Supplemental Materials

Supplemental Table 1. Strains used in this study.

Strain	Genotype
DDY4002	*MATa his3Δ200 leu2-3,112 ura3-52 MIF2-MYC::KanMX*
DDY4003	*MATa his3Δ200 leu2-3,112 ura3-52 CKA2-MYC::KanMX*
DDY4004	*MATa his3Δ200 leu2-3,112 ura3-52 CKA1-GFP::KanMX*
DDY4005	*MATa his3Δ200 leu2-3,112 ura3-52 CKB1-GFP::HIS3*
DDY4006	*MATa his3Δ200 leu2-3,112 ura3-52 CKA2-GFP::HIS3*
DDY4007	*MATa his3Δ200 leu2-3,112 ura3-52 CKB2-GFP::HIS3*
DDY4008	*MATa his3Δ200 leu2-3,112 ura3-52 CKA1-GFP::KanMX MTW1-RFP::HIS3*
DDY4009	*MATa his3Δ200 leu2-3,112 ura3-52 CKB1-GFP::HIS3 MTW1-RFP::HIS3*
DDY4010	*MATα his3Δ200 leu2-3,112 ura3-52*
DDY4011	*MATα his3Δ200 leu2-3,112 ura3-52 cka1Δ::CgURA3*
DDY4012	*MATα his3Δ200 leu2-3,112 ura3-52 cka1Δ::CgURA3 CKA2::NatR*
DDY4013	*MATα his3Δ200 leu2-3,112 ura3-52 cka1Δ::CgURA3 cka2-8::NatR*
DDY4014	*MATa his3Δ200 leu2-3,112 ura3-52 cka1Δ::CgURA3 CKA2::NatR SPC42-mCherry::HIS3 HIS3::pCu-LacI-GFP LacO::LEU2*
DDY4015	*MATa his3Δ200 leu2-3,112 ura3-52 cka1Δ::CgURA3 cka2-8::NatR SPC42-mCherry::HIS3 HIS3::pCu-LacI-GFP LacO::LEU2*
DDY4016	*MATa his3Δ200 leu2-3,112 ura3-52 PDS1-18MYC::LEU2 bar1Δ:: KanMX*
DDY4017	*MATa his3Δ200 leu2-3,112 ura3-52 dam1-ts PDS1-18MYC::LEU2 bar1Δ:: KanMX*
DDY4018	*MATa his3Δ200 leu2-3,112 ura3-52 cka1Δ::CgURA3 CKA2::NatR PDS1-18MYC::LEU2 bar1Δ:: KanMX*
DDY4019	*MATa his3Δ200 leu2-3,112 ura3-52 cka1Δ::CgURA3 cka2-8::NatR PDS1-18MYC::LEU2 bar1Δ:: KanMX*
DDY4020	*MATa his3Δ200 leu2-3,112 ura3-52 cka1Δ::CgURA3 cka2-8::NatR mad2Δ::CgLEU2 PDS1-18MYC::LEU2 bar1Δ:: KanMX*
DDY4021	*MATa his3Δ200 leu2-3,112 ura3-52 cka1Δ::CgURA3 CKA2::NatR mad1Δ::CgLEU2*
DDY4022	*MATα his3Δ200 leu2-3,112 ura3-52 cka1Δ::CgURA3 CKA2::NatR mad2Δ::CgLEU2*
DDY4023	*MATα his3Δ200 leu2-3,112 ura3-52 cka1Δ::CgURA3 cka2-8::NatR mad1Δ::CgLEU2*
DDY4024	*MATα his3Δ200 leu2-3,112 ura3-52 cka1Δ::CgURA3 cka2-8::NatR mad2Δ::CgLEU2*
DDY4025	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 MIF2::CgHIS3/MIF2*
DDY4026	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S325A)::CgHIS3/MIF2*
DDY4027	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S325D)::CgHIS3/MIF2*
DDY4028	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S54A, S98A, S154A)::CgHIS3/MIF2*
DDY4029	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S54A, S98A, S154A, S325A)::CgHIS3/MIF2*
DDY4030	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S54A, S98A, S154A, S325D)::CgHIS3/MIF2*
DDY4031	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S54D, S98D, S154D)::CgHIS3/MIF2*
DDY4032	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S54D, S98D, S154D, S325A)::CgHIS3/MIF2*

