

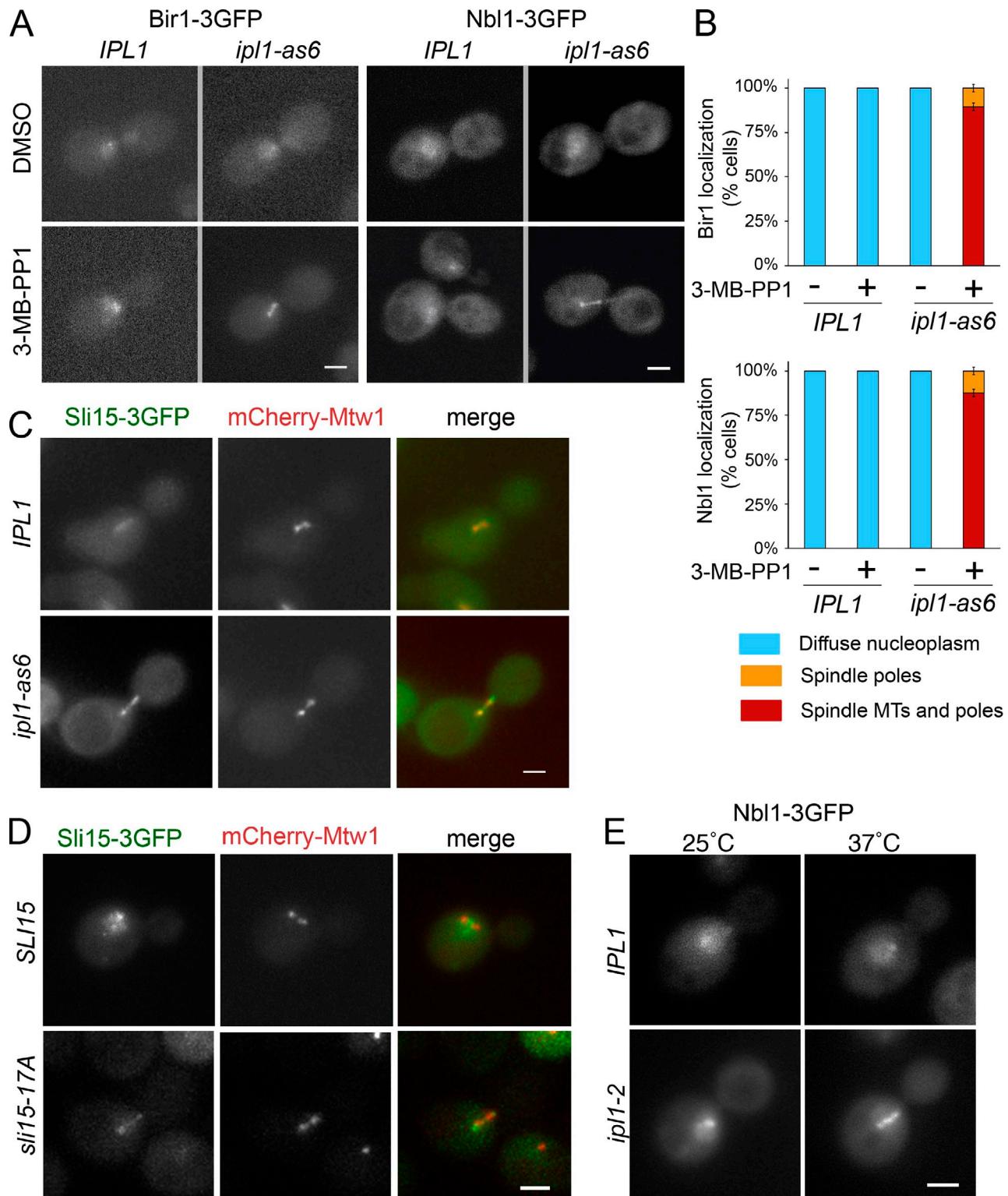
Nakajima et al., <http://www.jcb.org/cgi/content/full/jcb.201009137/DC1>

