Table S1. Oligonucleotides used in the study.

NCL-For	5’ CGCGGATCCATGGTGAAGCTCGCGAAGGCAG 3’
NCL-1XHA Rev	5’CCGCTCGAGCTAAGCGTAATCTGGAACATCGTATGGGTATTCAAACTTCGTCTTCTTTCC 3’
NCLΔRGG-1XHA	5’CCGCTCGAGCTAAGCGTAATCTGGAACATCGTATGGGTATTCACCCTTAGGTTTGGCCCAGTCC 3’
Primers for ddPCR	
ARS306 For	5’ CATACTAACGAGCGAGCAC 3’
ARS306 Rev	5’ CGAACTATTGGGATTGGGGG 3’
KanMX For	5’ CGGCAAAACAGCATTCCAG 3’
KanMX Rev	5’ CGTCATCAAAATCACTCGCATC 3’
STE50 For	5’ CTACAAACCAATCTCACCCATC 3’
STE50 Rev	5’ CCCCATAGCAAATGACCAAG 3’

Table S2. The strains and recombination rates shown in Figure 1.

Relevant Genotype	Recombination rate x 10^{-8} (95% C.I.)	
	GTOP	***GBTM***
WT	20.3** (18.2-24.4)	12.5** (9.3-15.6)
*top1*Δ	82.0 (68.2-127)	8.5 (7.5-13.3)
*nsr1*Δ	16.7 (13.8-20.1)	8.52 (7.4-11.2)
*top1*Δ*nsr1*Δ	25.8 (23.9-35.6)	6.11 (5.54-9.41)
top1-Y727F	669** (393-998)	16.3* (13.5-18.6)
*top1-Y727F nsr1*Δ	161** (111-250.2)	9.96** (8.95-12.5)
*rnh1*Δ*rnh201*Δ	287** (255-313)	86.9** (66.9-97.7)
*rnh1*Δ*rnh201*Δ *nsr1*Δ	298 (269-322)	109 (89.4-157)

For all fluctuation analyses, the number of cultures used are 36 unless indicated (*12 and ** 24). 95% C.I. – 95% confidence intervals were determined as described in the Materials and Methods.

Table S3. The strains and recombination rates shown in Figure S2.

Relevant Genotype	Recombination rate x 10^{-8} (95% C.I.)	
	GTOP	***GBTM***
WT	2.94 (2.58-3.4)	2.7 (2.51-3.02)
*top1*Δ	6.17 (5.38-7.39)	3.61 (3.19-4.06)
*nsr1*Δ	2.8 (2.35-3.19)	1.85 (1.56-2.71)
*top1*Δ*nsr1*Δ	3.04 (1.64-4.59)	2.25 (1.31-3.58)

For all fluctuation analyses, the number of cultures used are 24. 95% C.I. – 95% confidence intervals were determined as described in the Materials and Methods.

Table S4. The strains and recombination rates shown in Figure 2.

Relevant Genotype	Recombination rate x 10^{-8} (95% C.I.)			
	WT	*top1*Δ	*nsr1*Δ	*top1*Δ*nsr1*Δ
Sμ-GTOP	0.3 (0.261-0.533)	7.60 (3.82-22.7)	0.332 (0.332-0.515)	1.26 (0.433-1.95)
Sμ-GBTM	0.295 (0.257-0.463)	0.465 (0.258-0.811)	0.445 (0.346-1.22)	0.561 (0.275-1.24)
TCF3-GTOP	0.514 (0.291-0.614)	8.52 (6.7-11.7)	0.463 (0.299-1.12)	0.959 (0.569-1.43)
TCF3-GBTM	0.563 (0.336-0.762)	2.54 (1.02-3.73)	0.316 (0.316-0.568)	0.593 (0.33-1.03)

For all fluctuation analyses, the number of cultures used are 24. 95% C.I. – 95% confidence intervals were determined as described in the Materials and Methods.

Table S5. The strains and recombination rates shown in Figure 3B.

