Fig. S1

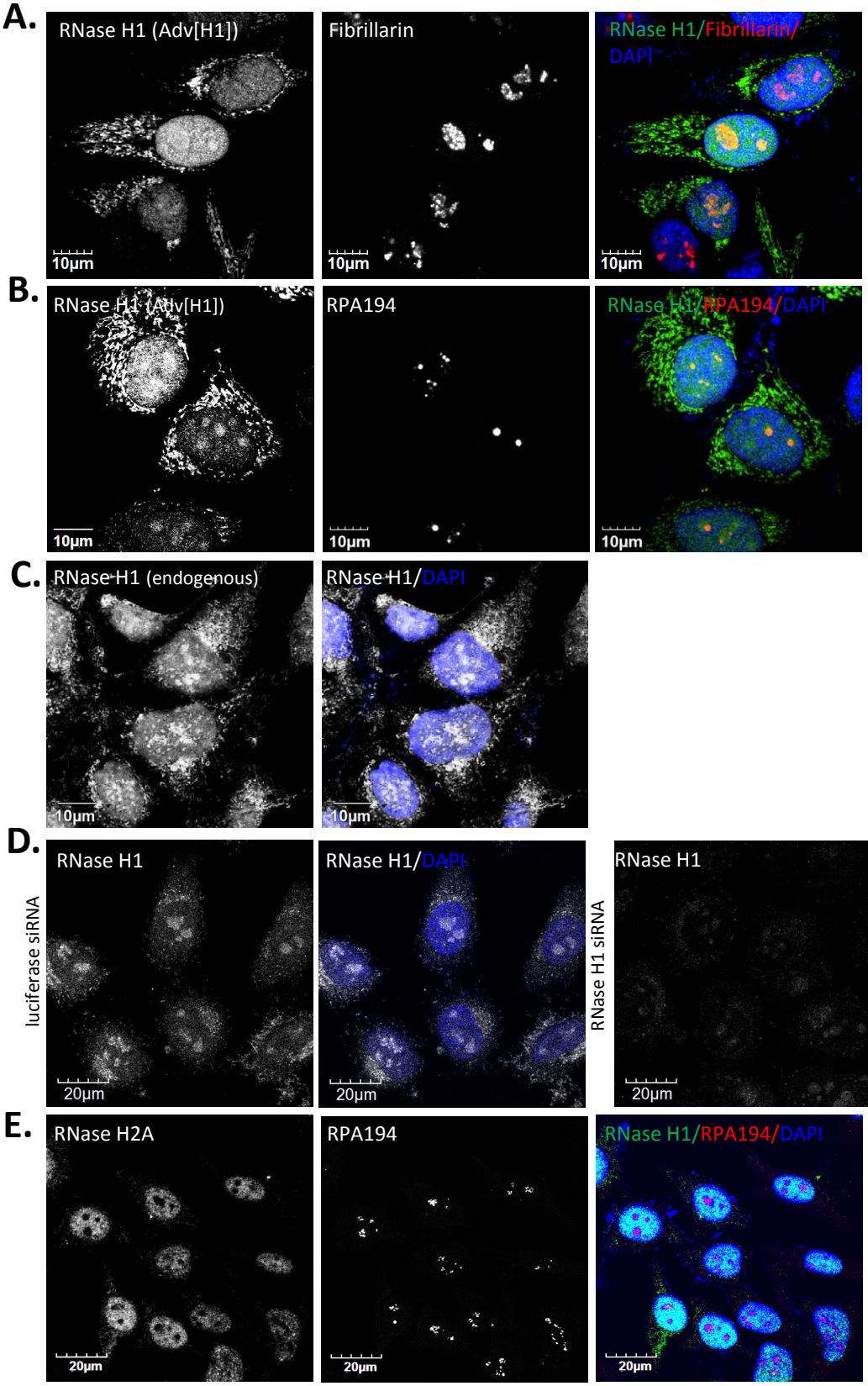

A.
RNase H1 (Adv[H1])
Fibrillarin
RNase H1/Fibrillarin/
DAPI
10μm
B.
RNase H1 (Adv[H1])
RPA194
RNase H1/RPA194/DAPI
10μm
C.
RNase H1 (endogenous)
RNase H1/DAPI
10μm
D.
luciferase siRNA
RNase H1
RNase H1/DAPI
RNase H1 siRNA
RNase H1
RNase H1/DAPI
20μm
E.
RNase H2A
RPA194
RNase H1/RPA194/DAPI
20μm

