

**Supplemental material ; Varela et al.**

**Supplemental Movie 1**

Strain GA-3604 (*BRN1-GFP pNOP1-CFP*) was grown at 30°C on SC medium to reach  $4 \times 10^6$  cells/ml. Progression through anaphase was analyzed by live microscopy at 10s intervals on a Zeiss LSM510 confocal microscope as described in Bystricky et al., 2005.

**Supplemental Movie 2**

Strain GA-2663 (*BRN1-GFP*) was grown at 30°C on SC and imaged by time-lapse microscopy using the Zeiss LSM510 as for Supplemental movie 1.

**Figure S1. Brn1 and Nop1 spiral during chromosome segregation.**

A) IF performed on exponentially growing cultures of strain GA-1656 (*brn1::BRN1-13 myc-KanMX6* in GA-180), with anti-Myc (green) and anti-Nop1 (red) antibodies to identify the nucleolus. Bar = 5µm.

B) Selected frames from a Zeiss LSM510 confocal time-lapse series of GA-3043 (*BRN1-GFP* (green), *CFP-TUB1* (red)) yeast cells as they progress from G2 to late mitosis. Imaging was performed as described for Movie1. Bar = 5µm.

C) and D) Selected frames from a Zeiss LSM510 confocal time-lapse series of yeast strain GA-3604 (*BRN1-GFP pNOP1-CFP*) as they progress from G2 to late mitosis (see movie 1). They illustrate the spiral movement of Brn1. Nop1 is not DNA bound and the spiral is less distinct in still images. Bar = 5µm.

E) IF as in A with anti-Myc (green) and anti-Top2 (red) on strain GA1656. The yellow signal shows the Brn1-Topo II colocalization. 3D image stacks were deconvolved and the two patterns were reconstructed in 3D using Imaris (Bitplane). Bar = 5µm.

**Figure S2. Brn1 stain is compact in the *net1-1* mutant**

A) Live microscopy of late G2-phase and anaphase cells from exponentially growing cultures of strains GA-3604 (*BRN1-GFP, pNOP1-CFP*) and GA-3266 (*net1-1 BRN1-GFP, pNOP1-CFP*) are shown. Cells were grown at 23 °C. Brn1 is hypercondensed in both G2 and anaphase and the rDNA appears to show early segregation.

B) Quantitation of phenotypic distribution of Brn1-GFP in interphase cells from exponentially growing cultures of strains GA-3604 (*BRN1-GFP*, *pNOP1-CFP*), GA-3266 (*net1-1 BRN1-GFP*, *pNOP1-CFP*) and GA-3350 (*sir2Δ BRN1-GFP*, *pNOP1-CFP*). Cells were grown at 23°C and incubated at 30°C for 2h before analysis by confocal Zeis LSM510. Cell morphology was used to differentiate G1 (no bud) from S phase (budded). A minimum of 100 cells was scored for each condition and strain. Examples of diffuse, semi.diffuse and compact Brn1 distributions are shown in for Brn1-GFP fluorescence to the right.

### **Figure S3. Brn1 belongs to the Condensin complex**

A) Western blot analysis of co-immunoprecipitates from asynchronous cultures of strains expressing the endogenous Brn1-13myc and Smc1-HA or Smc2-HA. Anti- myc (9E10) or HA (12CA5) antibodies were used. We recovered Brn1-13myc from whole cell extracts by IP and probed for Smc1 and Smc2 among the co-precipitating proteins. We detected strong association of Brn1-13Myc with Smc2, but not with Smc1. Similarly, Brn1 was recovered with HA-Smc2, and not HA-Smc1 by reverse IP, indicating that Brn1 belongs to Condensin and not Cohesin complex.

B) Lte1-HA distribution through the cell cycle. Exponentially growing cultures of strain GA-2975 (*LTE1-3HA BRN1-13Myc*) were fixed and analyzed by IF with anti-HA antibody. Micrographs show representative images and a control with no primary antibody on the right. Bars= 5µm.