Relevant Genotype	Plasmid	Recombination rate x 10^{-8} (95% C.I.)	
		GTOP*	***GBTM****
WT	Vec (*CEN-URA3*)	47.1 (45-50.8)	17 (12.9-24.2)
	pNSR1-Nsr1 (*CEN-URA3*)	57.5 (48-79)	26.7 (11.2-50.8)
	pNSR1-Nsr1ΔRGG (*CEN-URA3*)	35.2 (26 -43.5)	19.1 (16.5-21.8)
top1Δ	Vec (*CEN-URA3*)	270 (210-328)	35.9 (23-61.3)
	pNSR1-Nsr1 (*CEN-URA3*)	1170 (426-2190)	67.8 (41-106)
	pNSr1-Nsr1ΔRGG (*CEN-URA3*)	125 (60-273)	29.6 (20.9-43.5)
top1Δ nsr1Δ	Vec (*CEN-URA3*)	49.7 (45.5-69.3)	26.9 (19.8-36.6)
	pNSR1-Nsr1 (*CEN-URA3*)	424 (189-717)	21.7 (20.3-23.2)
	pNSR1-Nsr1ΔRGG (*CEN-URA3*)	37.4 (32.3-42)	22.5 (22.7-26)

Number of cultures used in the fluctuation analyses are **24 and ***36 as indicated. 95% C.I. – 95% confidence intervals were determined as described in the Materials and Methods.

Table S6. The strains and recombination rates shown in Figure 3C.

Relevant Genotype	Plasmid	*GTOP*
*top1*Δ *nsr1*Δ	Vec *(2μ-URA3)*	159 (124-176)
	pADH1-NTerm *(2μ-URA3)*	168 (134-206)
	pADH1-CTerm *(2μ-URA3)*	813 (561-992)
	pADH1-Nsr1*(2μ-URA3)*	2,030 (1,640-2,600)
*top1*Δ	Vec *(2μ-URA3)*	266 (138-471)
	pADH1-NTerm *(2μ-URA3)*	220 (192-299)
	pADH1-CTerm *(2μ-URA3)*	496 (312-819)

For all fluctuation analyses, the number of cultures used are 36. as indicated95% C.I. – 95% confidence intervals were determined as described in the Materials and Methods.

Table S7. The strains and recombination rates shown in Figure 4.

		Recombination rate x 10^{-8} (95% C.I.)	
Relevant Genotype	**Plasmid**	***GTOP****	***GBTM****
top1Δ nsr1Δ	Vec (*CEN-URA3*)	64.6 (24-93.8)	25.8 (22.6-33.8)
	pNSR1-NCL (*CEN-URA3*)	125 (117-184)	37 (29-43.3)
	pNSR1-NCLΔRGG (*CEN-URA3*)	89.3 (70.5-104.6)	30.6 (20.8-36.1)
top1Δ	Vec (*CEN-URA3*)	176 (135.3-226)	44 (33.3-64)
	pNSR1-NCL(*CEN-URA3*)	381 (347-427)	106 (62-211)
	pNSR1-NCLΔRGG (*CEN-URA3*)	183 (149-276)	40.2 (14.3--49.9)

Number of cultures used in the fluctuation analyses are **24 and ***36 as indicated. 95% C.I. – 95% confidence intervals were determined as described in the Materials and Methods.

Table S8. The strains and recombination rates shown in Figure S3D.

	Recombination rate x 10^{-8} (95% C.I.)	
Relevant Genotype	***GTOP***	***GBTM***
*top1*Δ	82.0 (68.2-127)	8.5 (7.5-13.3)
*top1*Δ *nsr1*Δ	25.8 (23.9-35.6)	6.11 (5.54-9.41)
*top1*Δ *nsr1*Δ*RGG*	28.7 (27.2-35.1)	8.7 (7.3-10.2)

For all fluctuation analyses, the number of cultures used are 36. 95% C.I. – 95% confidence intervals were determined as described in the Materials and Methods.

