

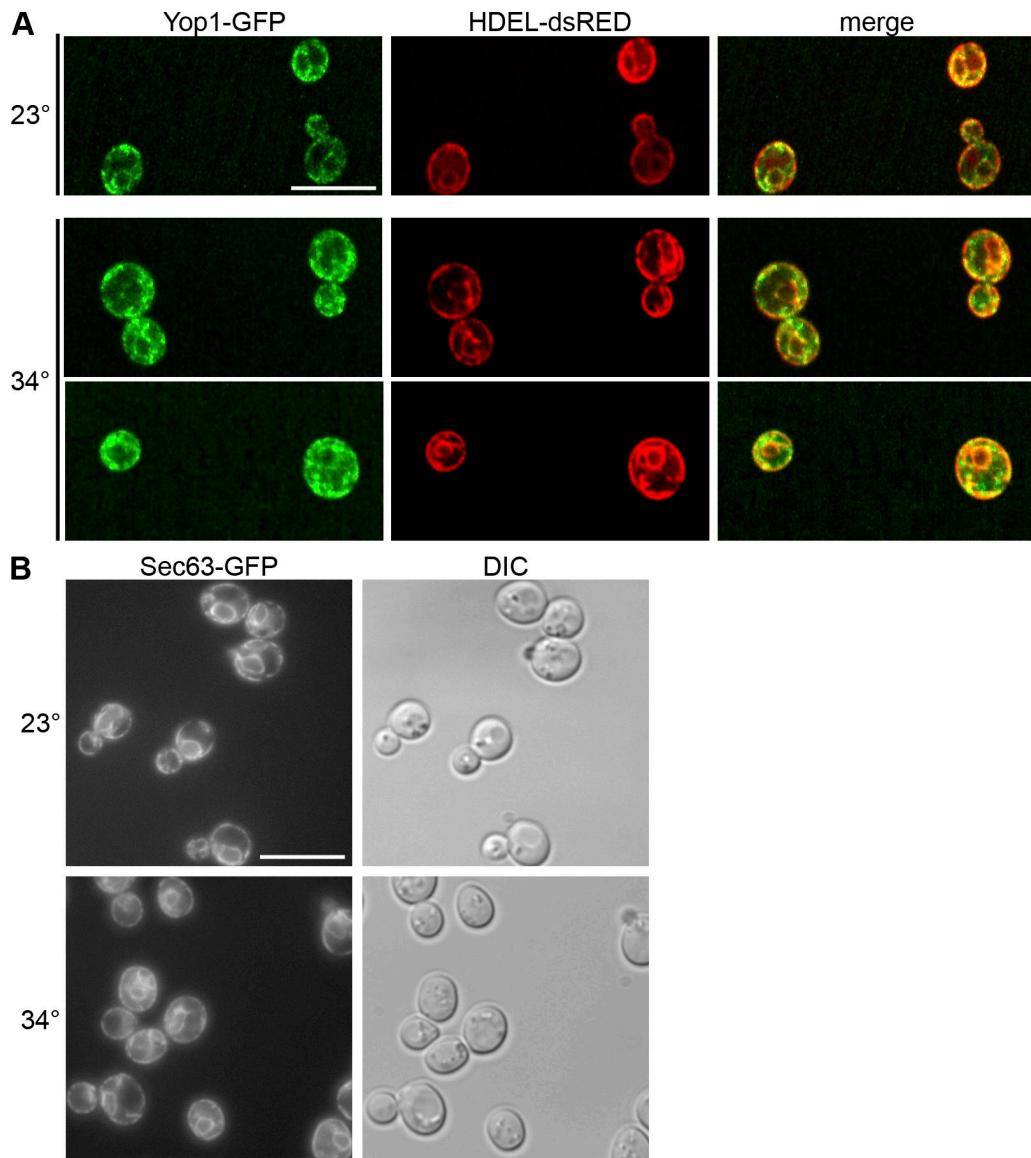
Dawson et al., <http://www.jcb.org/cgi/content/full/jcb.200806174/DC1>