Fig. S2

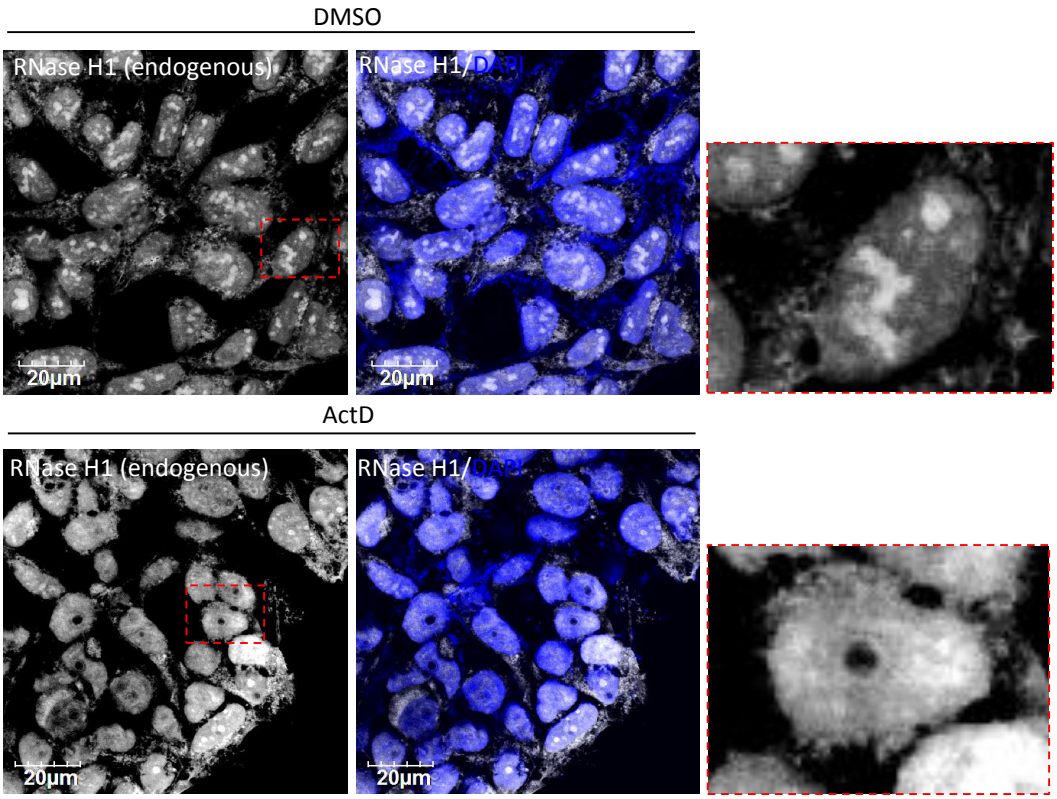

DMSO
RNase H1 (endogenous)
RNase H1/DAPI
20μm
20μm
ActD
RNase H1 (endogenous)
RNase H1/DAPI
20μm
20μm