Figure S1. *Ipl1* kinase activity is required for CPC exclusion from preanaphase spindles. (A) *Ipl1*/Aurora kinase inhibition relocalizes CPC components to the preanaphase spindle. Representative images of cells containing *IPL1* or *ipl1-as6* and *BIR1-3GFP* (left) or *NBL1-3GFP* (right), treated with DMSO (top) or 40 μ M 3-MB-PP1 (bottom). Cells were imaged 15 min after adding 3-MB-PP1 to preanaphase cells. Bar, 2 μ m. (B) Quantification of the observations represented in A. More than 200 cells from each treatment were analyzed using mCherry-Tub1 as a reference. (C) *Ipl1*/Aurora kinase inhibition relocalizes CPC components to the metaphase spindle. Representative images showing Sli15-3GFP and Mtw1-mCherry in cells containing *IPL1* (top) or *ipl1-as6* (bottom) treated with 40 μ M 3-MB-PP1. Cells were imaged 15 min after adding 3-MB-PP1 to metaphase cells synchronized with 10 mM methionine. Bar, 2 μ m. (D) sli15-17A relocalizes to the metaphase spindle. Representative images showing Mtw1-mCherry and either Sli15-3GFP or sli15-17A-3GFP. Bar, 2 μ m. (E) Defective *Ipl1*/Aurora kinase promotes relocalization of CPC components to the metaphase spindle. Representative images showing Nbl1-GFP in cells containing *IPL1* (top) or a ts allele of *IPL1*, *ipl1-2* (bottom; Chan and Botstein, 1993), exposed to either the permissive temperature (25°C) or the restrictive temperature (37°C) for 3 h. Bar, 2 μ m.