### **Figure S4. Analysis of telophase arrest in a *cdc15-2* strain**

A) Strain GA-3305 (*cdc15-2 BRN1-GFP*, *CFP-TUB1*) was arrested in metaphase using nocodazole at the permissive temperature. Cells were released at 37°C and samples were analyzed at the indicated time-points. The graph shows progression to the telophase arrest judged by the presence of long spindles.

B) Western blot showing samples taken from the culture in A at the timepoints indicated, probed for Cyclin B (Clb2), Tubulin (Tub1) and Sic1. A low level of Clb2 persists and Sic1 is unstable, consistent with an anaphase arrest.

**Supplemental Table 1 Yeast strains used in this study**

GA-180	MAT a <i>ade2-1 trp1-1 his3-11,15 ura3-1 leu2-3, 112 can1-100</i>	R. Rothstein (W303-1A)
GA-1656	<i>brn1::BRN1-13 myc-KanMX6</i> in GA-180	This work
GA-2663	<i>brn1::BRN1-GFP-KanMX6</i> in GA-180	This work
GA-2975	<i>brn1::BRN1-GFP-KanMX6 lte1::LTE1-3HA-URA3</i> in GA-180	
GA-3043	<i>lte1::CaURA brn1::BRN1-GFP-KanMX6 trp1-1::TRP1-CFP-TUB1</i> in GA-180	This work
GA-3045	<i>brn1::BRN1-13 myc-KanMX6 lte1::LTE1-3HA GFP-Tub1::URA3</i>	This work
GA-3263	<i>brn1::BRN1-GFP-KanMX6 ade2::ADE2-CFP-TUB1</i> in GA-180	This work
GA-3265	<i>cdc15-2 brn1::BRN1-GFP-KanMX6 ade2::ADE2-CFP-TUB1</i> in GA-180	This work
GA-3266	<i>net1-1 brn1::BRN1-GFP-KanMX6 pNOP1-CFP-ADE</i> in GA-180	This work
GA-3305	<i>brn1::BRN1-GFP-KanMX6 ade2-1::ADE2::CFP-TUB1</i> in GA-180	This work
GA-3327	<i>lte1::CaURA3</i> in GA-180	This work
GA-3330	<i>brn1::BRN1-GFP-KanMX6 trp1::TRP1-CFP-TUB1 pNOP1-CFP-ADE</i> in GA-180	This work
GA-3350	<i>brn1::BRN1-GFP-KanMX6 trp1::TRP1-CFP-TUB1 sir2::HIS3 pNOP1-CFP-ADE</i> in GA-180	This work
GA-3596	<i>lte1::CaURA brn1::BRN1-13 myc-KanMX6 ura3-1::URA3-GFP-TUB1 pNOP1-CFP-ADE</i> in GA-180	This work
GA-3599	<i>BRN1-13 myc-KanMx ura3-1::URA3-GFP-TUB1 pNOP1-CFP-ADE</i> in GA-180	This work
GA-3600	<i>BRN1-13myc-KanMx ura3-1::URA3-GFP-TUB1 cdc14::CDC14-3HA-CaURA3</i> in GA-180	This work
GA-3601	<i>lte1::CaURA brn1::BRN1-13myc-KanMx ura3-1::URA3-GFP::TUB1 cdc14::CDC14-3HA-CaURA3</i> in GA-180	This work
GA-3604	<i>brn1::BRN1-GFP-KanMX6 pNOP1-CFP-ADE</i>	This work
GA-3716	<i>brn1::BRN1-GFP-KanMX6 trp1-1::TRP1-CFP-TUB1</i> backcrossed twice to W303	This work
GA-3717	<i>tem1-1 brn1::BRN1-GFP-KMX6 trp1-1::TRP1-CFP-TUB1</i> crossed twice with W303	This work
GA-3779	<i>MAS1::lacO-URA3 ACS2::tetO-LEU2 ade2::HISp-CFP-LACI-URAp-TETR-YFP-ADE2</i> in GA-180	This work
GA-4114	<i>net1-1 MAS1::lacO-URA3 ACS2::tetO-LEU2 ade2::HISp-CFP-LACI-URAp-TETR-YFP-ADE2</i> in GA-180	This work
GA-3819	<i>cdc14-1 brn1::BRN1-GFP-kanMX6 ade2-1::ADE2::CFP-TUB1</i> in GA-180	This work
GA-4383	<i>brn1::BRN1-GFP-KMX6 trp1-1::TRP1-CFP-TUB1</i>	This work
GA-4864	<i>lte1::CaURA bub2::KanMX6 brn1::BRN1-GFP-KanMX6 trp1-1::TRP1-CFP-TUB1</i> in GA-180	This work
GA-4931	<i>Mat α cdc15-2 cdc28-as1-URA3 brn1::BRN1-13myc-KanMx</i> in GA180	This work
GA-5227	<i>net1::NET1-GFP-TRP1</i> (W303-1A background)	F. Uhlmann
GA-5228	<i>cdc15-2 net1::NET1-GFP-TRP1</i> (W303-1A background)	This work