SUPPLEMENTAL METHODS

DNA-Dynabeads Affinity Purification of Proteins

The DNA-Dynabeads affinity purification of proteins was carried out according to the previously described protocol (GAO *et al.* 2015) with some modifications. For DNA-conjugated Dynabeads preparation, biotinylated oligonucleotides SμG, SμG-M1 and SμG-M2 (Sigma) were incubated at 60°C overnight in the presence of 10 mM Tris pH 7.5 and 100 mM KCl and then conjugated to Streptavidin-Coupled M-280 Dynabeads® (Life Technologies) as per the manufacturer's instructions. Yeast cells from indicated backgrounds were used to prepare whole cell extracts by glass bead-mediated cell disruption. The oligo-conjugated beads were incubated with the yeast extract at 4 °C. After washing, proteins were eluted by boiling in 1XSDS-PAGE loading buffer followed by immunoblotting analysis using anti-FLAG antibody (Sigma; Cat# A8592).

Cell synchronization and Flow cytometry to measure DNA content.

Cell synchronization and sample collection was carried out as described in the Materials and Methods. For flow cytometry, yeast cells were fixed overnight in ice-cold 70% ethanol at 4°C and then re-suspended in 500μl of 50 mM sodium citrate (pH 7.2) containing RNase A and incubated for 2 hours at 37°C, followed by incubation with 50μl of 20 mg/ml Proteinase K. at 50°C for 1 - 2 hours. Cells were stained with SYTOX Green dye (4 μM in 50mM sodium citrate, pH 7.2; Thermo Scientific) for at least one hour and then analyzed using the BD FACS ARIA II SORP and FlowJo software (FlowJo, LLC).

REFERENCES

Gao, J., B. L. Zybailov, A. K. Byrd, W. C. Griffin, S. Chib *et al.*, 2015 Yeast transcription co-activator Sub1 and its human homolog PC4 preferentially bind to G-quadruplex DNA. Chem Commun (Camb) 51**:** 7242-7244.

Supplementary Figure Legends

Figure S1. The design of *pTET-lys2-GTOP* reporter. (**A**) The sequence of guanine-rich strand of the mouse SμG fragment inserted into the yeast genome for the construction of *pTET-lys2-GTOP* reporter is shown. The runs of 4 consecutive guanines are underlined and in bold. (**B**) The strand orientations of the SμG sequence in the *pTET-lys2-GTOP* and *-GBTM* cassettes relative to transcription are diagramed. In *GTOP* orientation, the strand containing guanine runs shown in **(A)** are on the non-transcribed strand favoring G4 DNA formation. In *GBTM* orientation, the same guanine runs are on the transcribed strand favoring base-pairing with the nascent RNA (red line). **(C)** A schematic drawing of the reporter used for the gross chromosomal rearrangement (GCR) analysis embedded in the left arm of chromosome 5, centromere-proximal to *CAN1* locus. *URA3* gene integrated telomere-proximal to *CAN1* locus is indicated.

Figure S2. Rates of recombination of the indicated strains under low transcription conditions. Error bars indicate 95% confidence intervals. Two rates are considered statistically different when the confidence intervals do not overlap. The rates, numbers of cultures used in fluctuation analyses, and 95% confidence intervals are listed in Table S3.

Figure S3. Effect of *nsr1*Δ deletion on cell growth and rRNA processing. (**A**) Overnight grown cells from indicated strains were grown to log phase in liquid YEPD media at 30 °C. Cells were then diluted ~1X10^6 cells/ml in fresh medium. Cell growth at 30 °C was monitored by measuring OD_{600}. The data represent averages of eight cultures with the error bars indicating the standard deviation. (**B**) Total RNAs were isolated from the depicted strains followed by northern blot probing with labeled oligos. The *SCR1* RNA serves as a loading control. (**C**) Ratio of 20S/35S in indicated strains normalized to the *WT* ratio. **(D)** Rates of recombination of *top1*Δ strains with genomic *NSR1*, *nsr1*Δ*RGG,* or *nsr1*Δ allele. Error bars indicate 95% confidence intervals. Two rates are considered statistically different when the confidence intervals do not overlap. The rates, numbers of cultures used in fluctuation analyses, and 95% confidence intervals are listed in Table S8.

