E08-05-0547 Bachant

Supplemental Figure 1. Chromosome Bridging in *top2* Mutants. WT (Y300) and *top2-4* (JBY335) strains were synchronized with α-factor, released at 35°C, and α-factor was restored after budding. WT (JBY1083) and *top2-SNM* (JBY1087) strains were processed in a similar manner but released at 30°C. (**A**) Micrographs show DAPI stained images of cells from different time points selected to depict early anaphase in WT cells (i) completion of chromosome segregation in late anaphase WT cells (ii), persistent chromatin stretching in *top2-4* and *top2-SNM* mutants (iii), the *top2-4* Cut^- phenotype (iv), and completed chromosome segregation in *top2-SNM* cells (v). Bar, 5μm. (**B**) Graphs depict kinetics of budding and anaphase chromatin stretching as evaluated by DAPI staining.

Supplemental Figure 2. (**A**) The loss rate of *CFIII URA3 SUP11*, a partially disomic chromosome (Spencer *et al.*, 1990), was determined in *TOP2* (JBY1407-1409; three experiments each), *top2-SNM* (JBY1411-1413; three experiments each), Δ*rad52* (JBY1423-1425; three experiments each) and Δ*rad52top2-SNM* (JBY1427, five experiments; JBY1428, four experiments) *CFIII* cells. (**B**) CFIII loss rate was also measured in Δ*mad2* (JBY1437-1442; two experiments each except 1438 and 1440 which had one experiment each) and Δ*mad2top2-SNM* (JBY1443-1450; two experiments each except 1443 and 1448 which had one experiment each) *CFIII* strains. Dots, individual data points; bars, median values. p values indicate the significance of differences between the indicated data sets as evaluated using Student's t-test.

Supplemental Figure 3. *CEN4-GFP* Separation in *top2-SNM* Mutants. Cells from mid-log phase cultures of *TOP2* (JBY577), *TOP2-HA* (JBY1057) and *top2-SNM-HA* (JBY1082) *CEN4-*

GFP strains were visualized by fluorescence microscopy using a number 4 neutral density filter. Pre-anaphase (*CEN4-GFP* separation ≤ 2 μm) medium to large budded cells were evaluated as having a single GFP dot, separated GFP foci, or *CEN4-GFP* separation with a filamentous aspect to the fluorescence. For each cell in which *CEN4-GFP* separation was observed, the extent to which the labeled chromatin was pulled apart was measured as precisely as possible, given the small distances and oscillation of the foci. Scoring was continued until *CEN4-GFP* distension was measured in 100 cells. (**A**) Images of two discrete foci in *TOP2-HA* cells and stretched GFP signals in the *top2-SNM-HA* strain. Brackets indicate the distance measured in each case. Bar 5 μm. (**B**) Scoring of cells into *CEN4-GFP* morphology categories. (**C**) The distribution of *CEN4-GFP* distension for each strain. p values indicate the significance of differences between the *TOP2-HA* and *top2-SNM-HA* distributions compared to the *TOP2* control.

Supplemental Figure 4. Topoisomer Distributions from Additional *loxCENlox* Excision Experiments. The strains, experimental methods and normalization procedure described in Figure 5 were used to examine loxCENlox topoisomer distributions in the presence of 2 mg/ml chloroquine for *cdc20TOP2*, *cdc20top2-4* and *cdc20top2-SNM* strains in the presence (+NZ) or absence (-NZ) of nocodazole. Overlays of topoisomer profiles derived from two additional experiments in the absence of nocodazole and one additional experiment in the presence of nocodazole are depicted. Blue, *cdc20TOP2* (denoted on graphs as WT); green, *cdc20top2-4*; red, *cdc20top2-SNM*. The overlay graphs shown in Figure 5B (which are from separate experiments) are also included here for comparison (5B). SC; (+) or (-) supercoiled forms. RC/NC; relaxed/nicked circle.