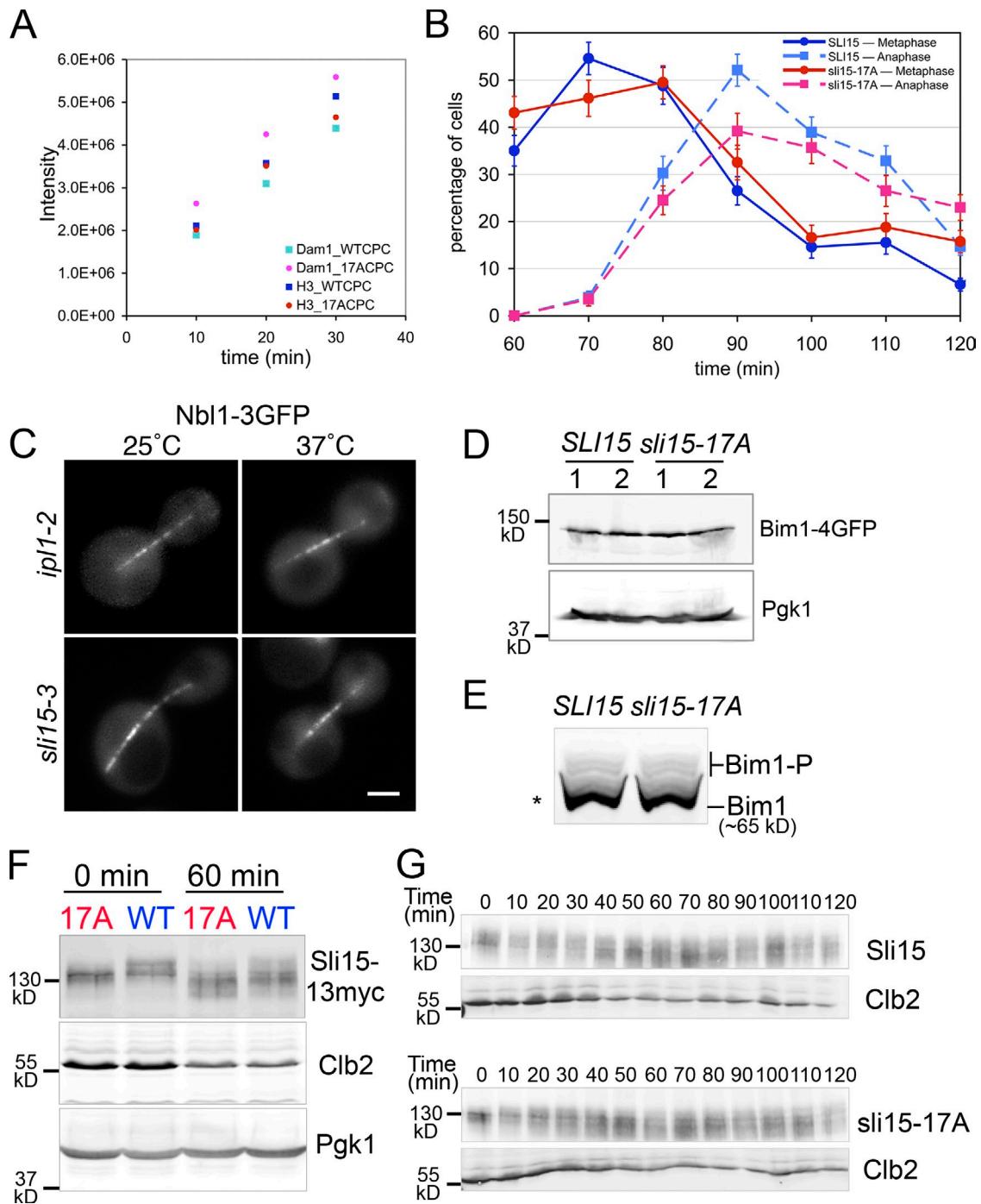


Figure S2. Reduced Ipl1/Aurora-mediated phosphorylation of Sli15/INCENP promotes CPC accumulation on the central spindle in anaphase. (A) CPC kinase reaction time course with the Dam1 complex and histone H3 as substrates. Incorporation of $\gamma^{[32]}\text{P}$ ATP was measured using ImageQuant software (GE Healthcare). The image of the reaction at 20 min is shown in Fig. 2 F. (B) Ipl1-dependent phosphorylation of Sli15 is required for normal cell cycle progression. Once released from G1 arrest, cells were fixed every 10 min. Cell cycle stage was determined based on the spindle and DNA morphology by indirect immunofluorescence of MTs using anti-tubulin antibody (YOL134; Accurate Chemical and Scientific Corporation) and DAPI staining. The percentage of the cells in metaphase and anaphase shows that *sli15-17A* cells had prolonged metaphase and anaphase relative to wild type. (C) Defective Ipl1/Aurora kinase promotes CPC targeting to the central spindle. Representative images showing Nbl1-GFP in cells containing a ts allele of *IPL1*, *ip11-2* (top; Chan and Botstein, 1993) or *sli15-3* (bottom; Kim et al., 1999) exposed to either the permissive temperature (25°C) or the restrictive temperature (37°C) for 3 h. Bar, 2 μm . (D) Bim1-4GFP is expressed at equivalent levels in wild-type and *sli15-17A* cells. (Top) Western blot of whole-cell lysates from wild-type and *sli15-17A* cells using anti-GFP antibody (Torrey Pines Biolabs). (Bottom) Western blot of the same membrane probed with anti-Pgk1 antibody (Invitrogen) as a loading control. The numbers indicate independent clones. (E) Bim1 phosphorylation levels in wild-type and *sli15-17A* cells. The electrophoretic mobility of Bim1-13myc was analyzed in a gel containing 30 μM phos-tag (Wako Chemicals USA) and 7% acrylamide. Asterisk indicates that the apparent molecular weight cannot be determined because of the nonlinear effects of the phos-tag gel on protein mobility. (F) Cell cycle-dependent electrophoretic mobility of Sli15 and Clb2. Cells expressing *SLI15-13myc* or *sli15-17A-13myc* were synchronized in S phase using hydroxyurea and released into normal media (time 0). Electrophoretic mobility of Sli15 was detected by Western blot using anti-myc antibody. Cell cycle stage was assessed using an anti-Clb2 antibody (Santa Cruz Biotechnology, Inc.). (G) The full time course from which the results in F are taken.