DDY4033	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S54D, S98D, S154D, S325D)::CgHIS3/MIF2*
DDY4034	*MATa his3Δ200 leu2-3,112 ura3-52 MIF2::CgHIS3 mCherry-TUB1::URA3 HIS3::pCu-LacI-GFP LacO::LEU2*
DDY4035	*MATa his3Δ200 leu2-3,112 ura3-52 mif2(S325A)::CgHIS3 mCherry-TUB1::URA3 HIS3::pCu-LacI-GFP LacO::LEU2*
DDY4036	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 MIF2-GFP::KanMX/MIF2*
DDY4037	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S325A)-GFP::KanMX/MIF2*
DDY4038	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S325D)-GFP::KanMX/MIF2*
DDY4039	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S54A, S98A, S154A)-GFP::KanMX/MIF2*
DDY4040	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S54A, S98A, S154A, S325A)-GFP::KanMX/MIF2*
DDY4041	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S54A, S98A, S154A, S325D)-GFP::KanMX/MIF2*
DDY4042	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S54D, S98D, S154D)-GFP::KanMX/MIF2*
DDY4043	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S54D, S98D, S154D, S325A)-GFP::KanMX/MIF2*
DDY4044	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 mif2 (S54D, S98D, S154D, S325D)-GFP::KanMX/MIF2*
DDY4045	*MATα his3Δ200 leu2-3,112 ura3-52 MIF2-GFP::KanMX*
DDY4046	*MATα his3Δ200 leu2-3,112 ura3-52 mif2 (S325A)-GFP::KanMX*
DDY4047	*MATα his3Δ200 leu2-3,112 ura3-52 mif2 (S325D)-GFP::KanMX*
DDY4048	*MATα his3Δ200 leu2-3,112 ura3-52 mif2 (S54A, S98A, S154A, S325A)-GFP::KanMX*
DDY4049	*MATα his3Δ200 leu2-3,112 ura3-52 mif2 (S54D, S98D, S154D)-GFP::KanMX*
DDY4050	*MATα his3Δ200 leu2-3,112 ura3-52 mif2 (S54D, S98D, S154D, S325A)-GFP::KanMX*
DDY4051	*MATα his3Δ200 leu2-3,112 ura3-52 mif2 (S54D, S98D, S154D, S325D)-GFP::KanMX*
DDY4052	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 NDC10::CgLEU2/NDC10*
DDY4053	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 ndc10(T106A)::CgLEU2/NDC10*
DDY4054	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 ndc10(T106D)::CgLEU2/NDC10*
DDY4055	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 ndc10(S4A, S189A, T627A, S895A)::CgLEU2/NDC10*
DDY4056	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 ndc10(S4D, S189D, T627D, S895D)::CgLEU2/NDC10*
DDY4057	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 ndc10(S4A, T106A, S189A, T627A, S895A)::CgLEU2/NDC10*
DDY4058	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 ndc10(S4D, T106D, S189D, T627D, S895D)::CgLEU2/NDC10*
DDY4059	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 NDC10-GFP::KanMX/NDC10*
DDY4060	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 ndc10(T106A)-GFP::KanMX /NDC10*
DDY4061	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 ndc10(T106D)-GFP::KanMX /NDC10*
DDY4062	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-*