Fig. S3

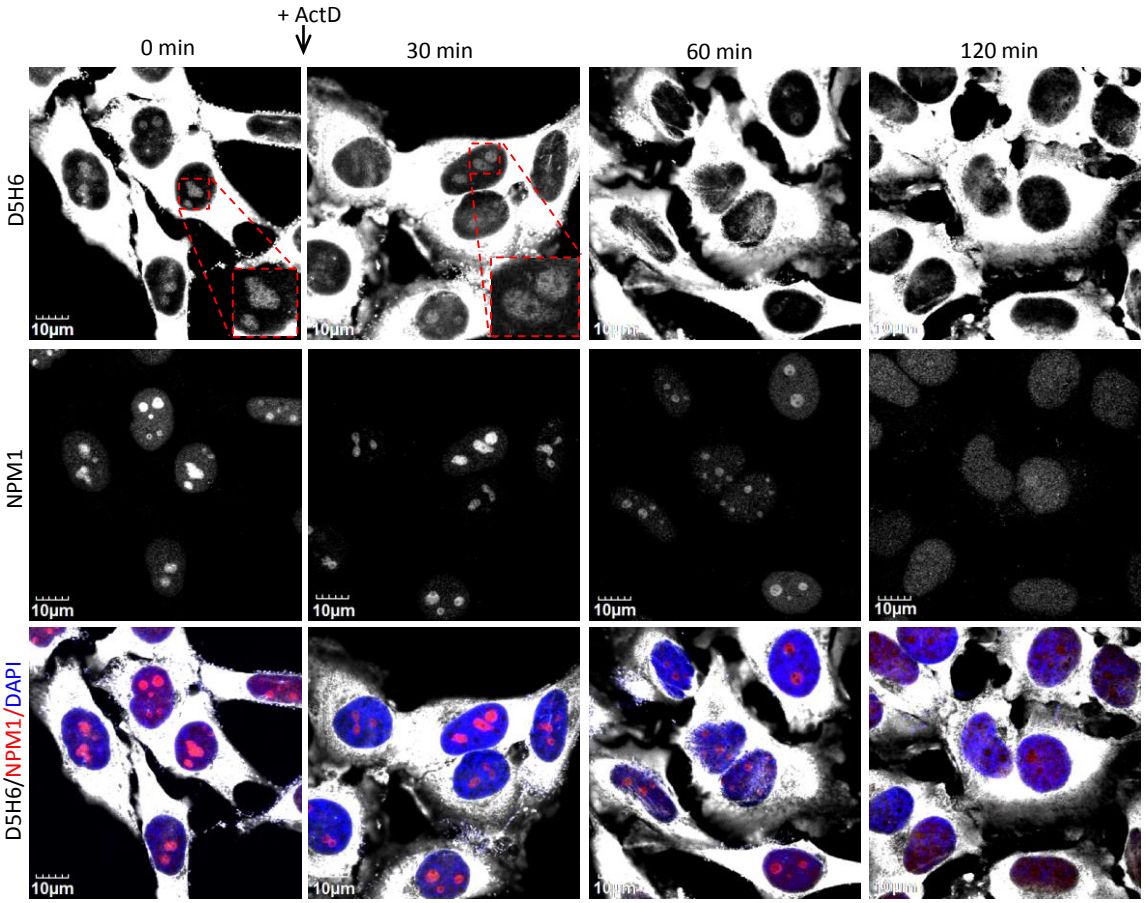
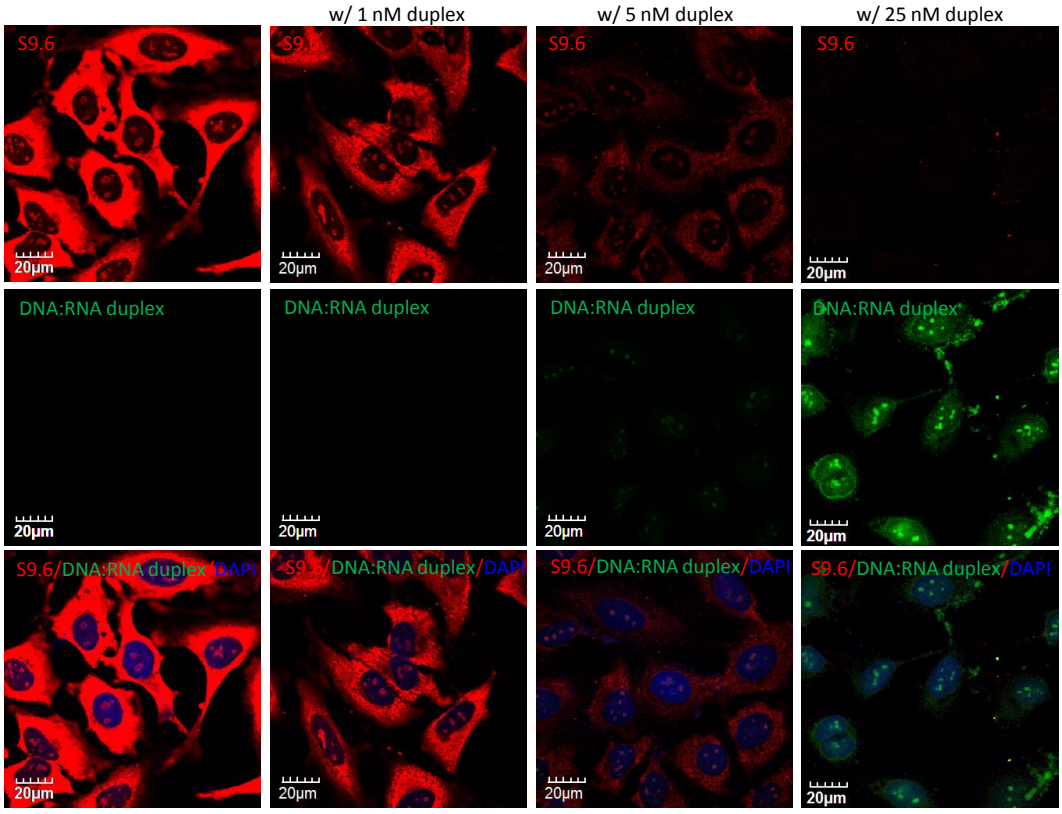
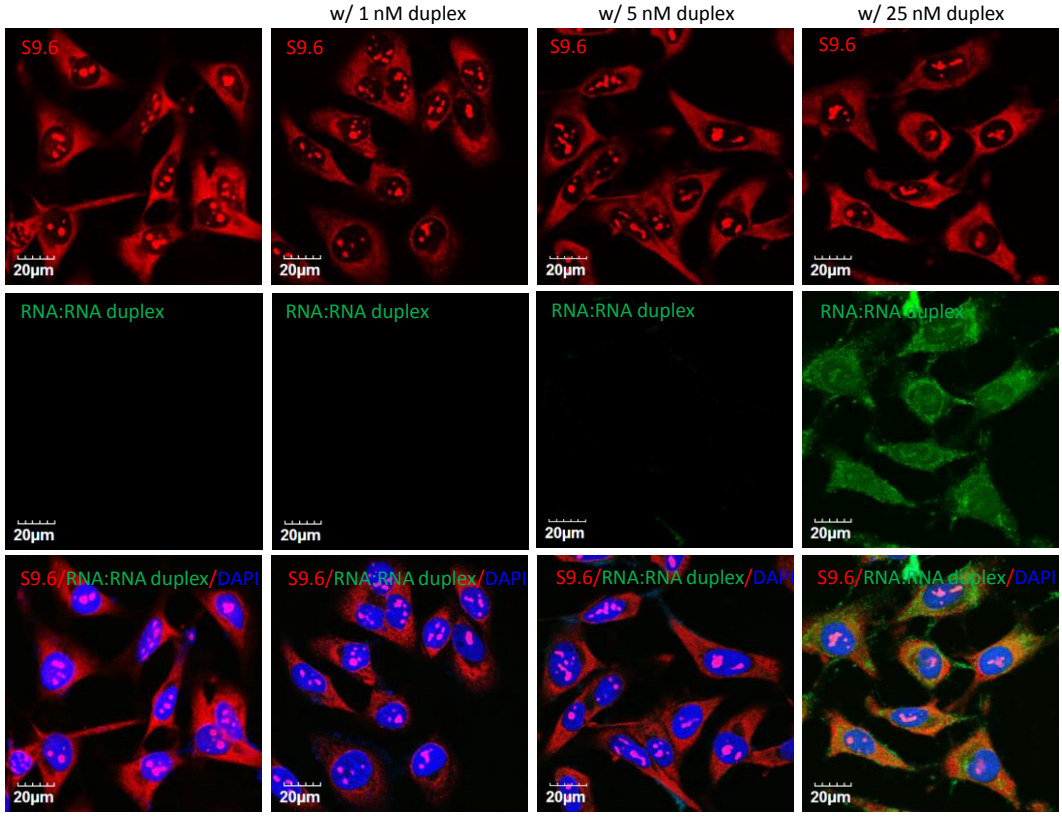


Fig. S4 A.


w/ 1 nM duplex
w/ 5 nM duplex
w/ 25 nM duplex
S9.6
DNA:RNA duplex
S9.6/DNA:RNA duplex/DAPI
20μm

B.


w/ 1 nM duplex
w/ 5 nM duplex
w/ 25 nM duplex
S9.6
RNA:RNA duplex
S9.6/RNA:RNA duplex/DAPI
20μm

C.

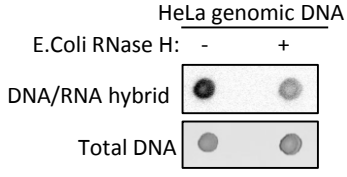
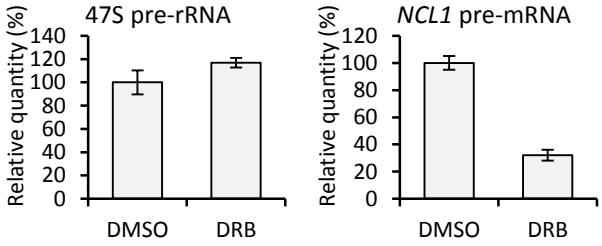

HeLa genomic DNA
E.Coli RNase H: - +
DNA/RNA hybrid
Total DNA

Fig. S5

A.