Figure S4. Nsr1 and Nsr1ΔRGG binding to G4 DNA *in vitro*.

(**A**) Sequence of the oligos used in pulldown assays. **(B)** For pull-down assays, the indicated, biotin-conjugated oligos were incubated with total yeast lysate from *top1Δ* cells expressing the C-terminal 3XFLAG tagged version of full length Nsr1 or Nsr1ΔRGG. αFLAG antibody was used to detect the respective proteins. **(C)** Quantification of % IP to input from (B); N=3.

Figure S5. (**A - D**) Flow cytometry analysis of the indicated strains containing the *pTET-lys2-GTOP* or -*GBTM* cassette. Cells were taken at the indicated timepoints (t = minutes after α-factor release) and stained with SYTOX green dye to measure DNA content.

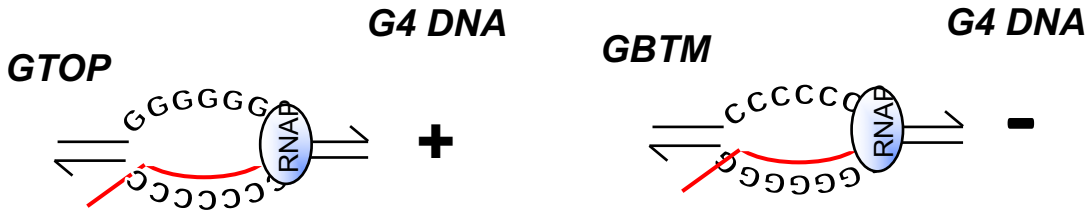
Figure S6. *GBTM* strains analyzed by ddPCR. Normalized copy number of (**A**) ARS306 (**B**) KanMX and **(C)** Ste50 loci. See Figure 6 legend for more detail.

Figure S1

(A) *SμG* sequence

TC GAGCTGAGCT GAGCTGGGTG AGCTGAGCTG AGCTGAGCTG GGTGAGCTGA
GCTGAGCTGA GCTGAGCTGG ***GTGAGCTGAG CTGAGCTGAG CTGAGCTGAG CTGAGCTGGG***
GTGAGCTGGG ***CTGAGCTGAG CTGAGCTGGG GTGAGCTGAG CTGAGCTGAG CTGAGCTGGG***
GTGAGCTGGG ***CTGAGCTGGG GT***GAGCTGGG ***CTGAGCTGAG CTGGGGTGAG CTGAGCTGAG***
CTGAGCTGAG CTGGGGTGAG CTGAGCTGAG CTGAGCTGGG GTGAGCTGAG CTGGGCT***GAG***
CTGAGCTGGG GTGAGCTGAG CTGAGCTGAG CTGAGCTGGG GTGAGCTGAG CTGAGCTGAG
CTGGGGTGAG CTGAGCTGGG GTGAGCTGAG CTGAGCTGGG GTGAGCTGAG CTGAGCTGAG
CTGGGGTGAG CTGAGCTGGG GTGAGCTGAG CTGAGCTGGG GTGAGCTGAG CTGAGCTGGG
GTGAGCTGAG CTGAGCTGGG GTGAGCTGAG CTGAGCTGGG GTGAGCTGAG CTAGGGTGAG
CTGGGCTGGG TGAGCTGGAG TGAGCTGAGC TGAGGTGAAC TGGGGTGAGC CGGATGTTTT
GAGTT***GAGCT GGGGT***AAGAT GAGCTGAACT GGGGTAAGAT GGGATGAGCT GTGGTGAGGG
GAGCTGGATT GAACTGAGCT GTGT***GAGCTG AGCTGGGGT***C AGCTGAGCAA GAGTGAGTAG
AGCTGGCTGG CCAAGAACCA AGAATCAATT AGGCTAAGTG AGCCAGATTG CGCTGGGA