	801/LYS2 ndc10(S4A, S189A, T627A, S895A)-GFP::KanMX/NDC10
DDY4063	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 ndc10(S4D, S189D, T627D, S895D)-GFP::KanMX/NDC10*
DDY4064	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 ndc10(S4A, T106A, S189A, T627A, S895A)-GFP::KanMX/NDC10*
DDY4065	*MATa/α his3Δ200/ his3Δ200 leu2-3,112/ leu2-3,112 ura3-52/ ura3-52 ade2-1/ADE2 lys-801/LYS2 ndc10(S4D, T106D, S189D, T627D, S895D)-GFP::KanMX/NDC10*
DDY4066	*MATa his3Δ200 leu2-3,112 ura3-52 ndc10Δ::KanMX* [p*RS315-NDC10*]
DDY4067	*MATa his3Δ200 leu2-3,112 ura3-52 ndc10Δ::KanMX* [p*RS315-ndc10(S4A, T106A, S189A, T627A, S895A)*]
DDY4068	*MATa his3Δ200 leu2-3,112 ura3-52 ndc10Δ::KanMX* [p*RS315- ndc10(S4D, T106D, S189D, T627D, S895D)*]
DDY4069	*MATa leu2 ura3-52 trp1 prb1-1122, pep4-3 pre1-451* [p*RS426-*P_{GAL1}*-MIF2-TEV-MYC*]
DDY4070	*MATa leu2 ura3-52 trp1 prb1-1122, pep4-3 pre1-451* [p*RS426-*P_{GAL1}*-NDC10-TEV-MYC*]
DDY4071	*MATa his3Δ200 leu2-3,112 ura3-52 ipl1-2 MIF2-MYC::KanMX*
DDY4072	*MATa his3Δ200 leu2-3,112 ura3-52 ipl1-2 NDC10-MYC::KanMX*

Sample Preparation for Mass Spectrometry Analysis

Trichloroacetic acid (TCA) precipitation: Protein samples were precipitated by mixing 1 volume of the sample solution (cold) with 1/3 volume of 100% (w/v) TCA (6.1 N, Sigma). The solution was mixed well to give a final TCA concentration of 25% and then left on ice for 3 hours. The sample was centrifuged for 30 minutes at 4°C and the supernatant aspirated leaving ~5-10 μl in the tube so as to not disturb the pellet. The pellet is washed twice with ice-cold acetone (500 μl each). After each wash, the solution was centrifuged for 10 minutes. The sample was then dried on a speed vacuum for 1-2 minutes or air-dried.

Digestion: Proteins were dissolved in 60 μl of 100 mM Tris-HCl pH 8.5 containing 8 M urea (Sigma). The protein was reduced by the addition of 500 mM *tris*(2-carboxyethyl) phosphine (TCEP) to a final concentration of 5 mM (incubated at room temperature for 20 minutes), followed by carboxyamidomethylation of cysteines by incubation at room temperature for 30 minutes in the dark with 500 mM iodoacetamide (final concentration 10 mM). Protein was prepared for analysis of phosphorylation sites by the method of MacCoss et al (MacCoss et al., 2002). The sample was split equally into three tubes. In one of the tubes the concentration of urea was then diluted 2-fold (to 4 M) by the addition of an equal volume of 100 mM Tris-HCl pH 8.5 and then subtilisin (Promega) was added at ~1:100 enzyme to substrate ratio (wt:wt) and incubated at 37°C for 4 hours in the dark. The other two samples were diluted 4-fold (to 2 M) and Elastase and Trypsin (Promega)

were added at ~1:100 enzyme to substrate ratio (wt:wt), respectively and both samples were then incubated at 37°C overnight in the dark. The resulting peptides from the three digests were dissolved in 20% acetonitrile (ACN) and 2% formic acid and combined into one tube. The sample was stored at -20°C prior to TiO_2 enrichment and LC-MS/MS analysis.

TiO_2 enrichment for phosphopeptide: A TiO_2 column was made by pressure-slurry packing TiO_2 (5-μ partisphere, Whatman, Clifton, NJ) into fused-silica capillary (250 μm i.d.) to a length of 2.5 cm. The peptide mixtures were pressure-loaded onto the column. The column was washed with buffer A and B (see the following section for buffer compositions) in succession and then phosphopeptides were eluted using 250 mM ammonium bicarbonate directly onto an analytical column with a 15 cm bed of 3 μm reversed phase (Aqua C18, Phenomenex, Torrance, CA) and a 5 μm tip. The column was then placed in-line with an Agilent 1200 quaternary HPLC pump (Palo Alto, CA) for mass spectrometry analysis.