Figure S1. Analysis of ER protein localization in *prp20-G282A* mutant cells. (A) Yop1-GFP mislocalizes in the *prp20-G282A* assembly mutant. Yop1-GFP and dsRed-HDEL localization in the *prp20-G282S* mutant background was visualized by direct laser-scanning confocal microscopy. The *prp20-G282S* *yop1-GFP* mutant (SWY4051) was grown to early log phase at 23°C and then shifted to 34°C for 5 h. Single-channel fluorescence for each protein is shown for each representative cell at each temperature, and the two-channel merged images are shown (right). (B) Sec63-GFP is not perturbed in the arrested *prp20-G282A* cells. The *prp20-G282A sec63-GFP* cells (SWY4116) were shifted to growth at 34°C for 5 h and visualized by direct fluorescence microscopy. DIC, differential interference contrast. Bars, 10 μm.

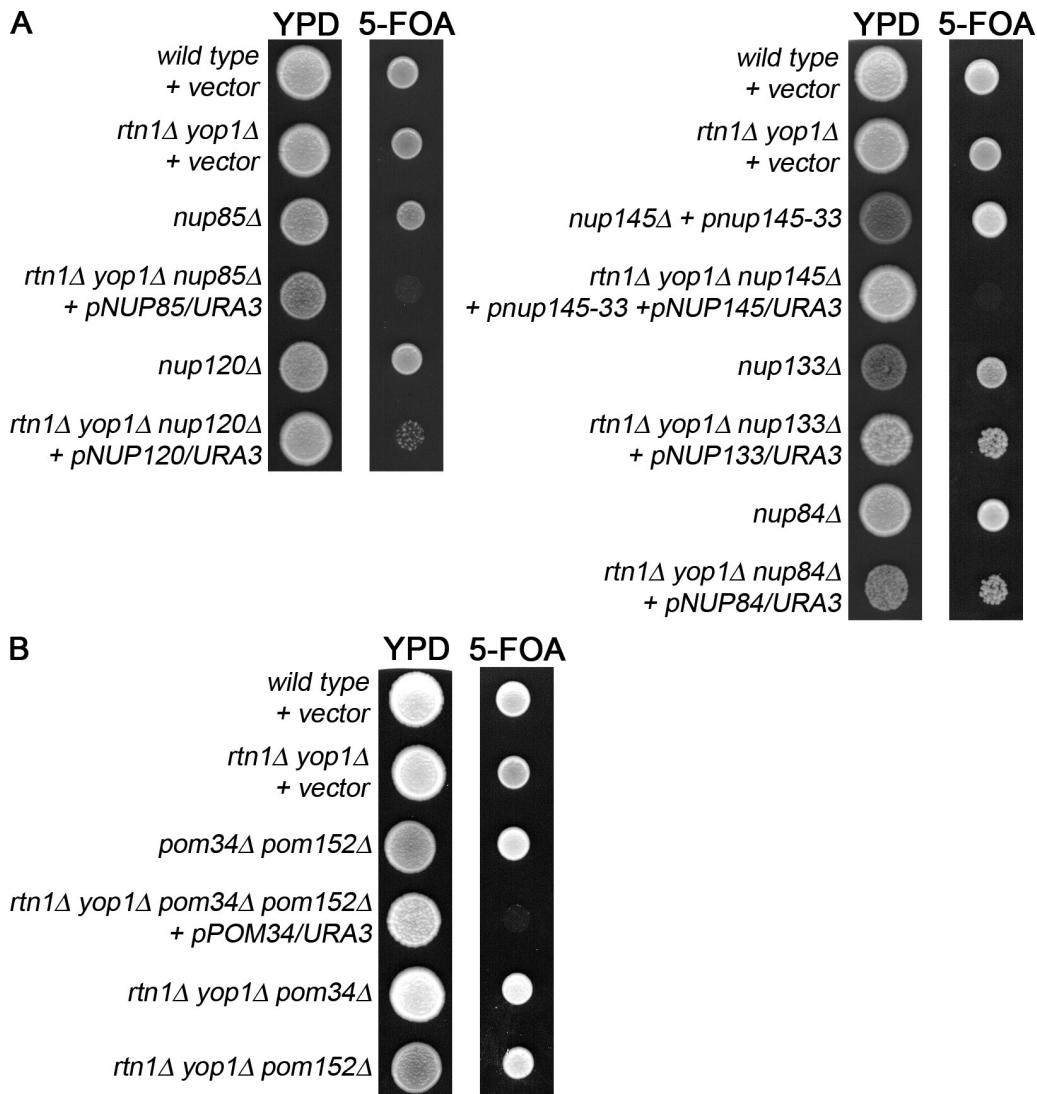


Figure S2. Analysis of yeast strain growth for the *rtn1Δ yop1Δ* double deletion combined with *nup* or *pom* mutants. (A) Mutants in genes encoding yNup84 subcomplex members genetically interact with the *rtn1Δ yop1Δ* mutant. The viable *rtn1Δ yop1Δ* mutant was combined with mutations in yNup84 subcomplex