Supplemental Figure 5. Analysis of *loxCENlox* Excision Products under Different Chloroquine Concentrations. *CEN* loop out products were isolated from *cdc20-1* (TWY286) and *cdc20-1top2-4* (TWY258) strains arrested at a non-permissive temperature in the absence of nocodazole (+ tension) as described for Figure 5. Identical genomic DNA samples (20 μg) from these strains were fractionated on gels containing 0, 2, 3, 4, and 5 μg/ml chloroquine and analyzed by Southern blotting. In these cases, electrophoresis was carried out for shorter periods of time (1X TAE running buffer with chloroquine in both the gel and running buffer; 2 ½ hours at 90V) and on smaller gels (10 x 7 cm, 1% agarose, 2 x 0.15 cm combs) than those used in Figure 5. As a result, *CEN* linking number variants are not resolved as clearly as in Figure 5 and Supplemental Figure 4. Samples isolated from *top2-4* cells appear to both relax and re-wind at a lower chloroquine concentration compared to *TOP2* controls.

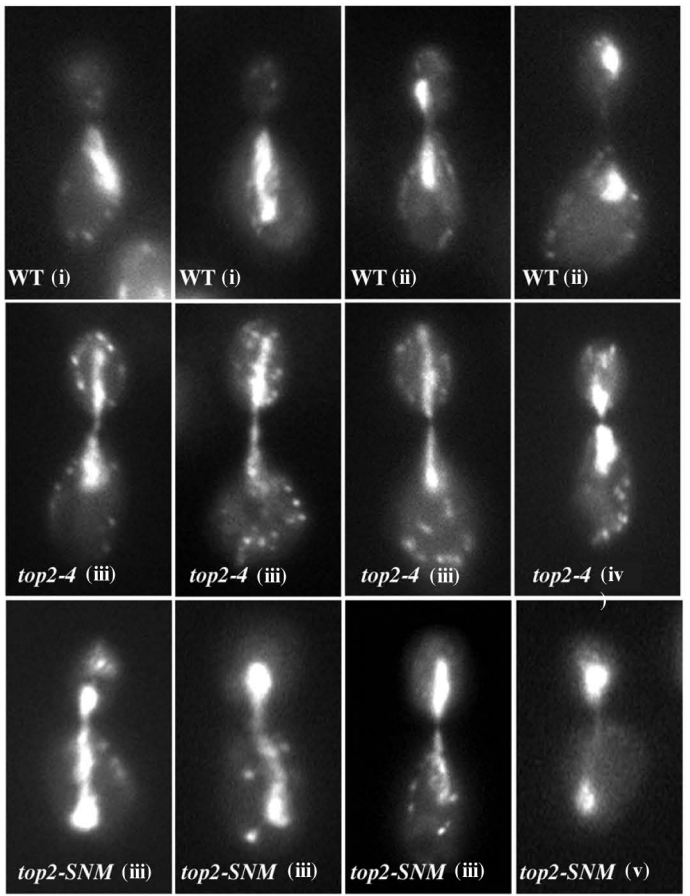
Supplemental Figure 6. Chromosome Segregation in *mtw1top2-SNM* Mutants. (**A**) The loss frequency of the non-essential *CFIII URA3 SUP11* chromosome fragment was determined in WT (JBY1407 and JBY1408; three experiments each), *mtw1-1* (JBY1541 and JBY1542; three experiments each), *mtw1-1top2-SNM* (JBY1548 and JBY1549; three experiments each) cultures grown at 34°C, a semi-permissive temperature for the *mtw1-1* allele. *CFIII* loss was determined by calculating the fraction of 5'-FOA resistant, Ade^- cells. Dots, individual data points; bars, median values. p value indicates the significance of the difference between the *mtw1* and *mtwtop2-SNM* data sets. (**B**) *mtw1-1* (SBY1646, two experiments; JBY1546, two experiments) and *mtw1-1top2-SNM* (JBY1554, two experiments; JBY1555, two experiments) *TRP1-GFP* strains were synchronized with α-factor, released at either 25°C or 35.5°C, and α-factor was restored after budding. At 35.5°, *mtw1-1* mutants exhibit a leaky pre-anaphase delay, and, five