HPLC conditions: The HPLC buffer solutions used were as follows: water/acetonitrile/formic acid (95:5:0.1, v/v/v) as buffer A, water/acetonitrile/ formic acid (20:80:0.1, v/v/v) as buffer B. The elution gradient was as follows: 10 min of 100% buffer A, a 5-min gradient from 0 to 15% buffer B, a 65-min gradient from 15 to 45% buffer B, a 15-min gradient from 45 to 100% buffer B, and 5 min of 100% buffer B.

Supplemental Figure Legends

Figure S1. CK2 subunits localize predominantly to the nucleus. (A) Localization of CK2 subunits. CK2 subunits were C-terminally tagged with GFP and integrated at their endogenous loci. All four subunits showed predominant nuclear localization. Cka1-GFP and Ckb1-GFP enriched at the nucleolar region. Three representative images of each strain were shown. (B) Cka1-GFP and Ckb1-GFP does not display kinetochore localization. Kinetochores were identified by Mtw1-RFP. Scale bar: 5 μm.

Figure S2. *In vitro* sites of Mif2 and Ndc10 phosphorylated by CK2. Mif2 and Ndc10 were purified from yeast and phosphorylated by CK2 *in vitro* as described above. The phosphorylated substrates were precipitated by 25% TCA. The phosphorylation sites

were analyzed by mass spectrometry as described in Materials and Methods. The predicted CK2 residues are shown in red, and the predicted Ipl1 residues in blue. The detected phosphorylated residues are underlined.

Figure S3. Mif2 and Ndc10 are phosphorylated by CK2 and Ipl1 independently. (A) The phosphorylation of Mif2-Myc by Ipl1 has no effect on the phosphorylation of Mif2-Myc by CK2, and *vice visa*. (B) Phosphorylation of Ndc10-Myc by Ipl1 has no effect on phosphorylation of Ndc10-Myc by CK2, and *vice visa*. Mif2-Myc or Ndc10-Myc was overexpressed and immobilized on protein G beads through binding to anti-Myc antibodies. The bead-bound Mif2-Myc or Ndc10-Myc was then subjected to a sequential phosphorylation. CK2+Ipl1: CK2 is the first kinase and Ipl1 is the second kinase. Ipl1+CK2: Ipl1 is the first kinase, and CK2 is the second kinase. a: the bead-bound substrates (~100 ng) was incubated with cold ATP and the first kinase (~2 ng) for 30 min. The first kinase was subsequently removed by washing the beads three times with a wash buffer (20 mM HEPES pH7.5, 1 mM EDTA, 1 M KCl). The beads were then incubated with [γ^{32}P]-ATP and the second kinase (~2 ng). b: the bead-bound substrate (~100 ng) was incubated with cold ATP and the first kinase (~2 ng) for 30 min. The first kinase was subsequently removed by washing the beads three times. The beads were then incubated with [γ^{32}P]-ATP alone. c: the bead-bound substrate (~100 ng) was incubated with cold ATP alone for 30 min. The cold ATP was subsequently removed by washing the beads three times. The beads were then incubated with [γ^{32}P]-ATP and the second kinase (~2 ng).

Figure S4. Substitution of Ser325 results in a reduced phosphorylation by CK2 *in vitro*.
Mif2-Myc and Mif2(S325A)-Myc were overexpressed and purified as described above. Substrates (~100 ng) were incubated with [γ^{32}P]-ATP in the presence of kinases (~2 ng). Top panel: autoradiography; lower panel: Coommassie staining.

Figure S5. Mif2 phospho-mutants localize similarly to wild-type Mif2. Viable *mif2* phospho-mutants were C-terminally tagged with GFP at their endogenous loci. CK2

phosphorylation site and putative Aurora B phosphorylation sites are indicated in red and blue, respectively. Representative images were shown. Scale bar: 5 μm.