members, and growth was assayed on 5-FOA media. Haploid strains bearing the designated chromosomal deletions and harboring the indicated URA3/CEN plasmid (SWY3811 + pRS316, SWY3876 + pSW3354, SWY3877 + pSW1079, SWY4050 + pSW351, SWY4099 + pSW610, and SWY4106 + pSW3459) were tested for growth compared with the respective parental strains on 5-FOA media at 23°C for 5 d. (B) An *rtn1Δ yop1Δ pom34Δ pom152Δ* quadruple-null strain is inviable. The haploid strain bearing *rtn1Δ yop1Δ pom34Δ pom152Δ* chromosomal deletions and harboring a POM34 URA3/CEN plasmid (SWY3878 with pSW1516) was tested for growth on 5-FOA media at 23°C for 5 d and compared with the indicated triple mutants and parental strains.

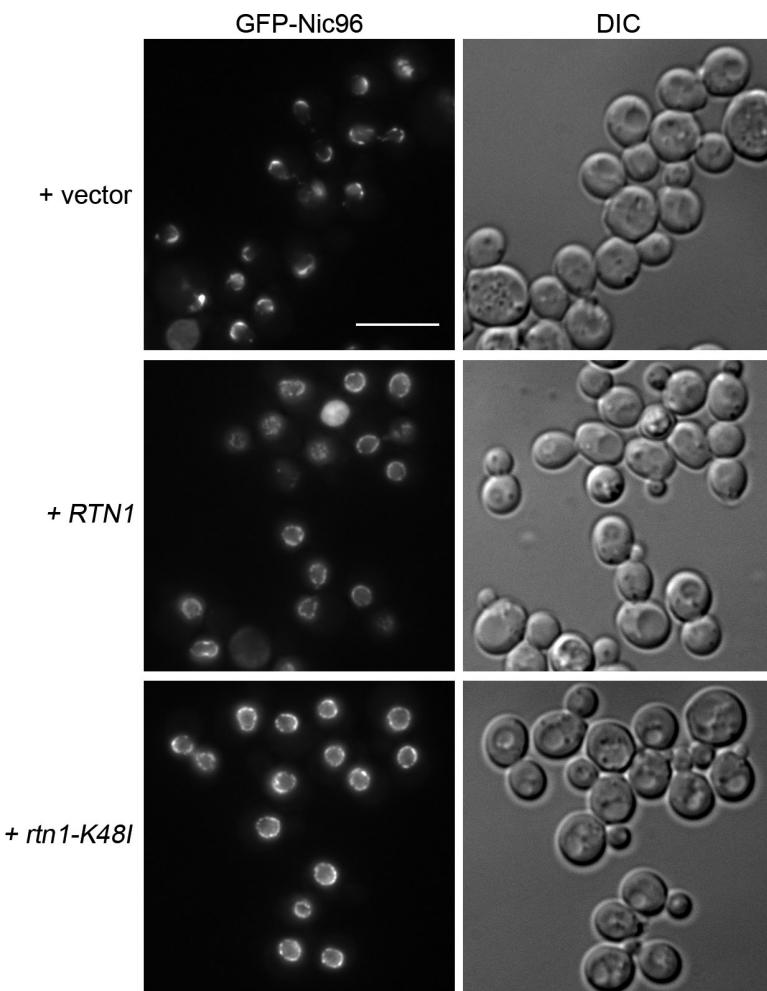


Figure S3. Exogenous expression of *RTN1* or *rtn1-K48I* rescues the *rtn1Δ yop1Δ Nup* mislocalization phenotype. The *rtn1Δ yop1Δ GFP-nic96* strain (SWY3931) was transformed with *LEU2* plasmids expressing *RTN1* (pSW3422) or *rtn1-K48I* (pSW3423). Direct fluorescence microscopy was conducted to evaluate GFP-Nic96 localization to NPC clusters. DIC, differential interference contrast. Bar, 10 μ m.