47S pre-rRNA
Relative quantity (%)
140
120
100
80
60
40
20
0
DMSO
DRB
NCL1 pre-mRNA
Relative quantity (%)
120
100
80
60
40
20
0
DMSO
DRB

B.

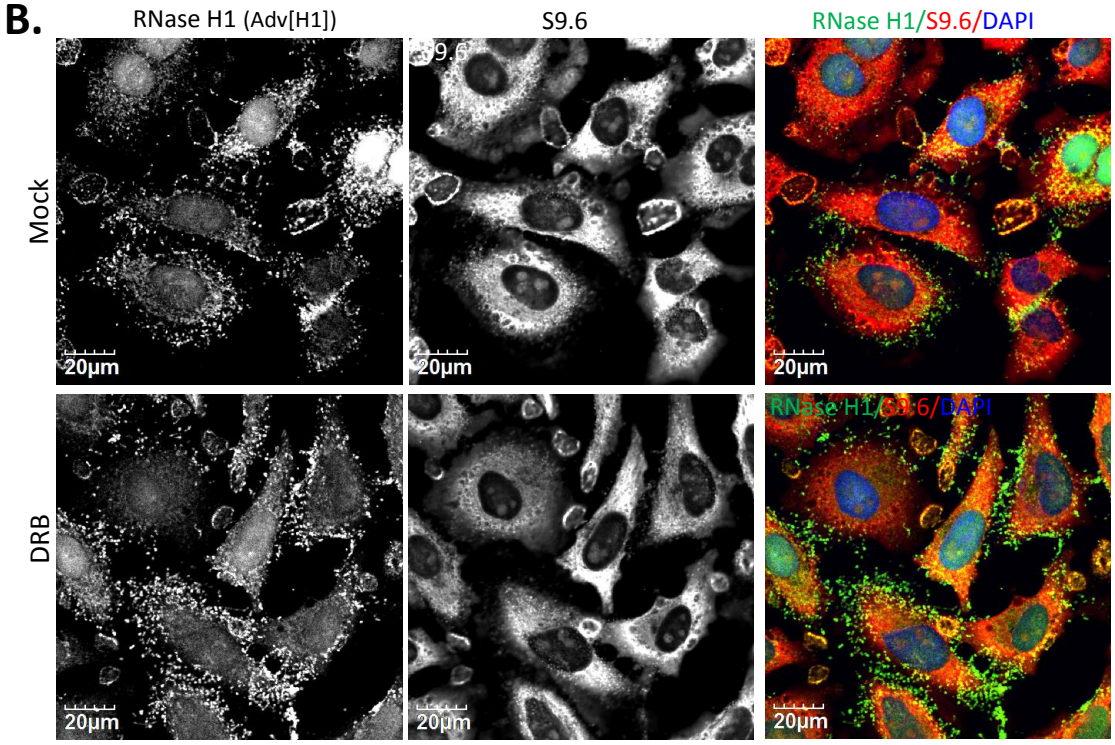

RNase H1 (Adv[H1])
S9.6
RNase H1/S9.6/DAPI
Mock
DRB
20μm

Fig. S6

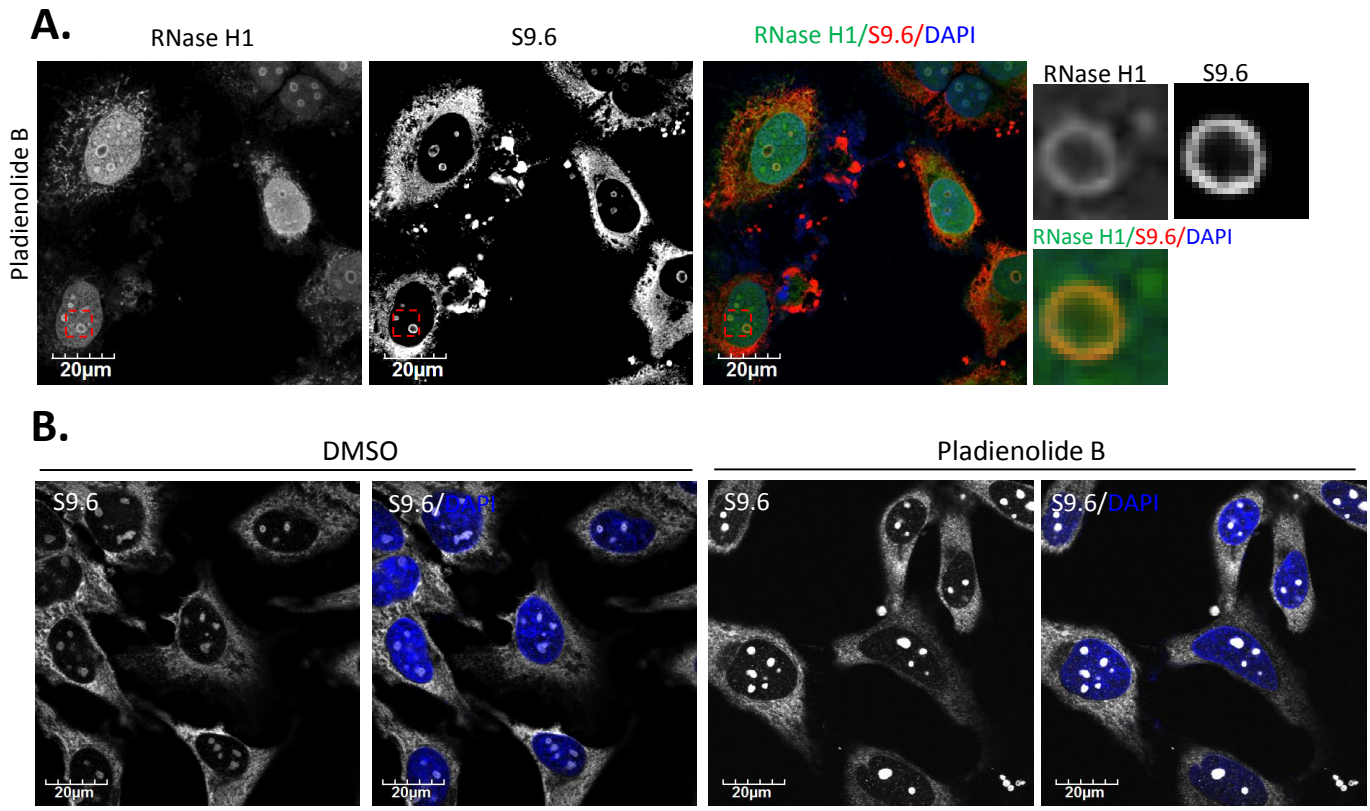

A.
RNase H1
S9.6
RNase H1/S9.6/DAPI
Pladienolide B
20μm
RNase H1
S9.6
RNase H1/S9.6/DAPI
B.
DMSO
Pladienolide B