(B) *GTOP vs. GBTM*



(C) GCR reporter configuration

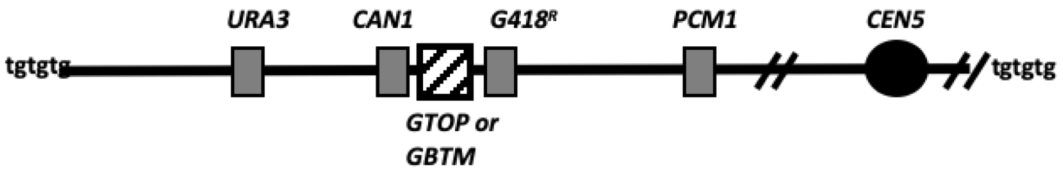


Figure S2

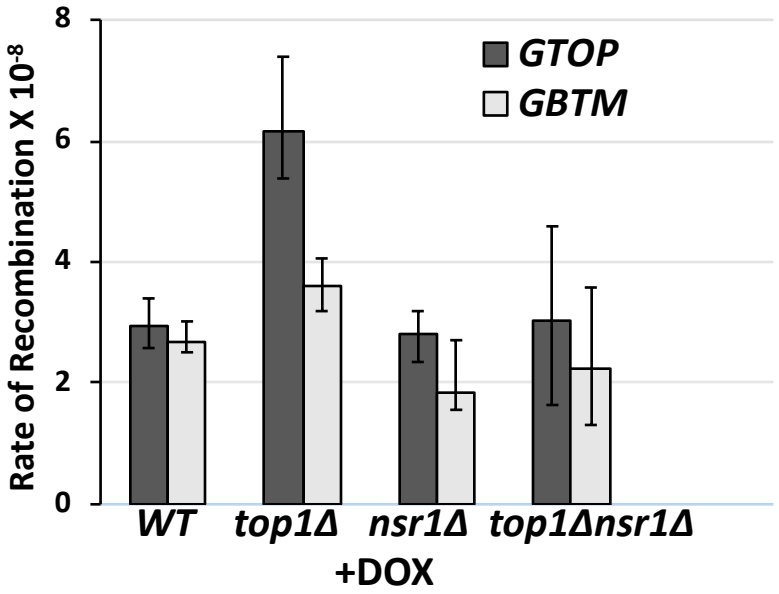

Rate of Recombination X 10-8
8
6
4
2
0
GTOP
GBTM
WT
top1Δ
nsr1Δ
top1Δnsr1Δ
+DOX

Figure S3.