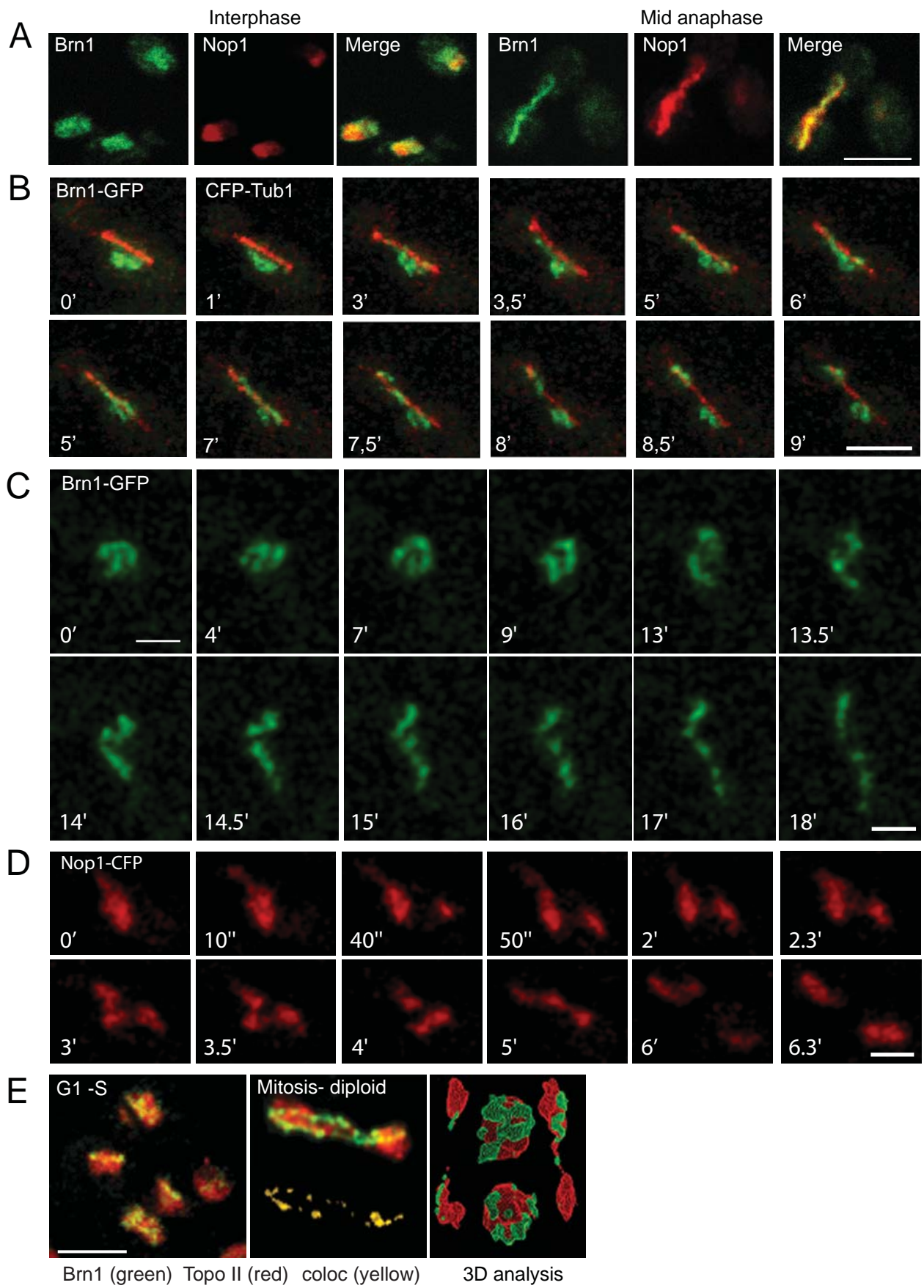


Figure S1, Varela et al.