S9.6
S9.6/DAPI
20μm

Fig. S7

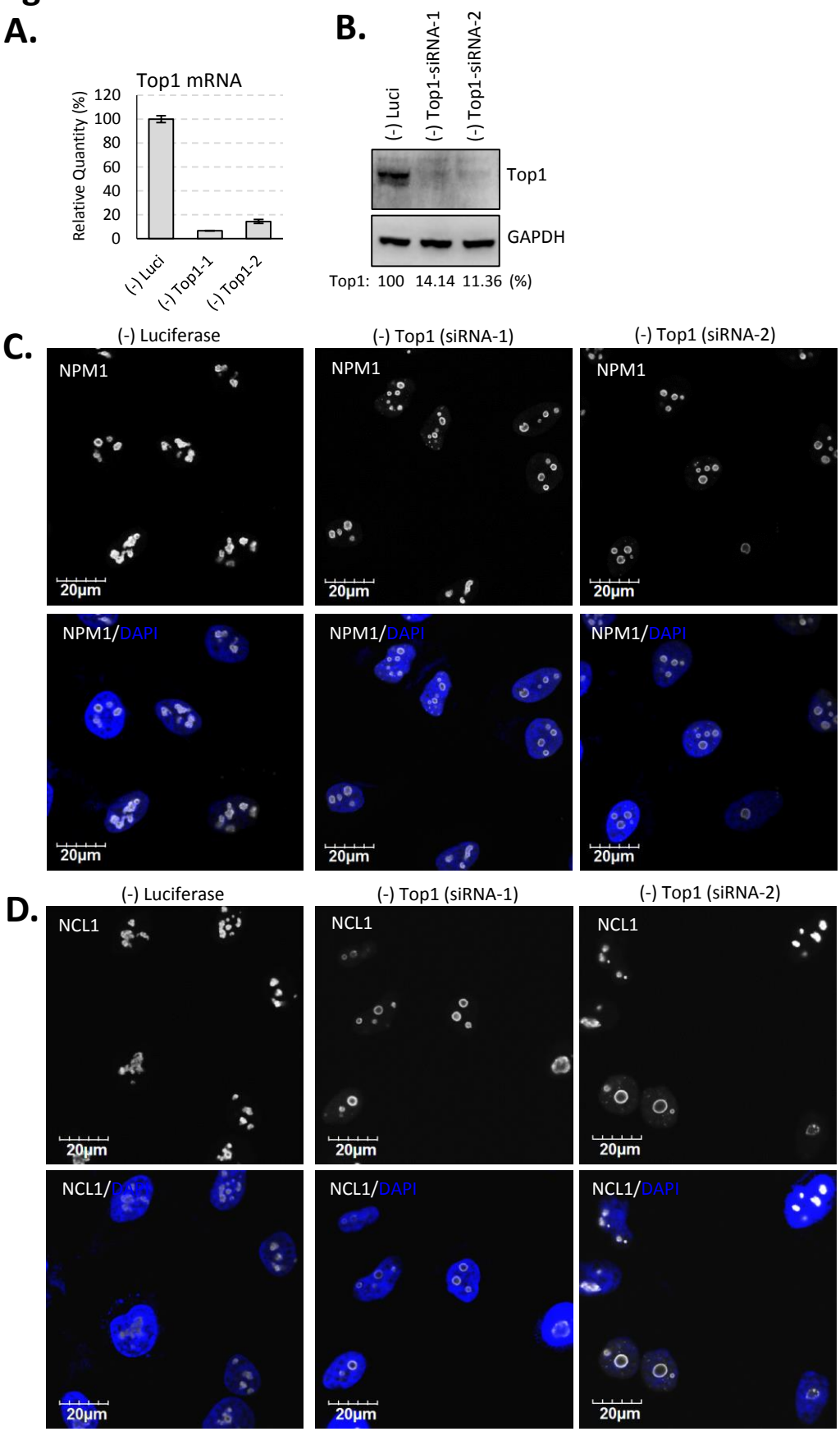

A.
Top1 mRNA
Relative Quantity (%)
120
100
80
60
40
20
0
(-) Luci
(-) Top1-1
(-) Top1-2
B.
(-) Luci
(-) Top1-siRNA-1
(-) Top1-siRNA-2
Top1
GAPDH
Top1: 100 14.14 11.36 (%)
C.
(-) Luciferase
(-) Top1 (siRNA-1)
(-) Top1 (siRNA-2)
NPM1
NPM1/DAPI
20μm
D.
(-) Luciferase
(-) Top1 (siRNA-1)
(-) Top1 (siRNA-2)
NCL1
NCL1/DAPI
20μm