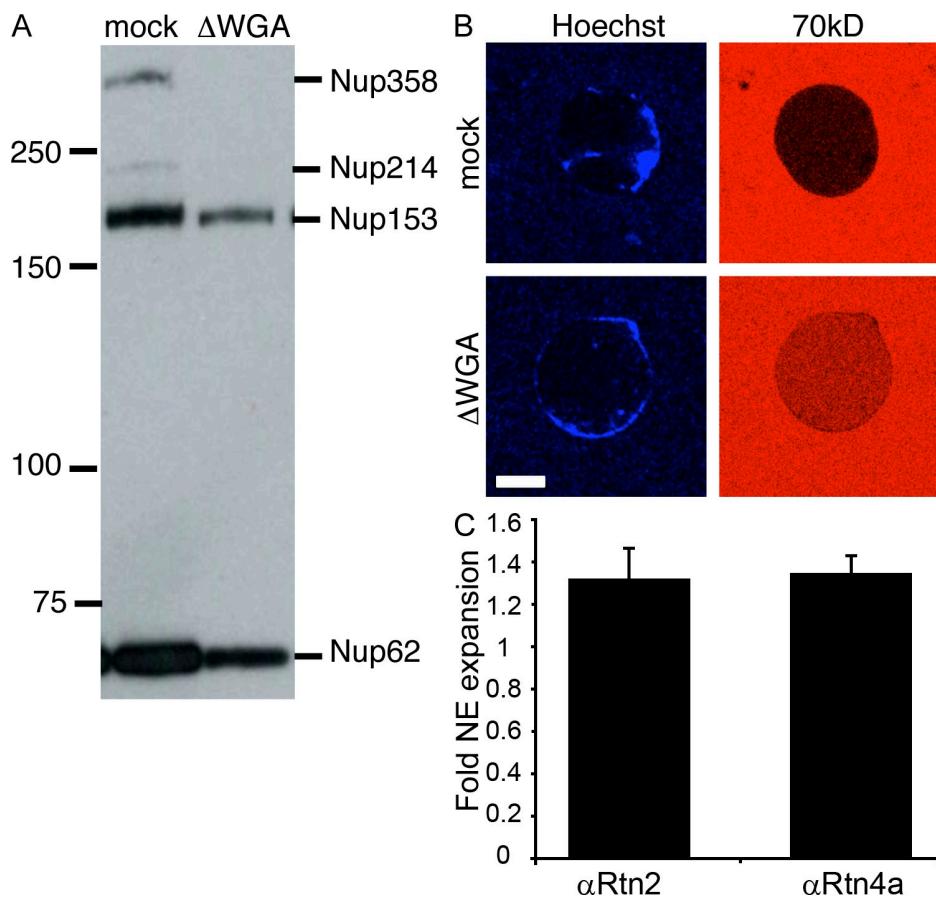


Figure S4. *Xenopus in vitro assembly assays.* (A and B) Immunodepletion of glycosylated Nups by WGA. (A) *Xenopus* cytosol was incubated with buffer (mock) or biotinylated WGA (Δ WGA) for 20 min, passed three times over streptavidin agarose beads, and analyzed by Western blotting using mAb414. Molecular mass is indicated in kilodaltons. (B) Sperm chromatin and membranes were incubated with mock- or WGA-depleted cytosol for 60 min. Fluorescently labeled 70-kD dextran was added to the reaction and imaged immediately using confocal microscopy. (C) NE expansion during assembly. Preassembled nuclei were incubated with extracts containing anti- (α) -Rtn2 and anti- (α) -Rtn4a antibodies for 20 min and imaged live by confocal microscopy using the fluorescent membrane dye DiIC₁₆(3). The NE surface area of nuclei at t = 0 and t = 20 min was calculated from the diameter of >50 spherical nuclei and plotted as fold increase from t = 0 (D'Angelo, M.A., D.J. Anderson, E. Richard, and M.W. Hetzer. 2006. *Science*. 312:440–443). Error bars represent standard deviation. Bar, 10 μ m.