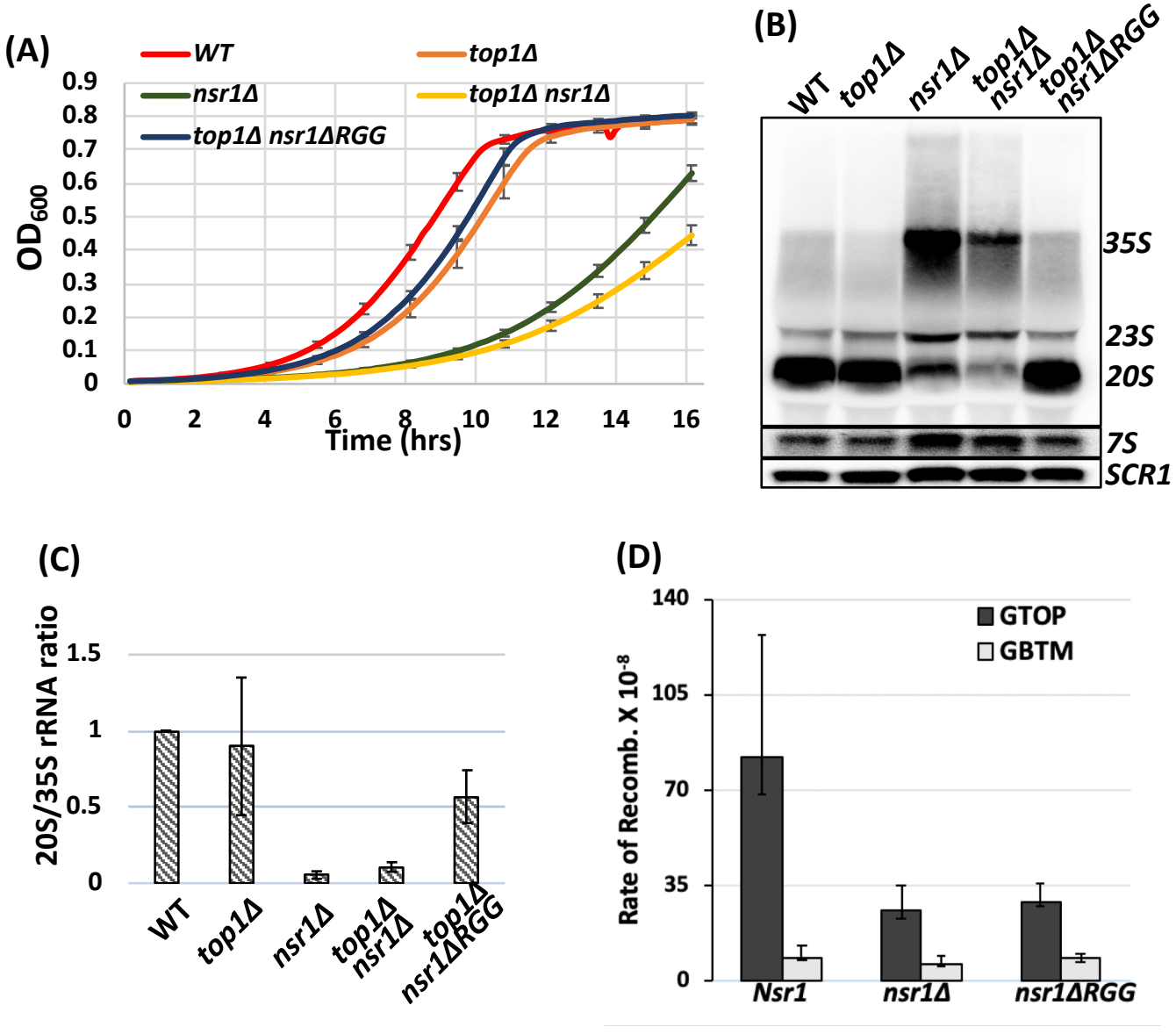
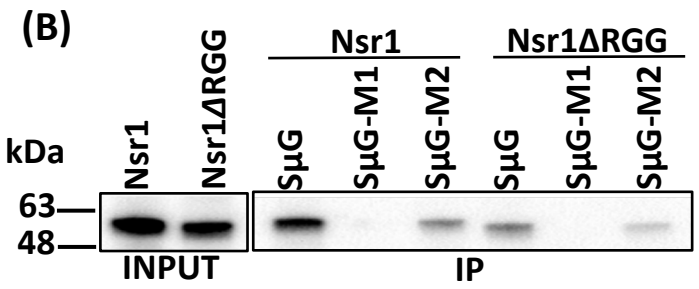

(A)
WT
top1Δ
nsr1Δ
top1Δ nsr1Δ
top1Δ nsr1ΔRGG
OD600
Time (hrs)
(B)
WT
top1Δ
nsr1Δ
top1Δ nsr1Δ
top1Δ nsr1ΔRGG
35S
23S
20S
7S
SCR1
(C)
20S/35S rRNA ratio
WT
top1Δ
nsr1Δ
top1Δ nsr1Δ
top1Δ nsr1ΔRGG
(D)
Rate of Recomb. X 10-8
GTOP
GBTM
Nsr1
nsr1Δ
nsr1ΔRGG

Figure S4.