Reference:

MacCoss, M.J., McDonald, W.H., Saraf, A., Sadygov, R., Clark, J.M., Tasto, J.J., Gould, K.L., Wolters, D., Washburn, M., Weiss, A*., et al.* (2002). Shotgun identification of protein modifications from protein complexes and lens tissue. Proceedings of the National Academy of Sciences of the United States of America *99*, 7900-7905.

Figure S1

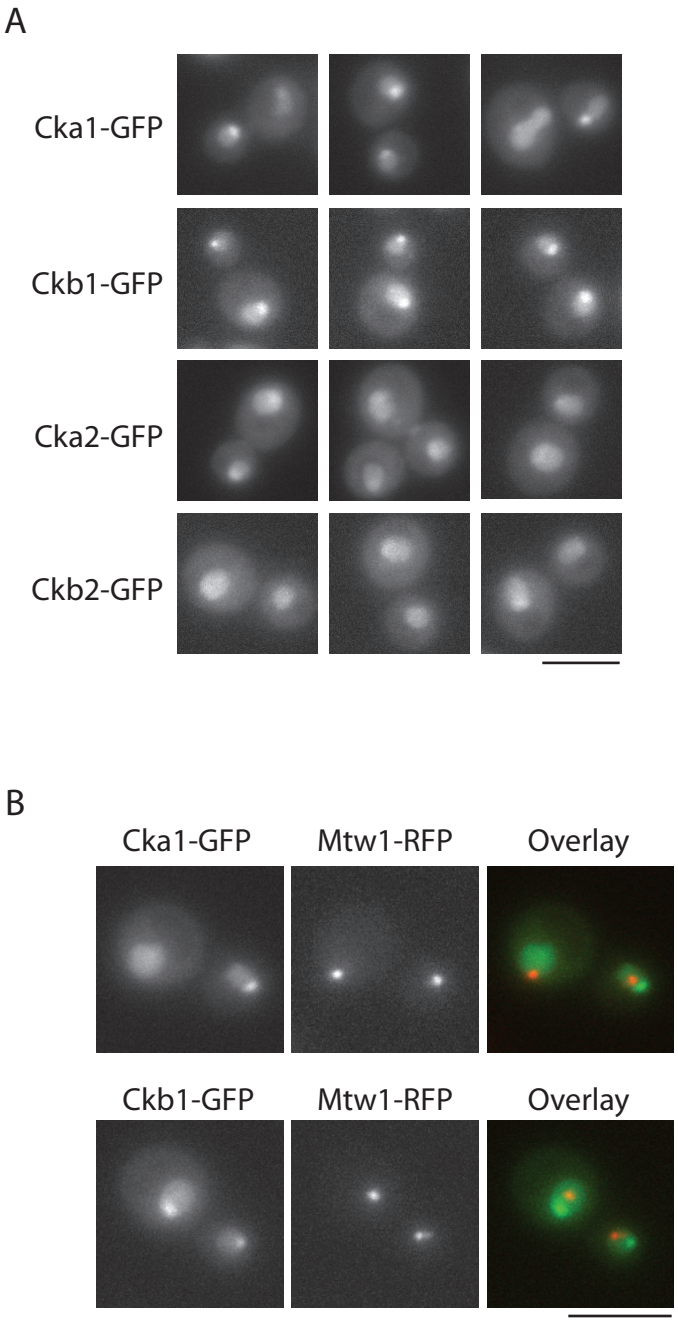

A
Cka1-GFP
Ckb1-GFP
Cka2-GFP
Ckb2-GFP
B
Cka1-GFP
Mtw1-RFP
Overlay
Ckb1-GFP
Mtw1-RFP
Overlay

Figure S2

Predicated CK2 sites are indicated in red, Ipl1 sites in blue. The phosphorylated residues by CK2 *in vitro* were underlined.