Table S1. Yeast strains used in this study

Strain	Genotype	Source
BY4742	<i>MATα ura3-52 his3Δ200 ade2-101 lys2-801</i>	Mortimer and Johnston, 1986
W303	<i>MATα/MATα ade2-1/ade2-1 ura3-1/ura3-1 his3-11,15/his3-11,15 trp1-1/trp1-1 leu2-3,112/leu2-3,112 can1-100/can1-100</i>	Thomas and Rothstein, 1989
AGY908	<i>MATα rat9(nup85)::HIS3 ura3-52 leu2Δ1 trp1Δ63 his3Δ200</i>	Goldstein et al., 1996
KBY52	<i>MATα nup1::LEU2 trp1 lys2 ura3 ade2 ade3 SNP1 + pLDB73</i>	Belanger et al., 1994
LGY103	<i>MATα nup159-1 (rat7-1) (t.s.) ura3-52 trp1Δ63 leu2Δ1</i>	Gorsch et al., 1995
LD463	<i>MATα nup82-3 (nle4-1) (t.s.) ura3 trp1 ade2 his3</i>	Kenna et al., 1996
nic96-1	<i>MATα HIS3::nic96 ade2 trp1 leu2 ura3 his3 + pUN100-LEU2-nic96-1 (t.s.)</i>	Zabel et al., 1996
NP170-11.1	<i>MATα nup170-1::HIS3 ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</i>	Aitchison et al., 1995
NP188-2.2	<i>MATα nup188-1::HIS ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</i>	Aitchison et al., 1995
PMY17	<i>MATα pom152-2::HIS3 ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</i>	Wozniak et al., 1994
yDBK166	<i>MATα brr6::HIS3/brr6-1:LEU2 ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112</i>	de Bruyn Kops and Guthrie, 2001
gle2 ⁻	<i>MATα gle2::his3 ade2 his3 leu2 trp1 ura3</i>	Bailer et al., 1998
Rtn1-GFP ^a	<i>MATα rtn1-GFP:HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Huh et al., 2003
Yop1-GFP ^a	<i>MATα yop1-GFP:HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Huh et al., 2003
Sec63-GFP ^a	<i>MATα yop1-GFP:HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Huh et al., 2003
nem1Δ ^b	<i>MATα nem1::Kan^r his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Winzeler et al., 1999
nup2Δ ^b	<i>MATα nup2::Kan^r his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Winzeler et al., 1999
nup53Δ ^b	<i>MATα nup53::Kan^r his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Winzeler et al., 1999
nup59Δ ^b	<i>MATα nup59::Kan^r his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Winzeler et al., 1999
nup60Δ ^b	<i>MATα nup60::Kan^r his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Winzeler et al., 1999
nup84Δ ^b	<i>MATα nup84::Kan^r his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Winzeler et al., 1999
nup120Δ ^b	<i>MATα nup120::Kan^r his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Winzeler et al., 1999
rtn1Δ ^b	<i>MATα rtn1::Kan^r his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Winzeler et al., 1999
rtn1Δ ^b	<i>MATα rtn1::Kan^r his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Winzeler et al., 1999
yop1Δ ^b	<i>MATα yop1::Kan^r his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Winzeler et al., 1999
SWY122	<i>MATα nup145ΔN(nup63-7)::LEU ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</i>	Wente and Blobel, 1994
SWY201	<i>MATα nup100::HIS3 ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112</i>	This study
SWY389	<i>MATα nup145::LEU2 ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 + pSW190</i>	Emtage et al., 1997
SWY423	<i>MATα nup133::HIS3 ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</i>	Bucci and Wente, 1998
SWY424	<i>MATα nup133::HIS3 ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</i>	Bucci and Wente, 1998
SWY519	<i>MATα ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 ade2-1::ADE2:ura3</i>	Bucci and Wente, 1997
SWY809	<i>MATα nup49ΔGLFG-GFP:TRP1 nup49-1::URA3 trp1-1 ura3-1 his3-11,15 leu2-3,112 can1-100 ade2-1::ADE2:ura3</i>	Bucci and Wente, 1997
SWY1353	<i>MATα snl1::HIS3 trp1-1 ura3-1 leu2-3,112 his3-11,15 ade2-1</i>	Ho et al., 1998
SWY1365	<i>MATα nup145::LEU2 ade2-1 trp1-1 ura3-1 leu2-3,112 his3-11,15 can1-100</i>	Emtage et al., 1997
SWY1695	<i>MATα GFP-nic96:HIS3 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 ade2-1::ADE2:ura3</i>	Bucci and Wente, 1998
SWY1722	<i>MATα nup57-E17 GFP-NIC96:HIS3 ade2-1:ADE2</i>	Bucci and Wente, 1998