(A)

SμG :	GAGCTGGGGTGAGCTGGGCTGAGCTGGGGTGAGCTGGGCTGAGCT
SμG-M1:	GAGCTGaGGTGAGCTGGGCTGAGCTGaGGTGAGCTGGGCTGAGCT
SμG-M2:	GAGCTGGGGTGAGCTGaGCTGAGCTGGGGTGAGCTGaGCTGAGCT



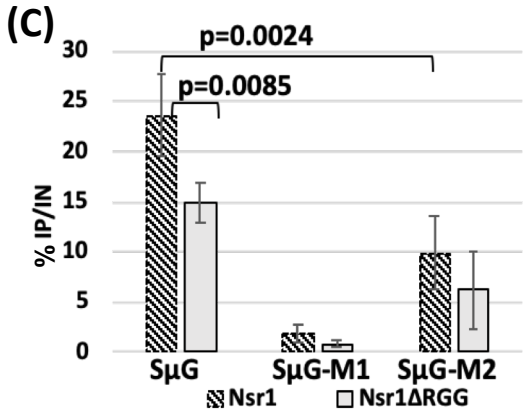
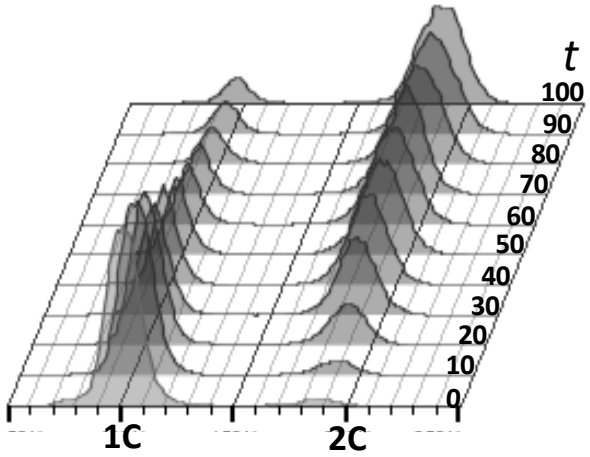
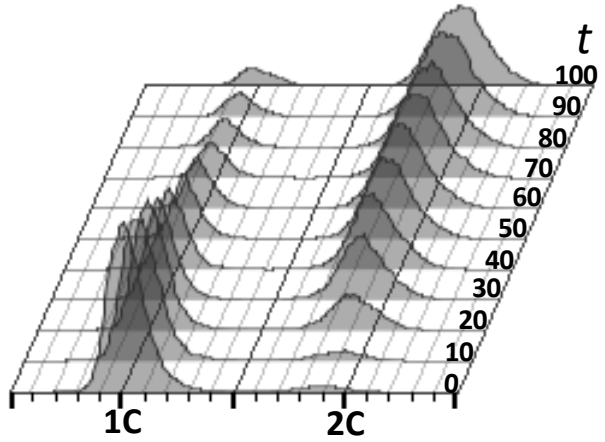


Figure S5.