Fig. S8

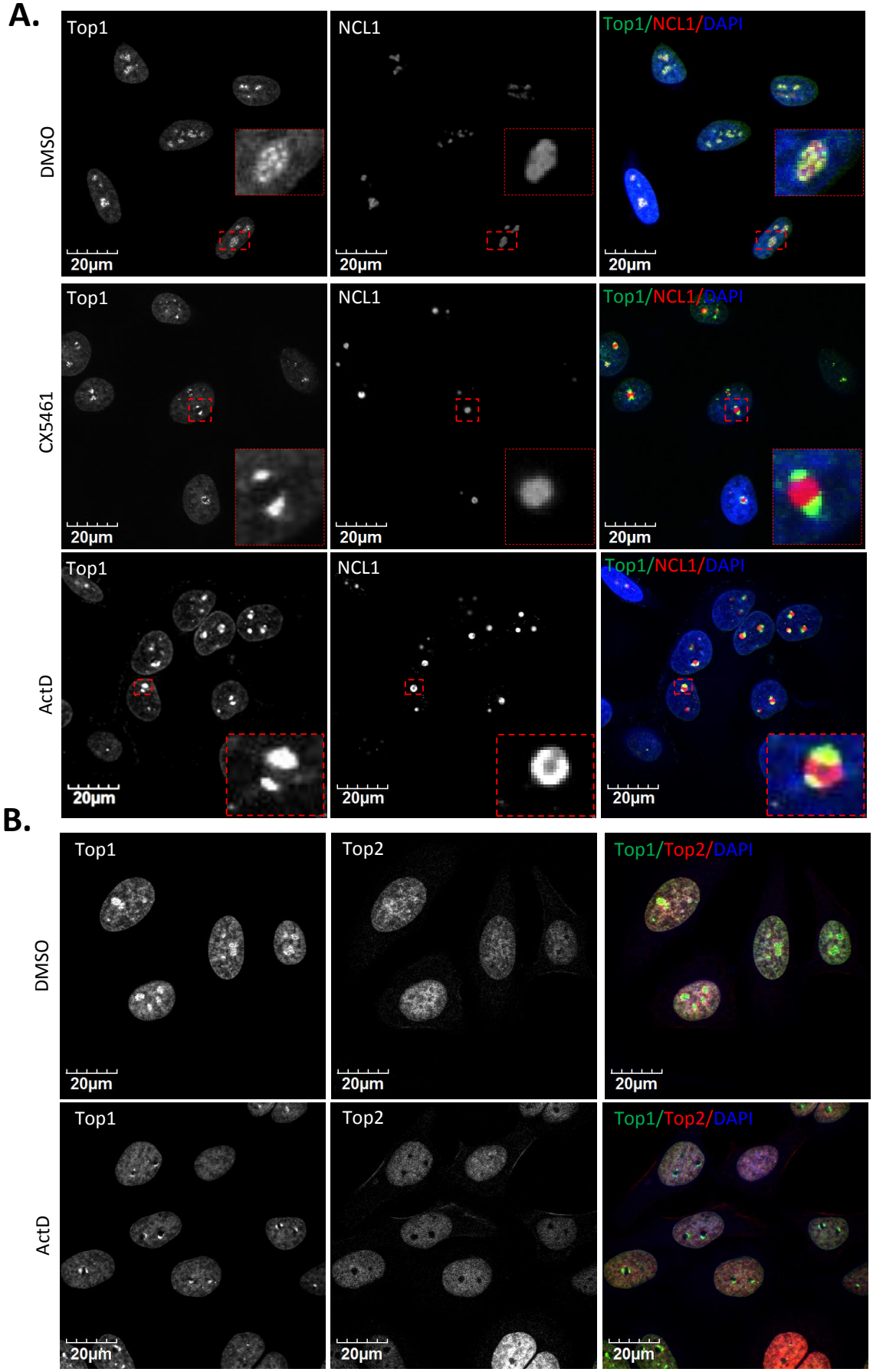

A.
DMSO
CX5461
ActD
Top1
NCL1
Top1/NCL1/DAPI
20μm
B.
DMSO
ActD
Top1
Top2
Top1/Top2/DAPI
20μm

Fig. S9

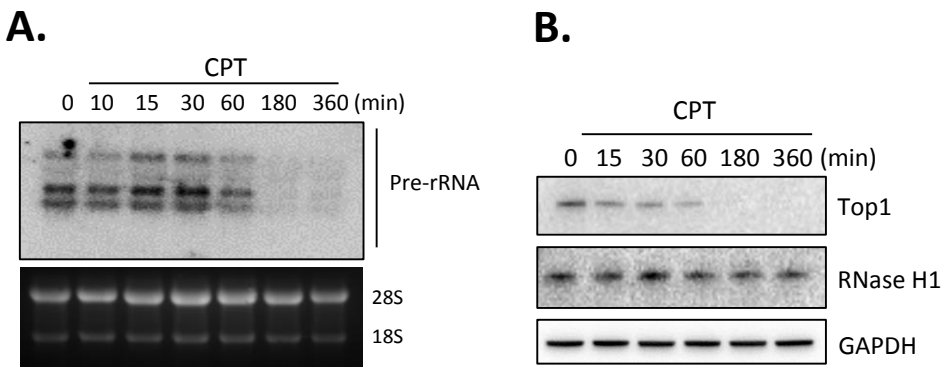
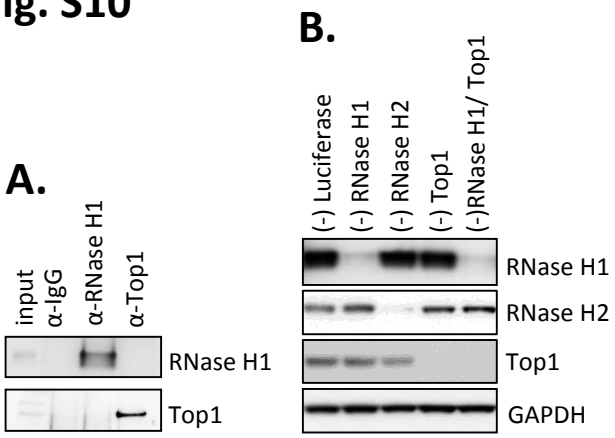