SWY2515	<i>MATα prp20-G282S GFP-nic96:HIS3 NUP170-GFP:URA3 trp1-1 ura3-1 his3-11,15 leu2-3,112 can1-100</i>	Ryan et al., 2003
SWY2518	<i>MATα prp20-G282S trp1-1 ura3-1 his3-11,15 leu2-3,112 can1-100 ade2-1::ADE2:ura3</i>	Ryan et al., 2003
SWY2565	<i>MATα pom34::spHIS5 ade2-1::ADE2 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</i>	Miao et al., 2006
SWY3139	<i>MATα pom34::spHIS5 nup59::G418 his3 ura3 leu2 met15 + pSW1516</i>	Miao et al., 2006
SWY3407	<i>MATα prp20-G282S GFP-nic96:HIS3 nup170-GFP:URA3 ura3-1 his3-11,15 leu2-3,112 lys2 can1-100 ade2-1::ADE2:ura3</i>	This study
SWY3671	<i>MATα prp20-G282S trp1-1 ura3-1 his3-11,15 leu2-3,112 can1-100 ade2-1::ADE2:ura3</i>	This study
SWY3673	<i>MATα prp20-G282S rtn1-GFP:HIS3 trp1-1 ura3-1 his3-11,15 leu2-3,112 ade2-1::ADE2:ura3</i>	This study
SWY3677	<i>MATα prp20-G282S yop1-GFP:HIS3 trp1-1 ura3-1 his3-11,15 leu2-3,112 ade2-1::ADE2:ura3</i>	This study
SWY3747	<i>MATα prp20-G282S rtn1-GFP:HIS3 trp1-1::dsRed-HDEL:Trp ura3-1 his3-11,15 leu2-3,112 can1-100 ade2-1::ADE2:ura3</i>	This study
SWY3748	<i>MATα prp20-G282S rtn1-GFP:HIS3 GFP-nic96:HIS3 trp1-1 ura3-1 his3-11,15 leu2-3,112 can1-100 ade2-1::ADE2:ura3</i>	This study
SWY3810	<i>MATα rtn1::Kan^R yop1::Kan^R ura3Δ0 leu2Δ0 met15Δ0 his3Δ1</i>	This study
SWY3811	<i>MATα rtn1::Kan^R yop1::Kan^R ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0</i>	This study
SWY3876	<i>MATα rtn1::Kan^R yop1::Kan^R nup85::HIS3 his3 ura3 leu2 + pSW3354</i>	This study
SWY3877	<i>MATα rtn1::Kan^R yop1::Kan^R nup120::Kan^R his3Δ1 ura3Δ0 leu2Δ0 + pSW1079</i>	This study
SWY3878	<i>MATα rtn1::Kan^R yop1::Kan^R pom34::spHIS5 pom152::HIS3 trp1 ura3 his3 leu2 lys2 + pSW1516</i>	This study
SWY3880	<i>MATα rtn1::Kan^R yop1::Kan^R nup49ΔGLFG-GFP:TRP1 nup49-1::URA3 his3 lys2 leu2</i>	This study
SWY3881	<i>MATα rtn1::Kan^R yop1::Kan^R pom34::spHIS5 his3 ura3 leu2 trp1-1</i>	This study
SWY3882	<i>MATα rtn1::Kan^R yop1::Kan^R pom152::HIS3 his3 ura3 leu2 lys2</i>	This study
SWY3898	<i>MATα rtn1::Kan^R yop1::Kan^R nup100::HIS3 lys2 ura3 leu2</i>	This study
SWY3931	<i>MATα rtn1::Kan^R yop1::Kan^R GFP-nic96:HIS3 ura3Δ0 leu2Δ0 his3Δ1 trp1-1</i>	This study
SWY4007	<i>MATα nup133::HIS3 rtn1-GFP:HIS3 his3 ura3 leu2 trp1-1</i>	This study
SWY4045	<i>MATα nup133::Kan^R yop1-GFP:HIS3 his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0</i>	This study
SWY4050	<i>MATα rtn1::Kan^R yop1::Kan^R nup133::HIS3 his3 leu2 ura3 lys2Δ0 + pSW351</i>	This study
SWY4051	<i>MATα prp20-G282S yop1-GFP:HIS3 trp1-1::dsRed-HDEL:Trp ura3-1 his3-11,15 leu2-3,112 ade2-1::ADE2:ura3</i>	This study
SWY4094	<i>MATα nup2::Kan^R rtn1::Kan^R yop1::Kan^R his3Δ1 leu2Δ0 ura3Δ0 + pSW3459</i>	This study
SWY4095	<i>MATα nup53::Kan^R rtn1::Kan^R yop1::Kan^R his3Δ1 leu2Δ0 ura3Δ0 + pSW3459</i>	This study
SWY4096	<i>MATα nup60::Kan^R rtn1::Kan^R yop1::Kan^R his3Δ1 leu2Δ0 ura3Δ0 + pSW3459</i>	This study
SWY4097	<i>MATα nic96::HIS3 rtn1::Kan^R yop1::Kan^R his3 leu2 trp1 ura3 + pUN100-LEU2-nic96-1 + pSW282</i>	This study
SWY4099	<i>MATα nup145::LEU2 rtn1::Kan^R yop1::Kan^R trp1 his3 leu2 ura3 + pSW190</i>	This study
SWY4101	<i>MATα nup57-E17 rtn1::Kan^R yop1::Kan^R trp1 his3 leu2 ura3 + pSW3459</i>	This study
SWY4102	<i>MATα nup145ΔN(nup63-7)::LEU2 rtn1::Kan^R yop1::Kan^R trp1 his3 leu2 ura3 + pSW190</i>	This study
SWY4103	<i>MATα gle2::HIS3 rtn1::Kan^R yop1::Kan^R his3 leu2 ura3 + pSW443</i>	This study
SWY4105	<i>MATα nup82-3 rtn1::Kan^R yop1::Kan^R his3 leu2 ura3 met15 + pSW3459</i>	This study
SWY4106	<i>MATα nup84::Kan^R rtn1::Kan^R yop1::Kan^R his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 + pSW3459</i>	This study
SWY4108	<i>MATα nup188-1::HIS3 rtn1::Kan^R yop1::Kan^R his3 leu2 met15Δ0 lys2Δ0 ura3 + pNUP188U</i>	This study
SWY4109	<i>MATα apq12::Kan^R rtn1::Kan^R yop1::Kan^R his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 + pSW3459</i>	This study
SWY4110	<i>MATα nem1::Kan^R rtn1::Kan^R yop1::Kan^R his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 + pSW3459</i>	This study
SWY4112	<i>MATα brr6::HIS3/brr6-1 rtn1::Kan^R yop1::Kan^R trp1 his3 leu2 met15Δ0 ura3 + pSW3459</i>	This study