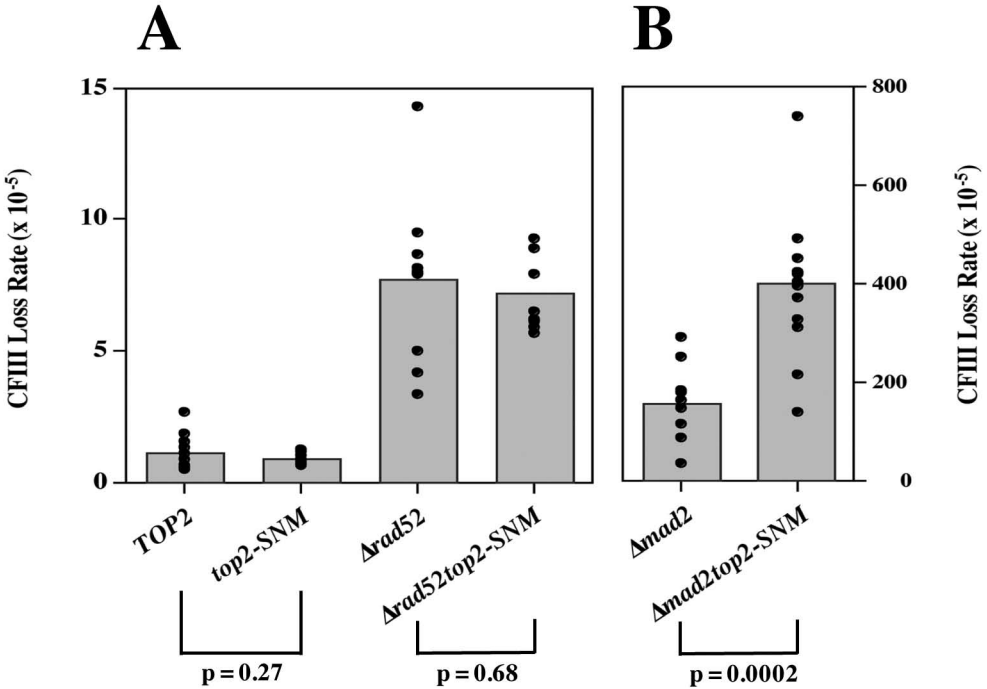
hrs after release, ~90% of *mtw1* and *mtw1top2-SNM* cells at both temperatures had completed mitosis and re-arrested as unbudded cells. These were then evaluated to determine the percentage of unbudded cells with one *TRP1-GFP* focus, indicating correct chromosome segregation, and the percentage exhibiting two foci, revealing a chromosome mis-segregation event. At least 100 cells were scored per sample. Graphs indicate the average and standard deviation of the combined experiments.

