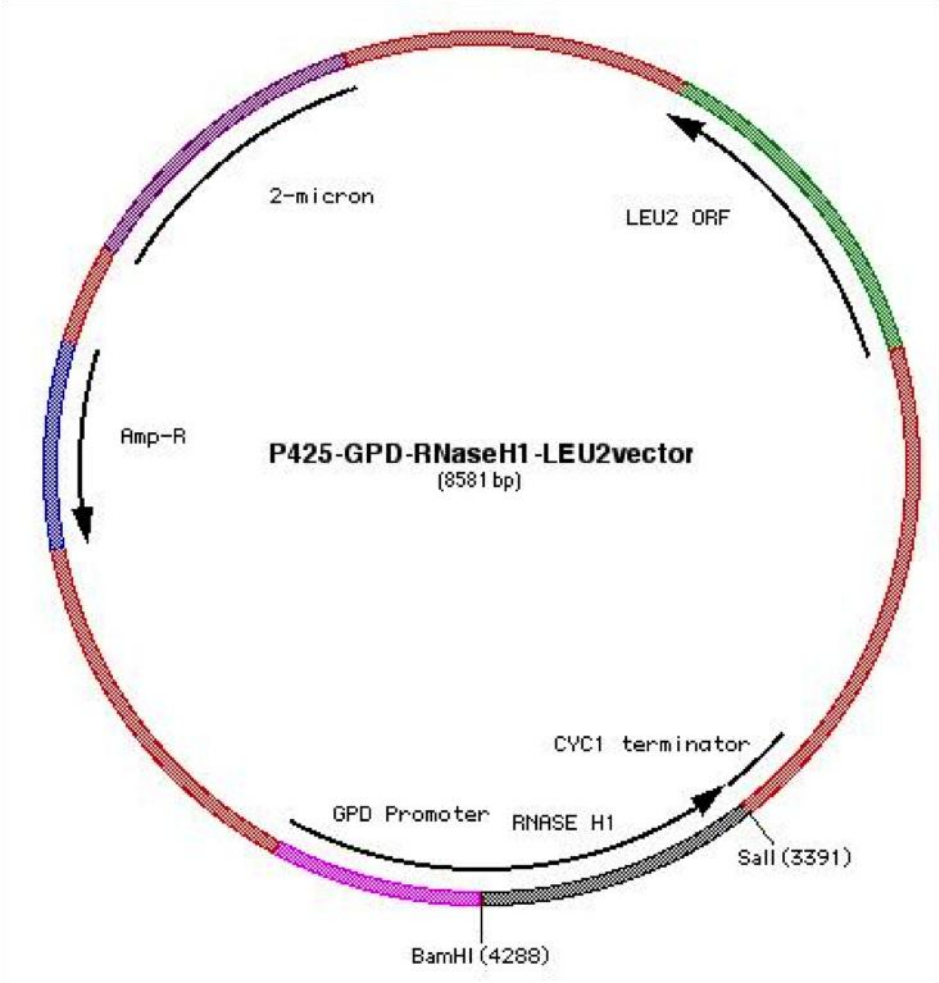


Figure S10. RNase H1 2μ Plasmid Map
The RNase H1 gene was cloned from cDNA prepared from RNA isolated from HeLa cells (GenBank number BT019670). RNase H1 was cloned into p425 GPD with the

restriction enzymes BamHI and SalI. Shown is the sequence of the RNase H1 gene cloned, and a map of the over-expression plasmid.