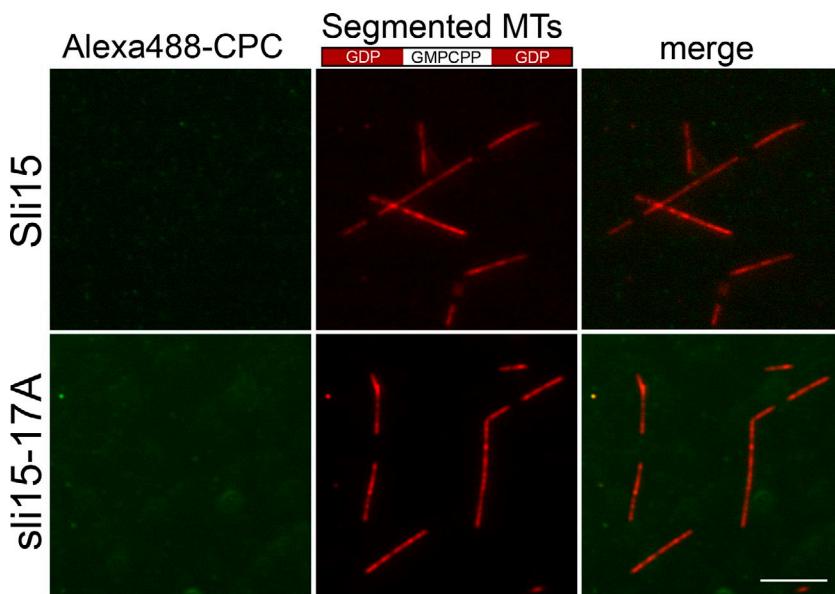
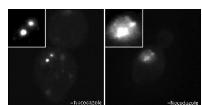


Figure S3. The Alexa 488-labeled CPC shows low affinity for rhodamine-labeled MTs. 0.2 μ M tubulin-derived MTs (see Materials and methods) were incubated with 2 nM Alexa 488-labeled CPC containing wild-type subunits (WT CPC) or sli15-17A (sli15-17A CPC). Bar, 5 μ m.



Video 1. Dynamic nuclear localization of the CPC is MT dependent. Diploid cells homozygous for *BIR1-3GFP* (at its endogenous locus and controlled by the endogenous promoter) were either treated with DMSO (right) or 15 μ g/ml nocodazole (left) for 1 h before imaging. Images were acquired continuously with an exposure time of 800 ms for 40 s using a microscope (model IX81; Olympus) operated by MetaMorph software (Molecular Devices). The display rate is 6 frames/s. Stills from this video appear in Fig. 1.