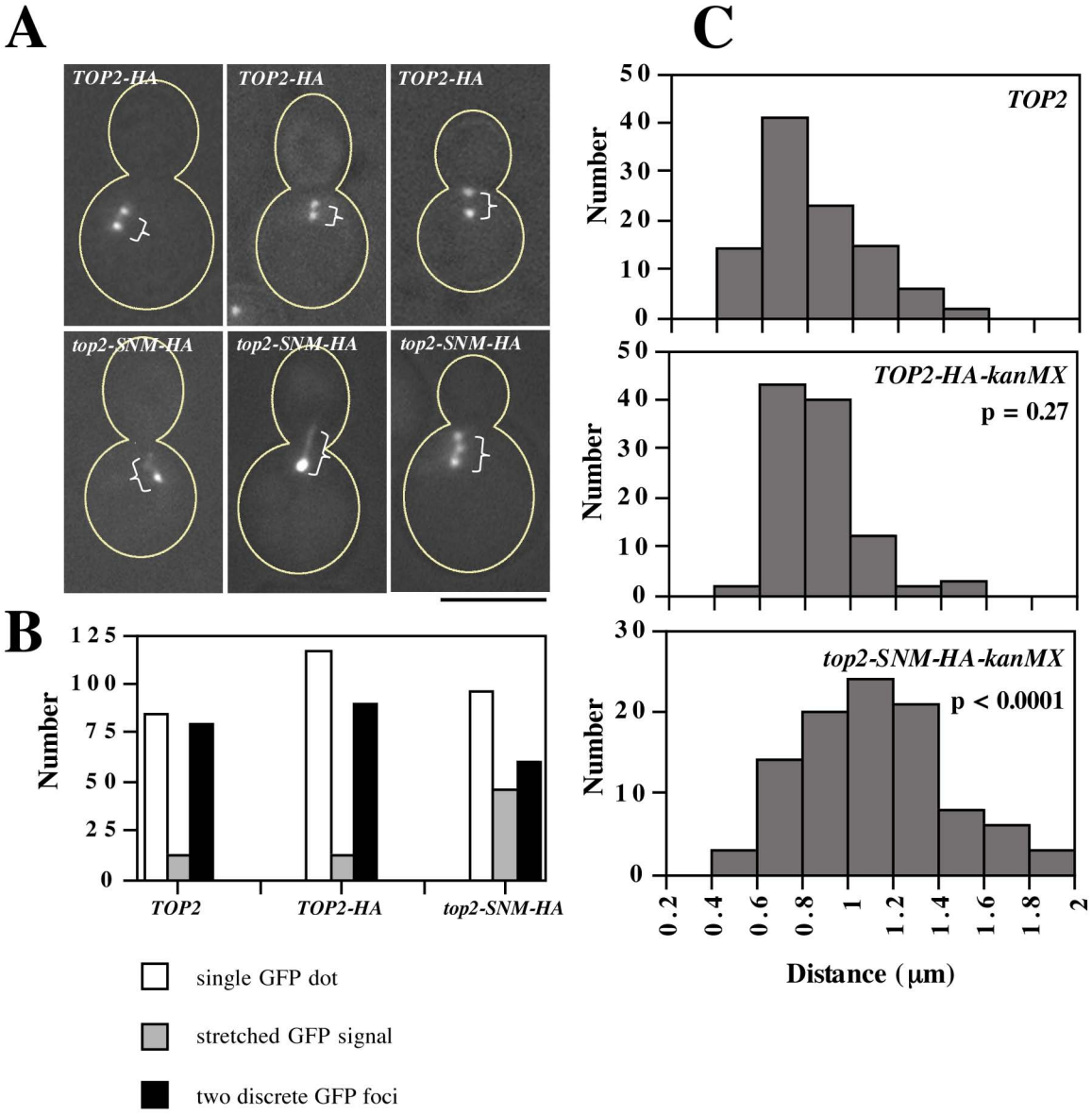
Supplemental Figure 7. Tension Checkpoint Integrity in *scc1top2* Mutants. WT (JBY649), *scc1-73* (JBY1562), *scc1-73ipl1-321* (JBY1570), *scc1-73top2-4* (JBY1569) and *scc1-73top2-SNM* (JBY1565) *PDS1-MYC* strains were released from α-factor at 37°C. α-factor was restored after budding. The percentage of large budded cells or (in separate experiments) nuclear Pds1-myc staining was determined at each time point.