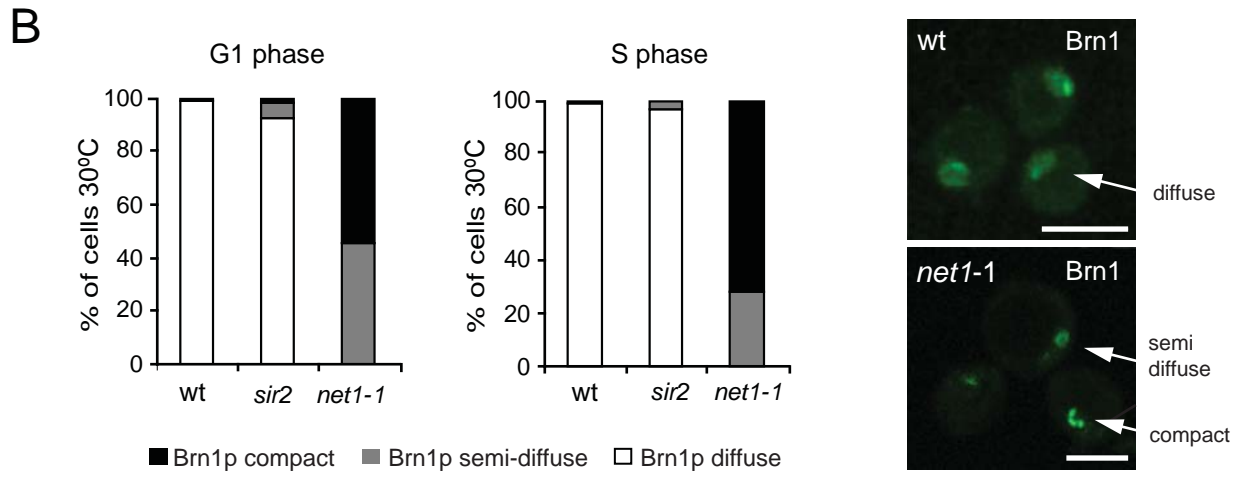
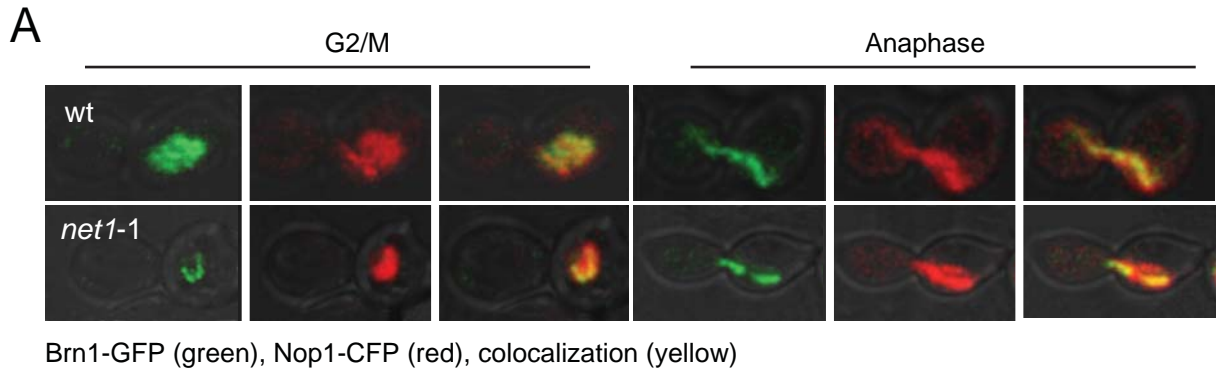