A.
CPT
0 10 15 30 60 180 360 (min)
Pre-rRNA
28S
18S
B.
CPT
0 15 30 60 180 360 (min)
Top1
RNase H1
GAPDH

Fig. S10


A.
input
α-IgG
α-RNase H1
α-Top1
RNase H1
Top1
B.
(-) Luciferase
(-) RNase H1
(-) RNase H2
(-) Top1
(-)RNase H1/ Top1
RNase H1
RNase H2
Top1
GAPDH

SUPPLEMENTAL INFORMATION

Supplemental Figure Legends:

Fig. S1 *Human RNase H1 accumulates in nucleoli*

A-B. Confocal immunofluorescent imaging of HeLa cells infected with $AdV_{[H1]}$ for 24 hours revealed co-localization of transiently overexpressed human RNase H1 with nucleolar markers **A**) fibrillarin and **B**) RPA194. **C.** Nucleolar localization was observed for endogenous RNase H1 in HEK293 cells. **D.** Depletion of RNase H1 by specific siRNA reduced the staining signals of endogenous RNase H1 in HeLa cells. **E.** Co-immunofluorescent staining of RNase H2A and RPA194 suggested that RNase H2A did not significantly localize to nucleoli.

Fig. S2 *RNase H1 is cleared from the nucleoli in cells treated with RNAP I inhibitor*

Representative confocal images of HEK293 cells treated with DMSO and with 0.02 μg/ml ActD for 2 h and stained for endogenous RNase H1.

Fig. S3 *Clearance of nucleolar R-loops upon treatment with ActD is confirmed using a second antibody specific for DNA:RNA hybrids*

Co-immunofluorescent staining of R-loops with D5H6 and anti-NPM1 in HeLa cells treated with 0.02 μg/ml ActD indicates that R-loops are cleared from nucleolar with 60 to 120 min of exposure to ActD.

Fig. S4 *S9.6 antibody staining specificity control.*

S9.6 antibody (2 μg/ml final concentration) was pre-incubated with (**A.**) synthetic DNA:RNA hybrids or (**B.**) duplex RNA at final concentrations of 1 nM, 5 nM, or 25 nM at room temperature for 15 min in IF blocking buffer. The pre-incubated antibody was then added to the fixed and permeabilized cells to stain for cellular DNA:RNA hybrids. The RNA strand of the hybrid is 6-carboxyfluorescein (6-FAM) labeled to allow the direct visualization of the hybrids in cells. Sequences of the hybrids used here are: DNA-5'-CTG CTA GCC TCT GGA TTT GA -3' and RNA- 5'-/6FAM/-UCA AAU CCA GAG GCU AGC AG-3'. The duplexed RNA shares the same sequences. **C.** Dot blot suggests that the S9.6 signals decrease significantly with the treatment of *E.coli* RNase H.

Fig. S5 *RNAP II inhibitor DRB does not affect nucleolar localization of RNase H1 and DNA:RNA hybrids*

A. qRT-PCR quantification of 47S pre-rRNA and *NCL1* pre-mRNA in HeLa cells treated with 10 μM DRB for 2 h. The error bars represent standard deviation from three parallel experiments. **B.** Co-immunofluorescent staining of R-loops and RNase H1 (overexpressed from $AdV_{[H1]}$) in HeLa cells treated with 10 μM DRB for 2 h.

Fig. S6 *Pladienolide B exposure causes co-migration of RNase H1 and DNA:RNA hybrids to the perinucleolar regions*

A. Representative images of co-immunofluorescent staining of endogenous RNase H1 and R-loops (using S9.6) in HeLa cells treated with pladienolide B (5 μM, 24 h) suggest the co-migration of RNase H1 and DNA:RNA hybrids to perinucleolar regions. **B.** Representative confocal images of HeLa cells treated with 5 μM pladienolide B and stained with S9.6 suggest that pladienolide B exposure elevates the levels of nucleolar DNA:RNA hybrids.

Fig. S7 *Depletion of Top1 causes morphological changes of nucleoli*

A. qRT-PCR of Top1 depletion by two different Top1 siRNAs. **B.** Western analysis of Top1 depletion by two different Top1 siRNAs. GAPDH serves as a loading control. **C-D.** Depletion of Top1 in HeLa cells resulted in smaller and more rounded nucleoli. Nucleoli were stained using (**C.**) anti-NPM1 or (**D.**) anti-NCL1 antibodies.

Fig. S8 *Nucleolar clearance of Top1 to perinucleolar caps occurs in HeLa cells treated with ActD or CX5461*

A. Representative images of immunofluorescent staining of Top1 and NCL1 in HeLa cells treated with DMSO, ActD (0.02 μg/ml, 2 h), or CX5461 (250 nM, 6 h). **B.** Representative images of immunofluorescent staining of Top1 and Top2 in HeLa cells treated with DMSO or ActD (0.02 μg/ml, 2 h).

Fig. S9 *Treatment of cells with CPT reduces levels of Top1 protein and pre-rRNA but does not affect levels of RNase H1 protein*

A. Northern analysis of levels of pre-rRNA after treatment of HeLa cells with 20 μM CPT for indicated times. Levels of mature 28S and 18S rRNA serve as loading controls. **B.** Western analysis of protein levels of RNase H1 and Top1 after treatment of HeLa cells with 20 μM CPT for indicated times. GAPDH serves as a loading control.