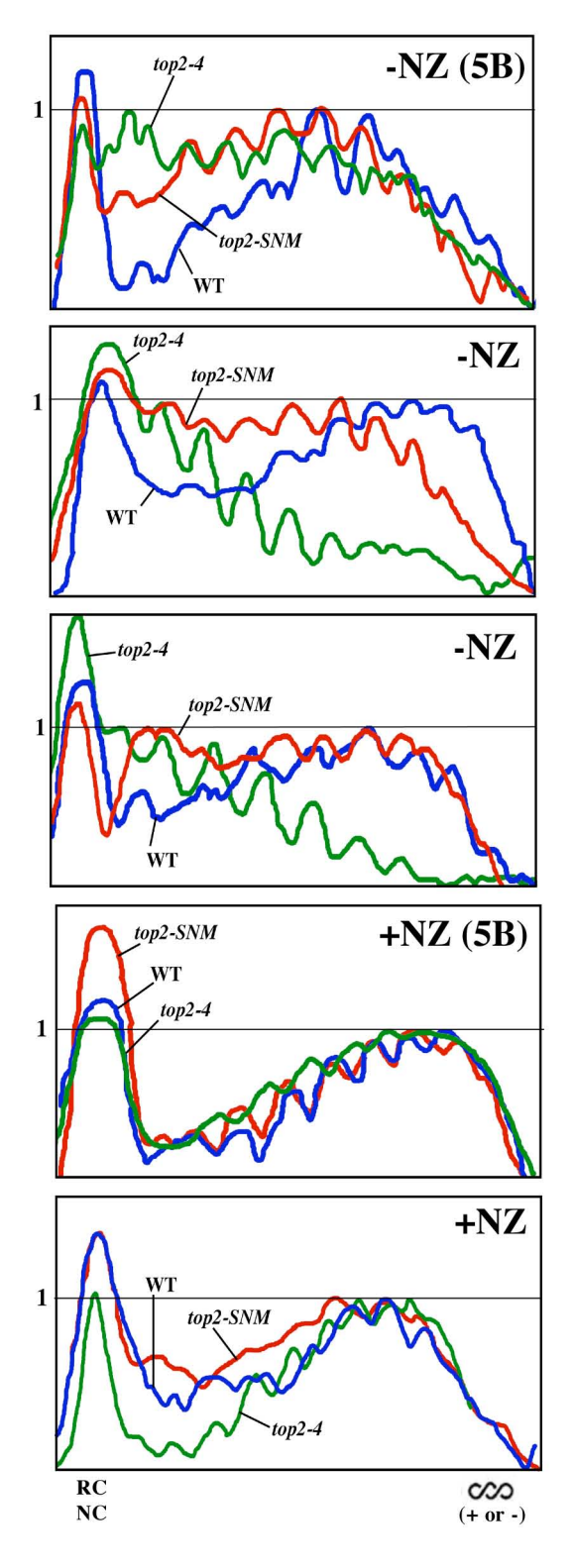
Supplemental Videos 1 and 2. *CEN4-GFP* dynamics in *cdc13* cells (JBY607). The first video shows *CEN4-GFP* stretching in a pre-anaphase arrested cell, followed by successful biorientation and compaction of the separated chromatin into two discrete, spherical GFP foci. This is the same cell depicted in Figure 2C. The second video shows a cell displaying bioriented *CEN4-GFP* foci at the time filming began. One of the *CEN4-GFP* foci can be observed to stretch, rapidly followed by an apparent recompaction of the chromatin. For imaging, cells were arrested at 32° for 2.5 hrs, transferred to agar media pads and placed on a temperature controlled stage at 32°C to maintain arrest. Image stacks were collected at 2 min intervals using 100X objective and a number 4 neutral density filter; each frame represents a maximum projection for each time point. The display rate is 2 frames/s.

