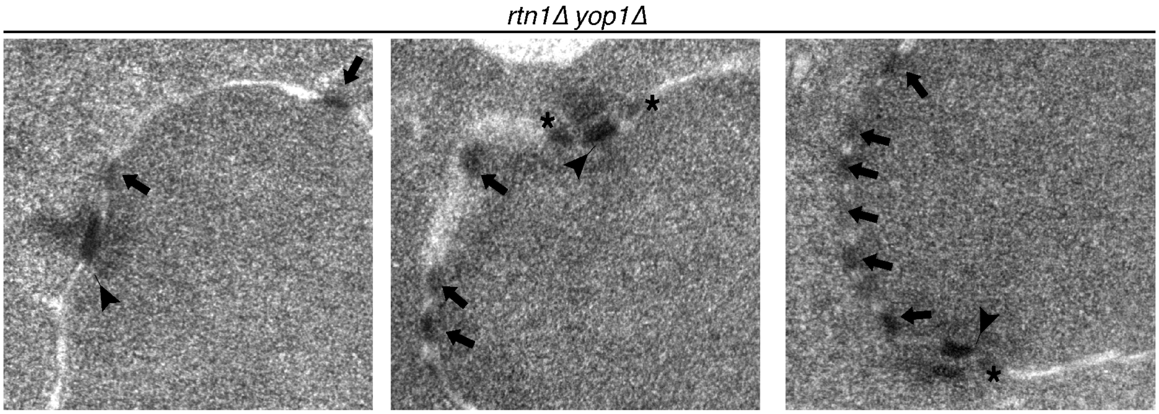

Figure S1 Deletion of *RTN1* and *YOP1* result in abnormalities in the SPB. *rtn1Δ yop1Δ* (SWY3811) cells were grown to early log phase at 23°C and processed for TEM. Scale bar, 100 nm. Arrowheads point to SPBs, arrows point to NPCs, asterisks indicate abnormal lobular structures on SPBs.

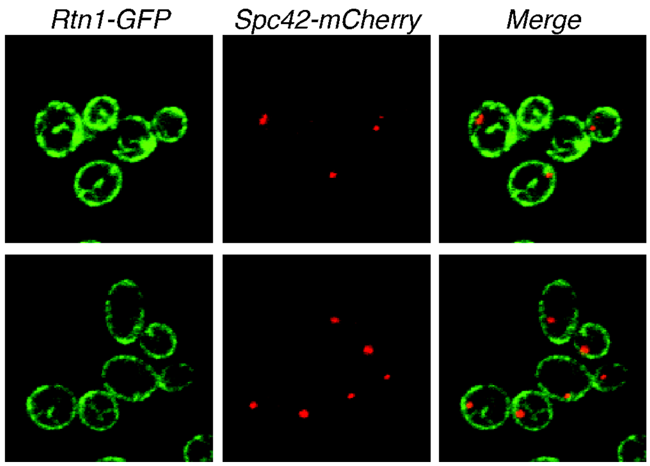

Figure S2 Rtn1 does not colocalize with SPBs. Asynchronous cultures of *nup120Δ RTN1-GFP*(SWY4047) expressing pSPC42-MCHERRY were grown to log phase and imaged. Scale bar, 2 µm

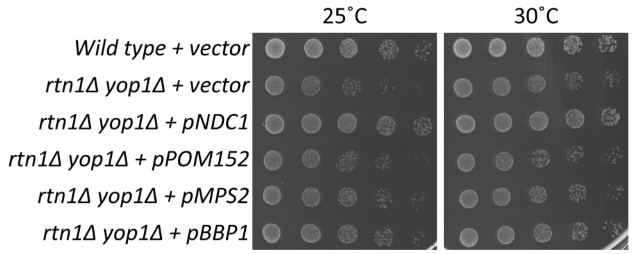

Figure S3 Overexpression of NDC1 results in rescue of *rtn1Δ yop1Δ growth* defects. Wildtype or *rtn1Δ yop1Δ* cells were transformed with plasmids expressing *NDC1*, *POM152*, *MPS2*, *BBP1*, or empty vector and grown to early log phase at 30°C in synthetic media lacking leucine. Strains were tested for growth at 25°C and 30°C.

Table S1 Yeast strains used in this study.