Table S1. Yeast strains and plasmids used in this study

Strain ID	Genotype	Reference
DDY1810	MAT _a leu2 ura3-52 trp1 prb1-1122 pep4-3 pre1-451	Cheeseman et al., 2001
DDY4001	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 BIR1-13myc::KanMX6	This paper
DDY4002	MAT _a his3Δ200 leu2-3,112 lys2-801 SLI15-3GFP::HIS3 ipl1-as6::LEU2 pdr5Δ::KanMX6 snq2Δ::cgHIS3 ura3-52::mCherry-TUB1::URA3	This paper
DDY4003	MAT _a his3Δ200 leu2-3,112 lys2-801 SLI15-3GFP::HIS3 pdr5Δ::KanMX6 snq2Δ::cgHIS3 ura3-52::mCherry-TUB1::URA3	This paper
DDY4004	MAT _a his3Δ200 leu2-3,112 lys2-801 BIR1-3GFP::HIS3 ipl1-as6::LEU2 pdr5Δ::KanMX6 snq2Δ::cgHIS3 ura3-52::mCherry-TUB1::URA3	This paper
DDY4005	MAT _a his3Δ200 leu2-3,112 lys2-801 BIR1-3GFP::HIS3 pdr5Δ::KanMX6 snq2Δ::cgHIS3 ura3-52::mCherry-TUB1::URA3	This paper
DDY4006	MAT _a his3Δ200 leu2-3,112 lys2-801 NBL1-GFP::KanMX6 ipl1-as6::LEU2 pdr5Δ::KanMX6 snq2Δ::cgHIS3	This paper
DDY4007	MAT _a his3Δ200 leu2-3,112 lys2-801 NBL1-GFP::KanMX6 pdr5Δ::KanMX6 snq2Δ::cgHIS3	This paper
DDY4008	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 SLI15-13myc::KanMX6 ipl1-as6::LEU2 pdr5Δ::KanMX6 snq2Δ::cgHIS3	This paper
DDY4009	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 SLI15-13myc::KanMX6 pdr5Δ::KanMX6 snq2Δ::cgHIS3	This paper
DDY4010	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 sli15-17A-13myc::KanMX6	This paper
DDY4011	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 sli15-13myc::KanMX6	This paper
DDY4012	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 sli15-17A-3GFP::HIS3::NAT ura3-52::mCherry-TUB1::URA3	This paper
DDY4013	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 SLI15-3GFP::HIS3::NAT ura3-52::mCherry-TUB1::URA3	This paper
DDY4014	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 lacO-CEN15(1.8)::URA3 HIS3::pCu-Lacl-GFP sli15-17A::NAT SPC42-GFP::KanMX6 PMET3-CDC20::HygMX6	This paper
DDY4015	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 lacO-CEN15(1.8)::URA3 HIS3::pCu-Lacl-GFP Sli15::NAT SPC42-GFP::KanMX6 PMET3-CDC20::HygMX6	This paper
DDY4016	MAT _a his3Δ200 leu2-3,112 lys2-801 sli15-17A::NAT ura3-52::GFP-TUB1::URA3 PMET3-CDC20::HygMX6	This paper
DDY4017	MAT _a his3Δ200 leu2-3,112 lys2-801 SLI15::NAT ura3-52::GFP-TUB1::URA3 PMET3-CDC20::HygMX6	This paper
DDY4018	MAT _a his3Δ200 leu2-3,112 lys2-801 ipl1-as6::LEU2 pdr5Δ::KanMX6 snq2Δ::cgHIS3 ura3-52::GFP-TUB1::URA3 PMET3-CDC20::HygMX6	This paper
DDY4019	MAT _a his3Δ200 leu2-3,112 lys2-801 pdr5Δ::KanMX6 snq2Δ::cgHIS3 ura3-52::GFP-TUB1::URA3 PMET3-CDC20::HygMX6	This paper
DDY4020	MAT _a his3Δ200 leu2-3,112 lys2-801 sli15-17A::NAT ipl1-as6::LEU2 ura3-52::GFP-TUB1::URA3 PMET3-CDC20::HygMX6	This paper
DDY4021	MAT _a his3Δ200 leu2-3,112 lys2-801 SLI15::NAT ipl1-as6::LEU2 ura3-52::GFP-TUB1::URA3 PMET3-CDC20::HygMX6	This paper
DDY4022	MAT _a his3Δ200 leu2-3,112 lys2-801 cdc14-2 Sli15-3GFP::HIS3 ipl1-as6::LEU pdr5Δ::KanMX6 snq2Δ::cgHIS3 ura3-52::mCherry-TUB1::URA3	This paper
DDY4023	MAT _a his3Δ200 leu2-3,112 lys2-801 cdc14-2 Sli15-3GFP::HIS3 pdr5Δ::KanMX6 snq2Δ::cgHIS3 ura3-52::mCherry-TUB1::URA3	This paper
DDY4024	MAT _a his3Δ200 leu2-3,112 lys2-801 cdc14-2 sli15-17A-3GFP::HIS3::NAT ipl1-as6::LEU pdr5Δ::KanMX6 snq2Δ::cgHIS3 ura3-52::mCherry-TUB1::URA3	This paper
DDY4025	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 sli15-17A::NAT ASE1-4GFP::KanMX6 ura3-52::mCherry-tub1::URA3	This paper
DDY4026	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 SLI15::NAT ASE1-4GFP::KanMX6 ura3-52::mCherry-tub1::URA3	This paper
DDY4027	MAT _a his3Δ200 leu2-3,112 lys2-801 sli15-17A::NAT ASE1-4GFP::KanMX6 ipl1-as6::LEU2 pdr5Δ::KanMX6 snq2Δ::cgHIS3 ura3-52::mCherry-TUB1::URA3	This paper
DDY4028	MAT _a his3Δ200 leu2-3,112 lys2-801 sli15-17A::NAT ASE1-4GFP::KanMX6 pdr5Δ::KanMX6 snq2Δ::cgHIS3 ura3-52::mCherry-TUB1::URA3	This paper
DDY4029	MAT _a his3Δ200 leu2-3,112 lys2-801 SLI15::NAT ASE1-4GFP::KanMX6 ipl1-as6::LEU2 pdr5Δ::KanMX6 snq2Δ::cgHIS3 ura3-52::mCherry-TUB1::URA3	This paper
DDY4030	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 NBL1-GFP::KanMX6	This paper
DDY4031	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 NBL1-GFP::KanMX6 ip1-2	This paper
DDY4032	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 NBL1-GFP::KanMX6 sli15-3	This paper
DDY4033	MAT _a his3Δ200 leu2-3,112 ura3-52 lys2-801 sli15-17A::NAT BIM1-4GFP::KanMX6 ura3-52::mCherry-TUB1::URA3	This paper