Fig. S10 *Endogenous RNase H1 IP and siRNA-mediated depletion of RNase H1*

A. Western analysis of immunoprecipitated endogenous RNase H1 and Top1. **B.** Western analysis confirmed the depletion of RNase H1 and Top1 by siRNAs.

Antibodies:

Western analysis of RNase H1, immunofluorescent (IF) staining of RNase H1 (except for Fig. 1A and S1A), and RNase H1-ChIP were all performed using a polyclonal anti-RNase H1 antibody raised in rabbit against the His-tagged partial human RNase H1 (amino acid 47–287). The sequence of the antigen is: MASHHHHHHS LVPRGSSASPE VSEGHENQHG QESEAKASKR LREPLDGDGHE SAEPYAKHMK PSVEPAPPVS RDTFSYMGDF VVVYTDGCCS SNGRRRPRAG IGVYWGPGHP LNVGIRLPGR QTNQRAEIHA ACKAIEQAKT QNINKLVLYT DSMFTINGIT NWVQGWKKNG WKTSAGKEVI NKEDFVALER LTQGMDIQWM HVPGHSGFIG NEEADRLARE GAKQSED. The antibody was purified as described previously (1,2). Staining of RNase H2A was performed using a polyclonal anti-RNase H2A antibody raised in rabbit against full-length human RNase H2A and purified as described previously (3).

Supplemental Table 1. Antibodies using in this study

Antibody	Company	Catalog number	Application
Top1	Abcam	ab85038	ChIP, Western blot
Top1	Abcam	ab197505	IF
DNA-RNA Hybrid [S9.6]	Kerafast	ENH001	IF, ChIP
DNA-RNA Hybrid [D5H6]	CovalAb	mab0105-P	IF
RNase H1	Abcam	ab56560	IF
NPM1	Abcam	ab24412	IF
BrdU	Abcam	ab6326	IF
Flag	Sigma-Aldrich	F3165	IF
RPA194	Santa Cruz	sc-46699	IF
NCL1	Abcam	ab22758	IF, western
NCL1 -AF647	Abcam	ab202709	IF
Fibrillarin-AF488	Abcam	ab184817	IF
Fibrillarin	Abcam	ab4566	IF
Top2	Abcam	ab201504	IF
p54nrb	Santa Cruz	sc-376865	Western blot
PSPC1	Abcam	ab104230	Western blot
PARP1	Santa Cruz	sc-8007	Western blot
Ku70	Abcam	ab3114	Western blot
FEN1	Santa Cruz	sc-28355	Western blot

PCNA	Abcam	ab29	Western blot
RPA70	Calbiochem	NA13	Western blot
p32	Santa Cruz	sc-48795	Western blot
SSBP1	Santa Cruz	sc67101	Western blot
p21	Abcam	ab7960	Western blot
γ-tubulin	Sigma-Aldrich	T5168	Western blot
GAPDH	Sigma-Aldrich	G8795	Western blot

ChIP-qPCR:

Supplemental Table 2. Primer probe sequences using in this study for ChIP-qPCR

#1 Forward	TCATCCTGGCCGTCTGAG
#1 Reverse	GAAAGGATCGCGGCAGAG
#1 Probe	CGGCCGAATTCGTTTCCGAGTC
#2 Forward	GGTATATCTTTCGCTCCGAGTC
#2 Reverse	TCACCGTAGGCCAGAG
#2 Probe	AGAGGACAGCGTGTCAGCAATAACC
#3 Forward	AAAAGCCTTCTCTAGCGATCTG
#3 Reverse	CATAACGGAGGCAGAGACAG
#3 Probe	TCCGGTACCCCCAAGGCAC
#4 Forward	CCGCGCTCTACCTTACCT
#4 Reverse	CGACCAAAGGAACCATAACTG
#4 Probe	TGAGCCATTCGCAGTTTCACTGTACC
#5 Forward	GCGAGAGCCGAGAACTCG
#5 Reverse	ACCAACGACACGCCCTTC
#5 Probe	TCTCTCTCCCCCCCGTCTCC
#6 Forward	TTTGCTGTCTCGTCTGGC
#6 Reverse	TCCACCGCTAAGAGTCGT
#6 Probe	CGCCCTCGCCAAATCGACCTC
#7 Forward	GACACTTCGAACGCACTTG
#7 Reverse	CTCAGACAGGCGTAGCCCCG
#7 Probe	GATTGATCGGCAAGCGAC
#8 Forward	CAGGTCTCCAAGGTGAACAG
#8 Reverse	CTTAGAGCCAATCCTTATCCCG
#8 Probe	CAGGTCTCCAAGGTGAACAG
#9 Forward	TGT TCC CGT GAG AGT GAT TTC
#9 Reverse	GCC TGA CTC CAT TTC GTA TTT TC
#9 Probe	TC ACG TCT GTC ATC CCG AGG TCA

Supplemental Reference:

1. Wu, H., Lima, W.F. and Crooke, S.T. (2001) Investigating the structure of human RNase H1 by site-directed mutagenesis. *The Journal of biological chemistry*, **276**, 23547-23553.
2. Wu, H., Lima, W.F., Zhang, H., Fan, A., Sun, H. and Crooke, S.T. (2004) Determination of the role of the human RNase H1 in the pharmacology of DNA-like antisense drugs. *The Journal of biological chemistry*, **279**, 17181-17189.
3. Wu, H., Sun, H., Liang, X., Lima, W.F. and Crooke, S.T. (2013) Human RNase H1 is associated with protein P32 and is involved in mitochondrial pre-rRNA processing. *PloS one*, **8**, e71006, 71001-71015.