SWY4114	<i>MATα nup159-1/rat7-1 rtn1::Kan^R yop1::Kan^R his3 leu2 ura3 + pLG4</i>	This study
SWY4115	<i>MATα nup116-5::HIS3 rtn1::Kan^R yop1::Kan^R his3 lys2Δ0 leu2 ura3 + pSW131</i>	This study
SWY4116	<i>MATα prp20-G282S sec63-GFP his3 leu2 met15Δ0 ura3Δ0</i>	This study
SWY4117	<i>MATα nup133::HIS3 nic96-mCherry:HygB rtn1-GFP:HIS3 trp1-1 his3 leu2 ura3</i>	This study
SWY4118	<i>MATα spo7::spHIS5 rtn1::Kan^R yop1::Kan^R ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 + pSW3459</i>	This study
SWY4119	<i>MATα nup170-1::HIS3 rtn1::Kan^R yop1::Kan^R his3 ura3 leu2 + pSW3459</i>	This study
SWY4120	<i>MATα nup59::Kan^R rtn1::Kan^R yop1::Kan^R ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 + pSW3459</i>	This study
SWY4121	<i>MATα snl1::HIS3 rtn1::Kan^R yop1::Kan^R trp1-1 lys2Δ0 his3 leu2 ura3 + pSW574</i>	This study
SWY4122	<i>MATα nup1::IEU2 rtn1::Kan^R yop1::Kan^R trp1 ura3 ade2 ade3 + pLDB73</i>	This study
SWY4124	<i>MATα rtn1::Kan^R his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 nic96-mCherry:HygB</i>	This study
SWY4125	<i>MATα nic96-mCherry:HygB rtn1-GFP:HIS3 trp1-1 his3 leu2 ura3</i>	This study