Mif2

MDYMKLGLKSRKTGIDVKQDIPKDEYSMENIDDFFKDDETSLISMRRKSRRK**S**SLFLPST
LNGDTKNVLPPFLQS**Y**K**S**QDDEVVQSPSGKGDGSRR**S**SLLSHQSNFLSPANDFEPIEEEP
EQEENDIRGNDFATPITQKLSKPTYKRKYSTRY**S**LDTSESPSVRL**T**PDRITNKNVYSDVP
DLVADEDDDDRVNTSLNTSDNALLEDELEDDGFIPESEEDGDYIESDSSLDSGSDSASDS
DGDNTYQEVEEEAEVNTNDNEDDYIRRQASDVVRTDSIIDRNGLRKSTRVKVAPLQYWRN
EKIVYKRKSNKPVLDIDKIVT**Y**DE**S**EDEEEILAAQRRKKQKKKPTPTRPYNYVP**T**GRPRG
RPKKDPNAKENLIPEDPNEDIIERIESGGIENGEWLKHGILEANVKISDTKEETKDEIIA
FAPNL**S**QTEQVKDTKDENFALEIMFDKHKEYFASGILKLPAISGQKKLSNSFRT**Y**I**T**FHV
IQGIVEVTVCKNKFL**S**VKGS**T**FQIPAFNEYAIANRGNDEAKMFFVQVTVSEDANDDNDKE
LDSTFDTFG*

Ndc10

MRS**S**ILFLLKLMKIMDVQQQQEAMS**S**EDRFQELVDSLKPRTAHQYKTYYTKYIQWCQLNQ
IIPTPEDNSVNSVPYKDLPISAELIHWFLLD**T**LITDDKPGEKREE**T**EDLDEEEENSFKIA
TLKKIIGSLNFLSKLCKVHENPNANIDTKYLESVTKLHTHWIDSQKAITTNETNNTNTQV
LCPPLLKV**S**LNLWNPETNHLSEKFFKTCSEKLRFLVDFQLRSYLNLSFEERSKIRFGSLK
LGKRDRDAIIYHKVTHSAEKKDTPGHHQLLALLPQDCPFICPQTTLAAYLYLRFYGIPSV
SKGDGFPNLNADENGSLLQDIPILRGKSLTTYPREETFSNYYTTVFRYCHLPYKRREYFN
KCNLVYPTWDEDTFRTFFNEENHGNWLEQPEAFAFPDKIPFDFKKIMNFKSPYTSYSTNA
KKDPFPPPKDLLVQIFPEIDEYKRHDYEGLSQNSRDFLDLMEVLRERFLSNLPWIYKFFP
NHDIFQDPIFGNSDFQSYFNDKTIH**S**KG**S**PILSFDILPGFNKIYKNKTNFYSLLIERP**S**Q
LTFA**SS**HNPDTHPTQKQE**S**EGPLQMSQLDTTQLNELLKQQSFEYVQFQTLSNFQILLSVF
NKIFEKLEMKKSSRGYILHQLNLFKI**T**LDERIKKSKIDDADKFIRDNQPIKKEENIVNED
GPNTSRRTKRPKQIRLLSIAD**SS**DE**SST**EDSNVFKKDGESIEDGAYGENEDENDSEMQEQ
LKSMINELINSKISTFLRDQMDQFELKINALLDKILEEKVTRIIEQKLGSHTGKFSTLKR
PQLYMTEEHNVGFDMEVPKKLRTSGKYAETVKDNDDHQAMSTTASP**S**PEQDQEAKSYTDE
QEFMLDK**S**ID**S**IEGIILEWF**T**PNAKYANQCVHSMNKSGNKSWRANCEALYKERK**S**IVEFY
IYLVNHESLDRYKAVDICEKLRDQNEGSFSRLAKFLRKWRHDHQNSFDGLLVYLSN*