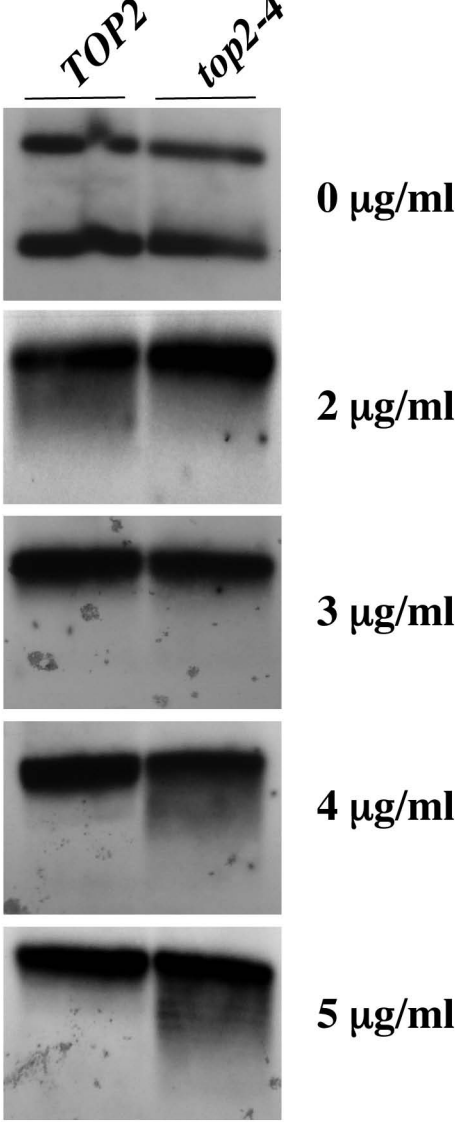
Supplemental Videos 3 and 4. *CEN4-GFP* dynamics in *cdc13top2-SNM* mutants (JBY1100). The first video shows continuous *CEN4-GFP* stretching in a pre-anaphase arrested cell. This is the same cell depicted in Figure 2D. The second video shows two cells displaying stretched configurations of *CEN4-GFP*. Interestingly, towards the latter part of the movie *CEN4-GFP* fibers in the right cell appear to be pulled along different vectors. Cells were imaged and videos prepared as described for Supplemental Videos 1 and 2.

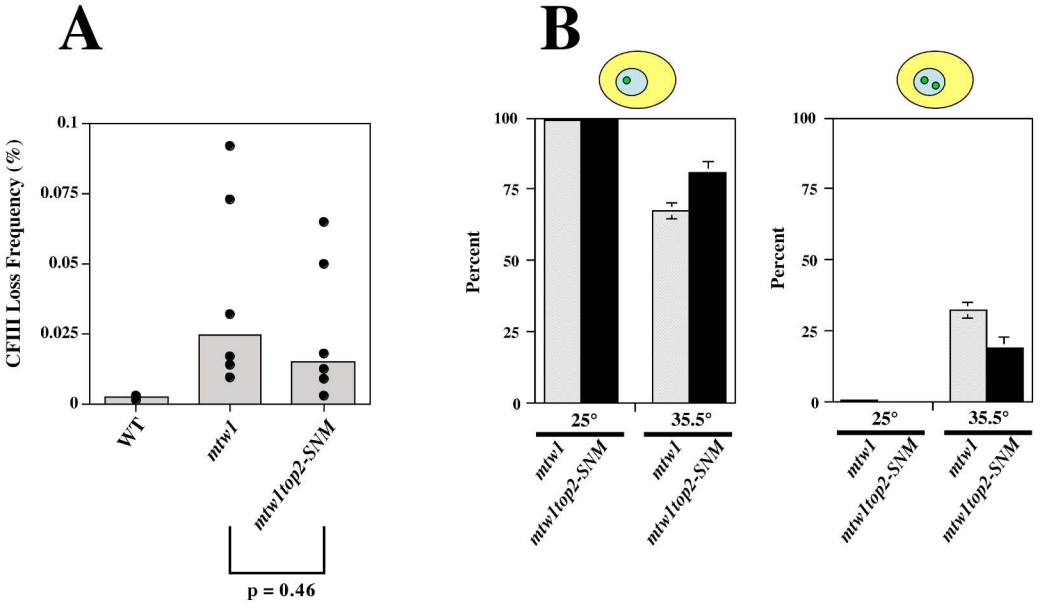

A
WT (i)
WT (i)
WT (ii)
WT (ii)
top2-4 (iii)
top2-4 (iii)
top2-4 (iii)
top2-4 (iv)
top2-SNM (iii)
top2-SNM (iii)
top2-SNM (iii)
top2-SNM (v)
B
Budded Cells
Stretched Anaphase Chromatin
Total Cells Scored (%)
Time (min)
WT
top2-4
top2-SNM