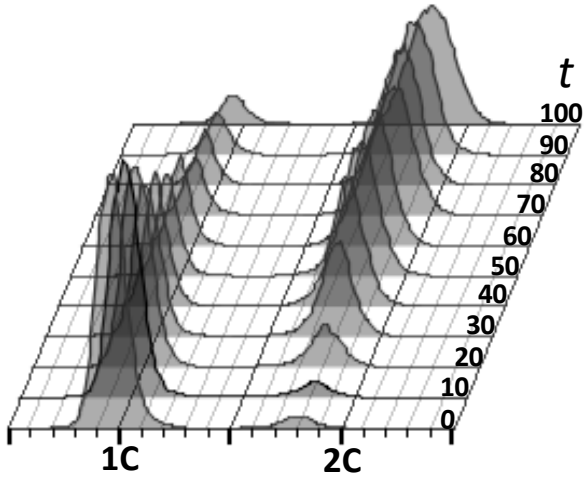
(A) *top1Δ Nsr1 (GTOP)*



(B) *top1Δ nsr1-ΔRGG (GTOP)*



(C) *top1Δ Nsr1(GBTM)*



(D) *top1Δ nsr1-ΔRGG (GBTM)*

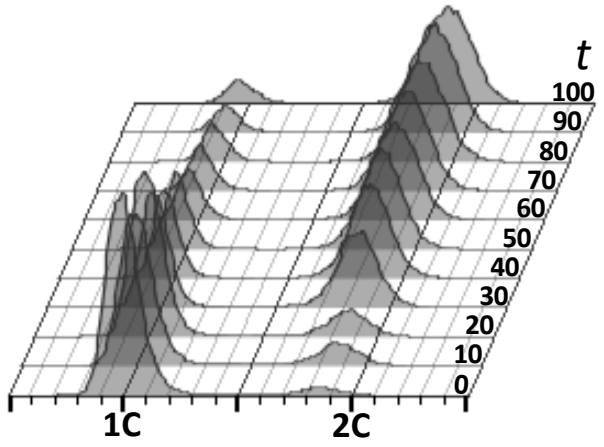
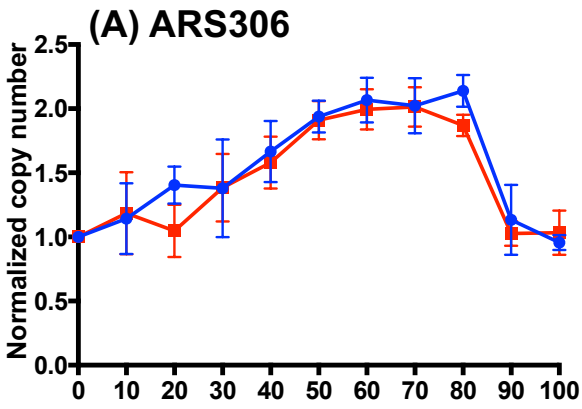
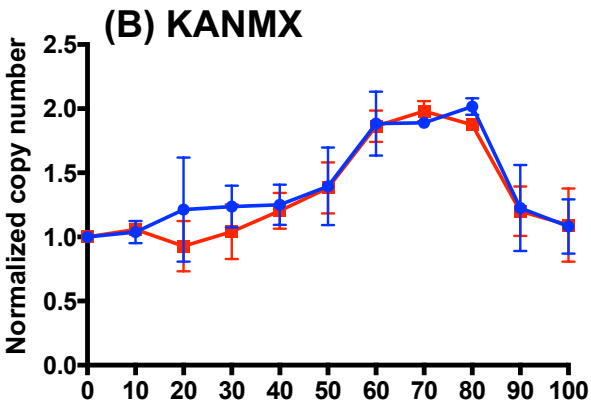
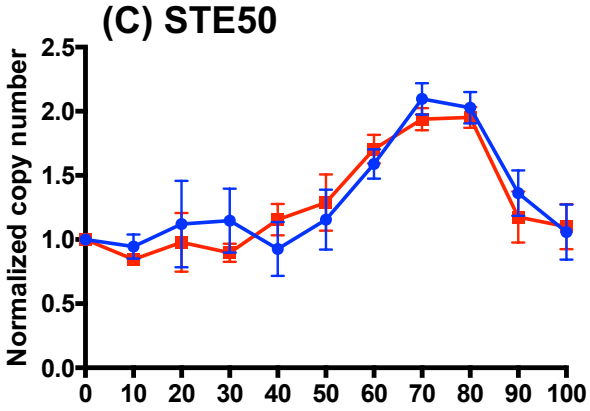


Figure S6.







For (A), (B), and (C) ;

nsr1ΔRGG *NSR1*