Figure S3

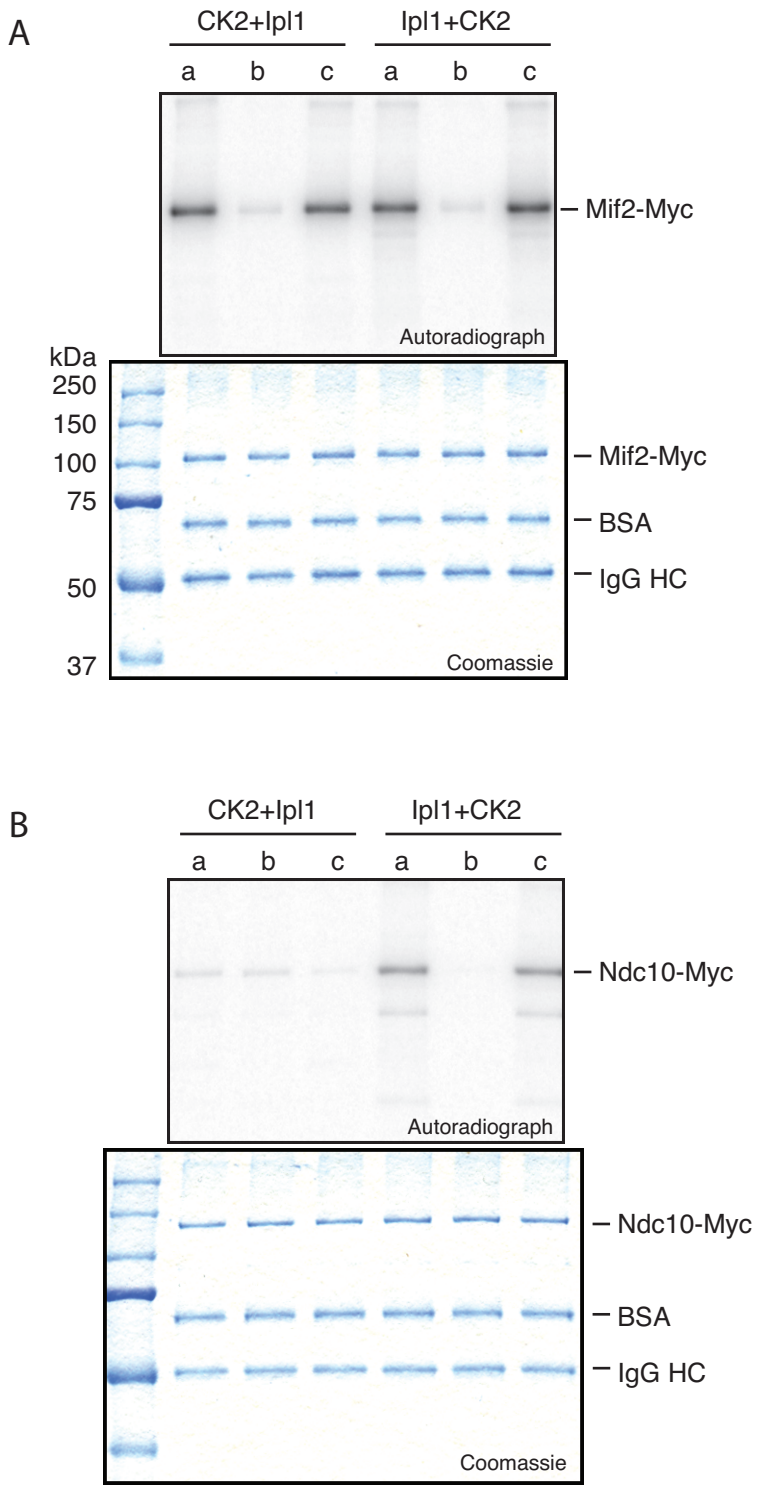

A
CK2+Ipl1
Ipl1+CK2
a
b
c
a
b
c
Mif2-Myc
Autoradiograph
kDa
250
150
100
75
50
37
Mif2-Myc
BSA
IgG HC
Coomassie
B
CK2+Ipl1
Ipl1+CK2
a
b
c
a
b
c
Ndc10-Myc
Autoradiograph
Ndc10-Myc
BSA
IgG HC
Coomassie