Strain	Genotype	Source
BY4741	*MATa his3Δ1 leu2Δ0 LYS2 met15Δ0 ura3Δ0*	(Mortimer and Johnston 1986)
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 MET15 ura3Δ0*	(Mortimer and Johnston 1986)
Bbp1-GFP	*MATa BBP1-GFP:HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	(Huh *et al.* 2003)
Ndc1-GFP	*MATa NDC1-GFP:HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	(Huh *et al.* 2003)
Rtn1-GFP	*MATa RTN1-GFP:HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	(Huh *et al.* 2003)
Ndc1-TAP	*MATa NDC1-TAP:HIS3 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	(Ghaemmaghami *et al.* 2003)
nup120Δ	*MATa nup120::KanR his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	(Winzeler *et al.* 1999)
nup133Δ	*MATa nup133::KanR his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	(Winzeler *et al.* 1999)
SLJ001	*MATa bar1::hisG;ura3-1;leu2-3,112;trp1-1;his3-11,15;ade2-1;can1-100;GAL+*	This Study
SLJ173	*MATα bar1::hisG;ura3-1;leu2-3,112;trp1-1;his3-11,15;ade2-1;can1-100;GAL+*	This Study
SLJ1433	*MATa trp1::GAL-myc-SPC42-TRP1*	(Jaspersen *et al.* 2002)
SLJ3828	*MATa yop1::HygR rtn1::KanR trp1::GAL-myc-SPC42-TRP1*	This Study
SLJ5572	*MATa his3Δ200 trp1-901 leu2-3,112 ade2 LYS2::(lexAop)4-HIS3 ura3::(lexAop)8-lacZ ade2::(lexAop)8-ADE2 GAL4*	This Study
SLJ5975	*MATα NDC1-3×HA-HIS3MX6:*	This Study
SLJ5976	*MATa YOP1-3×FLAG-KanR*	This Study
SLJ5977	*MATα NDC1-3×HA-HIS3MX6 YOP1-3×FLAG-KanR*	This Study
SLJ5572	*MATa his3Δ200 trp1-901 leu2-3,112 ade2 LYS2::(lexAop)4-HIS3 ura3::(lexAop)8-lacZ (lexAop)8-ADE2 GAL4*	Dual Biotech NMY51
SWY3810	*MATa rtn1::KanR yop1::KanR ura3Δ0 leu2Δ0 met15Δ0 his3Δ1*	(Dawson *et al.* 2009)
SWY3811	*MATα rtn1::KanR yop1::KanR ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*	(Dawson *et al.* 2009)
SWY4047	*MATα nup133::KanR RTN1-GFP:HIS3 ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*	(Dawson *et al.* 2009)
SWY4522	*MATa NDC1-GFP:HIS3 his3Δ1 met15Δ0 ura3Δ0 leu2Δ0::DsRed-HDEL:LEU2*	This Study
SWY4616	*MATα GFP-TUB3 his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	This Study
SWY4617	*MATa GFP-TUB3 his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	This Study
SWY4636	*MATα NDC1-TAP:HIS3 RTN1-GFP:HIS3 his3Δ1 leu2Δ0 ura3Δ0*	This Study
SWY4637	*MATa NDC1-TAP:HIS3 RTN1-GFP:HIS his3Δ1 leu2Δ0 ura3Δ0*	This Study
SWY4725	*MATα rtn1::KanR yop1::KanR NIC96-GFP:HIS3 met15Δ0 his3Δ1 leu2Δ0 ura3Δ0*	This Study
SWY4877	*MATα rtn1::KanR yop1::KanR GFP-TUB3 his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	This Study
SWY4878	*MATα rtn1::KanR yop1::KanR GFP-TUB3 his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	This Study
SWY4906	*MATa rtn1::KanR yop1::KanR leu2Δ0::DsRed-HDEL:LEU2 ndc1-GFP:HIS3 ura3Δ0*	This Study

SWY4934	*MATa rtn1::KanR yop1::KanR GFP-TUB3 his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0*	This Study
SWY4935	*MATa rtn1::KanR yop1::KanR GFP-TUB3 his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	This Study
SWY4950	*MATa rtn1::KanR yop1::KanR BBP1-GFP:HIS3 NIC96-mcherry:HYGB his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0*	This Study
SWY4970	*MATa NIC96-mcherry:HYGB BBP1-GFP:HIS3 his3Δ1 leu2Δ0 ura3Δ0*	This Study
SWY4971	*MATa nup120::KanR NIC96-mcherry:HYGB BBP1-GFP:HIS3 his3Δ1 leu2Δ0 ura3Δ0*	This Study
SWY4972	*MATa rtn1::KanR yop1::KanR SEC63-GFP:HIS3 his3Δ1leu2Δ0::DsRED-HDEL:LEU2 ura3Δ0*	This Study
SWY5033	*MATα nup133::KanR NIC96-mcherry:HYGB BBP1-gfp:HIS3 his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 met15Δ0*	This Study

* All strains beginning with "SLJ" are derivatives of W303 and all strains beginning with "SWY" are derivatives of S288C.