Figure S2, Varela et al.

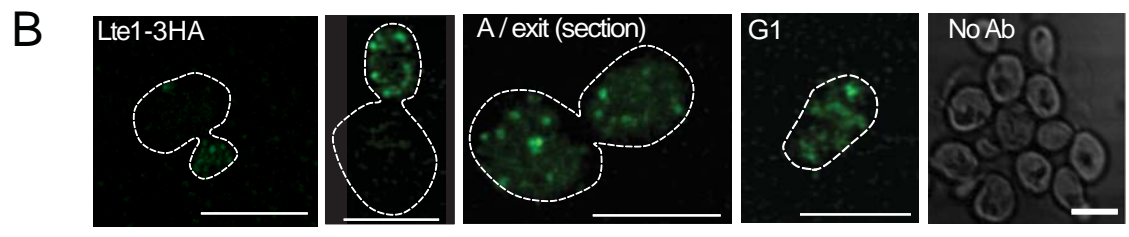
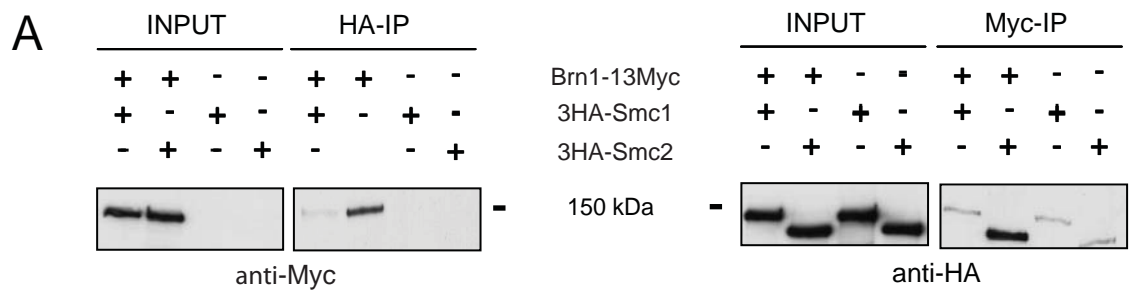


Figure S3, Varela et al

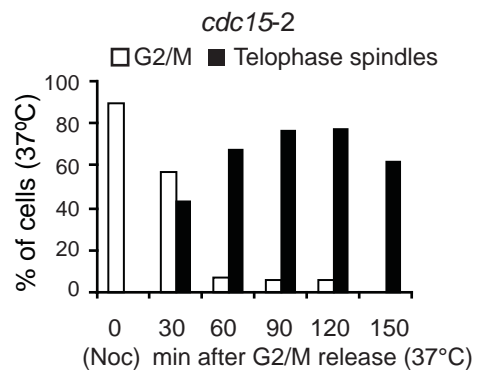
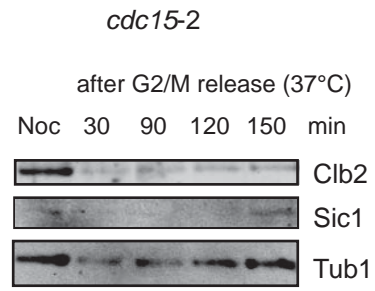
**A****B**

Figure S4, Varela et al.