t.s., temperature sensitive.

^aGFP fusion strains obtained from the Yeast GFP Fusion Collection (Invitrogen; Huh et al., 2003).

^bGene deletion strains obtained from the Yeast Knockout deletion collection (Open Biosystems; Winzeler et al., 1999).

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Table S2. Plasmids used in this study

Plasmid name	Description	Source
pRS316	CEN/URA3	Sikorski and Hieter, 1989
pRS315	CEN/LEU2	Sikorski and Hieter, 1989
dRed-HDEL	trp1::dsRed-HDEL:TRP1 integration plasmid	Bevis et al., 2002
pBS35	mCHERRY/HYGB integration plasmid	Shaner et al., 2004
pLDB73	NUP1/CEN/URA3/ADE3	Belanger et al., 1994
pLG4	RAT7/CEN/URA3	Gorsch et al., 1995
pRAT9.15	NUP85/CEN/TRP1	Goldstein et al., 1996
pNS167	NAB2 NLS fused to GFP under MET25 promotor URA3	Shulga et al., 2000
pNUP188U	NUP188/CEN/URA3	Aitchison et al., 1995
pRS315.NDC1	NDC1/CEN/LEU2	Chial et al., 1998
pUN100-LEU2-nic96-1	nic96-1/CEN/LEU2	Zabel et al., 1996
pSW131	NUP116/CEN/URA3	Wente and Blobel, 1994
pSW132	NUP100/CEN/URA3	Wente and Blobel, 1994
pSW190	NUP145/CEN/URA3	Emtage et al., 1997
pSW229	POM152/CEN/LEU2	This study
pSW282	NIC96/CEN/URA3	This study
pSW300	nup145-R4 (aa 1–1,012)	Emtage et al., 1997
pSW351	NUP133/CEN/URA3	This study
pSW443	GLE2/CEN/URA3	This study
pSW574	SNL1/CEN/URA3	Ho et al., 1998
pSW575	SNL1/CEN/LEU2	Ho et al., 1998
pSW610	nup145-33 (aa 593–893)	Emtage et al., 1997
pSW1079	NUP120/CEN/URA3	This study
pSW1516	POM34/CEN/URA3	Miao et al., 2006
pSW1517	POM34/CEN/LEU2	Miao et al., 2006
pSW3354	NUP85/CEN/URA3	This study
pSW3420	RTN1-GFP/CEN/LEU2	This study
pSW3421	rtn1-K48I-GFP/CEN/LEU2	This study
pSW3422	RTN1/CEN/LEU2	This study
pSW3423	rtn1-K48I/CEN/LEU2	This study
pSW3459	RTN1/CEN/URA3	This study
pSW3460	loxP-HIS5-loxP integration plasmid	This study

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