DAWSON, T. R., M. D. LAZARUS, M. W. HETZER and S. R. WENTE, 2009 ER membrane-bending proteins are necessary for de novo nuclear pore formation. J Cell Biol 184: 659-675.

GHAEMMAGHAMI, S., W. K. HUH, K. BOWER, R. W. HOWSON, A. BELLE *et al.*, 2003 Global analysis of protein expression in yeast. Nature 425: 737-741.

HUH, W. K., J. V. FALVO, L. C. GERKE, A. S. CARROLL, R. W. HOWSON *et al.*, 2003 Global analysis of protein localization in budding yeast. Nature 425: 686-691.

JASPERSEN, S. L., T. H. GIDDINGS and M. WINEY, 2002 Mps3p is a novel component of the yeast spindle pole body that interacts with the yeast centrin homologue Cdc31p. J Cell Biol 159: 945-956.

MORTIMER, R. K., and J. R. JOHNSTON, 1986 Genealogy of principal strains of the yeast genetic stock center. Genetics 113: 35-43.

WINZELER, E. A., D. D. SHOEMAKER, A. ASTROMOFF, H. LIANG, K. ANDERSON *et al.*, 1999 Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science 285: 901-906.

Table S2 Plasmids used in this study.

Plasmid	Genotype	Source
dRed-HDEL	*trp1::DsRED-HDEL:TRP1* integration plasmid	(Bevis *et al.* 2002)
pBS35	*mCHERRY/HYGB* integration plasmid	(Shaner *et al.* 2004)
pRS315	CEN/*LEU2*	(Sikorski and Hieter 1989)
pRS425	2μ/*LEU2*	(Christianson *et al.* 1992)
pRS315.NDC1	*NDC1*/CEN/*LEU2*	(Chial *et al.* 1998)
PSJ906	*SPC42-mCHERRY-HIS*/*LEU2*	This Study
PSW863	*POM152*/2μ/*LEU2*	(Miao *et al.* 2006)
PSW3422	*RTN1*/CEN/*LEU2*	(Dawson *et al.* 2009)
PSW3673	*APQ12*/2μ/*LEU2*	This Study
PSW3674	*BBP1*/2μ/*LEU2*	This Study
PSW3675	*BRR6*/2μ/*LEU2*	This Study
PSW3676	*MPS2*/2μ/*LEU2*	This Study
PSW3592	*leu2Δ0::DsRED-HDEL:LEU2* integration cassette	This Study

Bevis, B. J., A. T. Hammond, C. A. Reinke and B. S. Glick, 2002 De novo formation of transitional ER sites and Golgi structures in Pichia pastoris. Nat Cell Biol 4: 750-756.

Chial, H. J., M. P. Rout, T. H. Giddings and M. Winey, 1998 Saccharomyces cerevisiae Ndc1p is a shared component of nuclear pore complexes and spindle pole bodies. J Cell Biol 143: 1789-1800.

Christianson, T. W., R. S. Sikorski, M. Dante, J. H. Shero and P. Hieter, 1992 Multifunctional yeast high-copy-number shuttle vectors. Gene 110: 119-122.

Dawson, T. R., M. D. Lazarus, M. W. Hetzer and S. R. Wente, 2009 ER membrane-bending proteins are necessary for de novo nuclear pore formation. J Cell Biol 184: 659-675.

Miao, M., K. J. Ryan and S. R. Wente, 2006 The integral membrane protein Pom34p functionally links nucleoporin subcomplexes. Genetics 172: 1441-1457.

Shaner, N. C., R. E. Campbell, P. A. Steinbach, B. N. Giepmans, A. E. Palmer *et al.*, 2004 Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. Nat Biotechnol 22: 1567-1572.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics 122: 19-27.