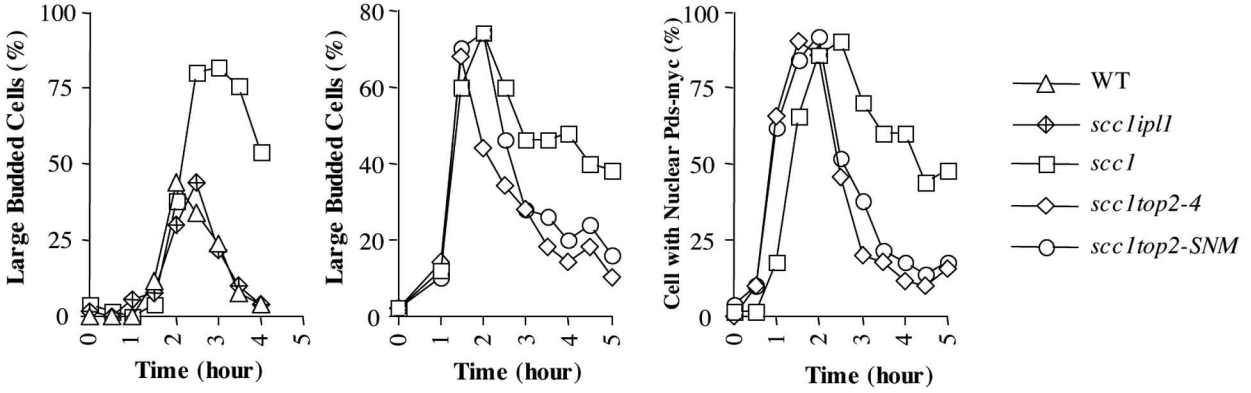

A
B
CFIII Loss Rate (x 10-5)
15
10
5
0
800
600
400
200
0
TOP2
top2-SNM
Δrad52
Δrad52top2-SNM
Δmad2
Δmad2top2-SNM
p = 0.27
p = 0.68
p = 0.0002


A
TOP2-HA
TOP2-HA
TOP2-HA
top2-SNM-HA
top2-SNM-HA
top2-SNM-HA
B
Number
125
100
75
50
25
0
TOP2
TOP2-HA
top2-SNM-HA
single GFP dot
stretched GFP signal
two discrete GFP foci
C
TOP2
TOP2-HA-kanMX
p = 0.27
top2-SNM-HA-kanMX
p < 0.0001
Number
50
40
30
20
10
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
Distance (μm)


-NZ (5B)
top2-4
1
top2-SNM
WT
-NZ
top2-4
top2-SNM
1
WT
-NZ
top2-4
top2-SNM
1
WT
+NZ (5B)
top2-SNM
WT
1
top2-4
+NZ
1
WT
top2-SNM
top2-4
RC
NC
(+ or -)


TOP2
top2-4
0 μg/ml
2 μg/ml
3 μg/ml
4 μg/ml
5 μg/ml


A
CFIII Loss Frequency (%)
0.1
0.075
0.05
0.025
0
WT
mtw1
mtw1top2-SNM
p = 0.46
B
Percent
100
75
50
25
0
25°
35.5°
mtw1
mtw1top2-SNM


Large Budded Cells (%)
100
75
50
25
0
0 1 2 3 4 5
Time (hour)
Large Budded Cells (%)
80
60
40
20
0
0 1 2 3 4 5
Time (hour)
Cell with Nuclear Pds-myc (%)
100
75
50
25
0
0 1 2 3 4 5
Time (hour)
WT
scc1ipl1
scc1
scc1top2-4
scc1top2-SNM