Table S1. Yeast strains and plasmids used in this study (Continued)

Strain ID	Genotype	Reference
DDY4034	MAT α his3Δ200 leu2-3,112 ura3-52 lys2-801 SLI15::NAT BIM1-4GFP::KanMX6 ura3-52::mCherry-TUB1::URA3	This paper
pDD2301	pFastbacdual::IPL1::SLI15-CBP-TEV-ZZ Amp ^R	Nakajima et al., 2009
pDD2302	pFastbacHTb::BIR1 Amp ^R	Nakajima et al., 2009
pDD2221	pET24b::NBL1	This paper
pDD2222	PFastBacDUAL::IPL1::sli15-17A-cTAP	This paper
pDD2223	PFastBacDUAL::ipl1-K133R::SLI15-cTAP	This paper
pDD2224	pRS426::PGal-CIN8-TEV-Zmyc	This paper

All yeast strains generated for this study are derived from the S288C strain.

References

- Cheeseman, I.M., C. Brew, M. Wolyniak, A. Desai, S. Anderson, N. Muster, J.R. Yates, T.C. Huffaker, D.G. Drubin, and G. Barnes. 2001. Implication of a novel multiprotein Dam1p complex in outer kinetochore function. *J. Cell Biol.* 155:1137–1145. doi:10.1083/jcb.200109063
- Nakajima, Y., R.G. Tyers, C.C. Wong, J.R. Yates III, D.G. Drubin, and G. Barnes. 2009. Nbl1p: a Borealin/Dasra/CSC-1-like protein essential for Aurora/Ipl1 complex function and integrity in *Saccharomyces cerevisiae*. *Mol. Biol. Cell.* 20:1772–1784. doi:10.1091/mbc.E08-10-1011