Figure S4

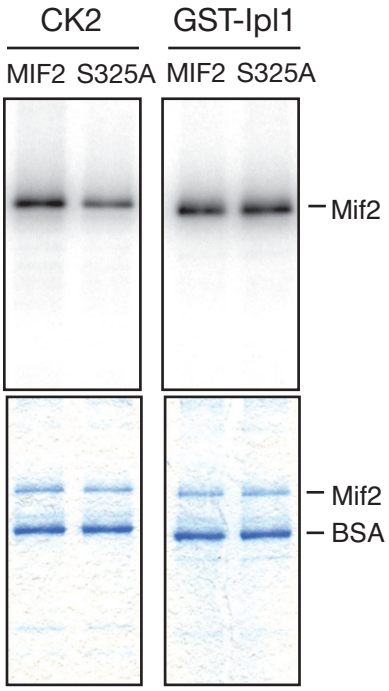
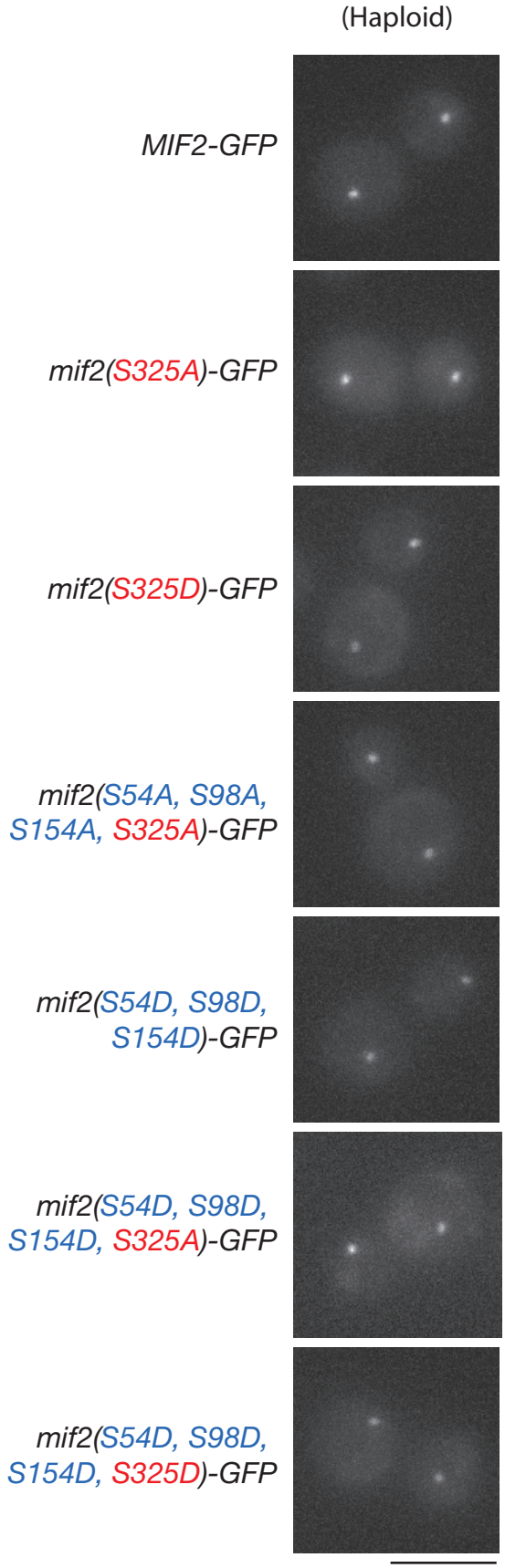


Figure S5


(Haploid)
MIF2-GFP
mif2(S325A)-GFP
mif2(S325D)-GFP
mif2(S54A, S98A, S154A, S325A)-GFP
mif2(S54D, S98D, S154D)-GFP
mif2(S54D, S98D, S154D, S325A)-GFP
mif2(S54D, S98D, S154D, S325D)-GFP