Supplementary Material: Malvezzi et al., 2012 "A structural basis for kinetochore recruitment of the Ndc80 complex via two distinct centromere receptors"

Figures S1-S6 with Figure legends

Supplementary Table 1 (Yeast strains used in this study)

Supplementary Table 2 (Accession numbers)

Supplementary Experimental procedures

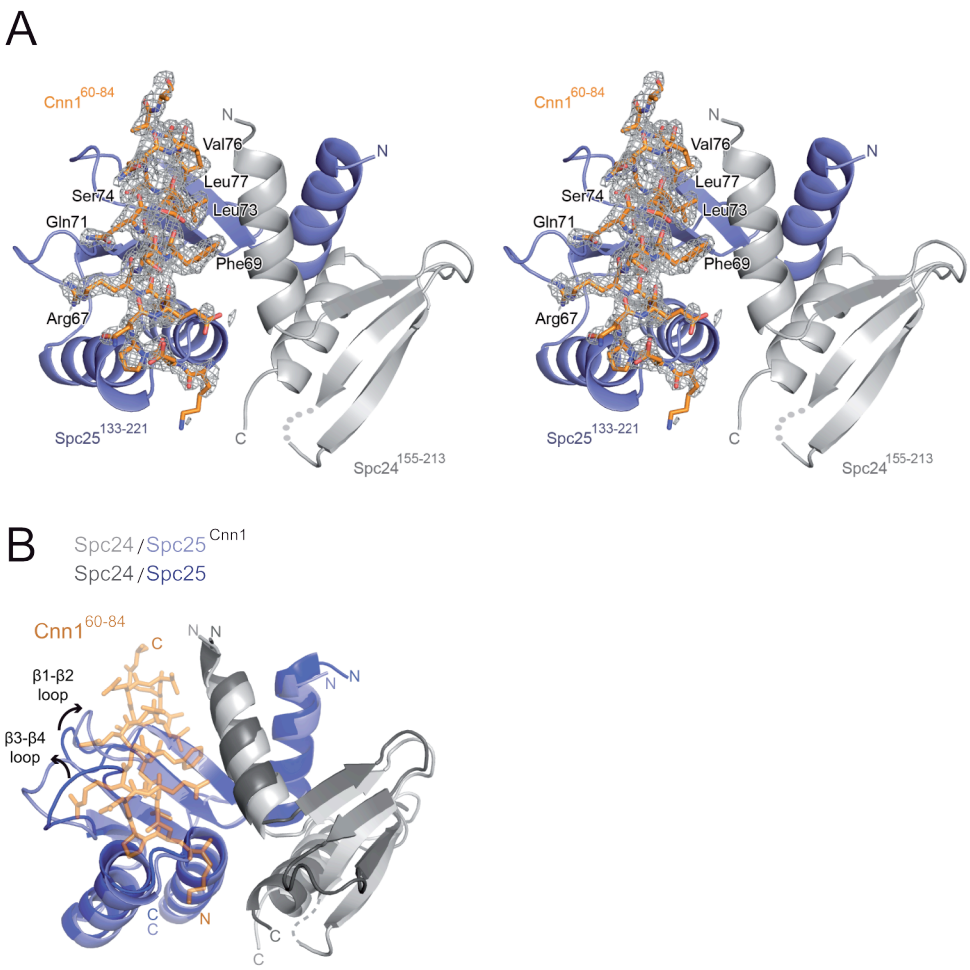


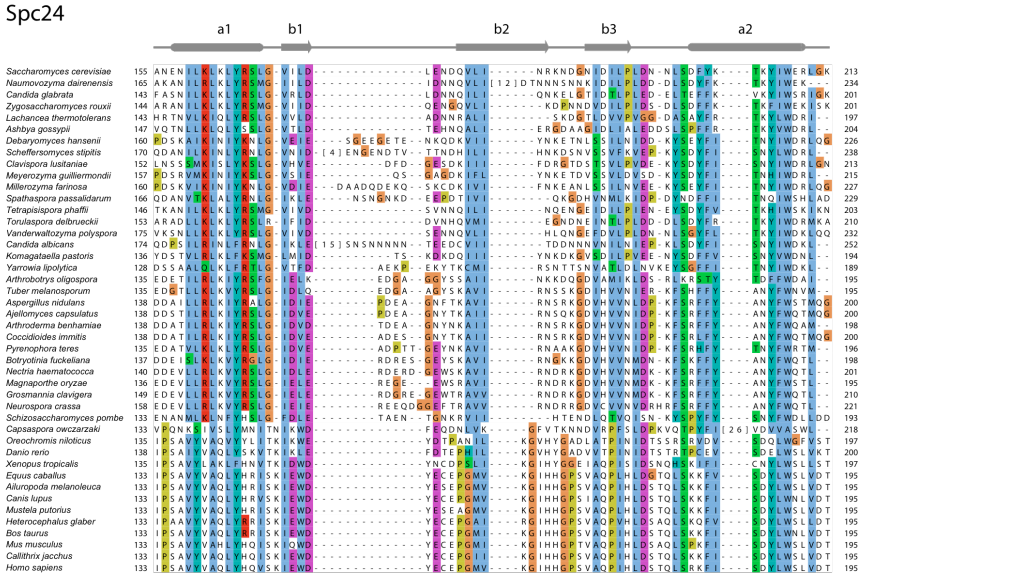
Figure S1 Malvezzi et al., 2012

Figure S1: Electron density of the Cnn1 peptide bound to Spc24-25 and Spc24-25 changes upon Cnn1-binding.

A: Stereo view of the $2F_o$-F_c difference Fourier map (contoured at 1.0 σ and displayed as grey cage) of the Spc24$^{155-213}$-25$^{133-221}$-Cnn1$^{60-84}$ crystal structure located at the Spc24-25 interface and calculated without the Cnn1 peptide. Spc24 and Spc25 are represented according to their secondary structure, in light grey and slate blue, respectively. Cnn1 is shown as sticks with C in orange, N in blue and O in red. The labels refer to the residues of Cnn1 involved in Spc24-25

binding and observable in the figure. Ser74 is also labeled. A dotted line indicates the residue Lys184 of Spc24 not visible in the electron density (see experimental procedures). **B**: Superposition of $Spc24^{155-213}$-$25^{133-221}$-$Cnn1^{60-84}$ (Spc24-25^{Cnn1}) and $Spc24^{155-213}$-$25^{133-221}$ (Spc24-25; Wei et al., 2006), performed on $Spc25^{133-221}$. The secondary structure of Spc24 and Spc25 in Spc24-25^{Cnn1} is shown in light grey and slate blue, respectively; in the Spc24-25 crystal structure, in dark grey and blue, respectively. Cnn1 is displayed in orange sticks (transparency 60%). The arrows indicate the movement of the β1-β2 and β3-β4 loops upon Cnn1-binding.

Spc24



Spc25

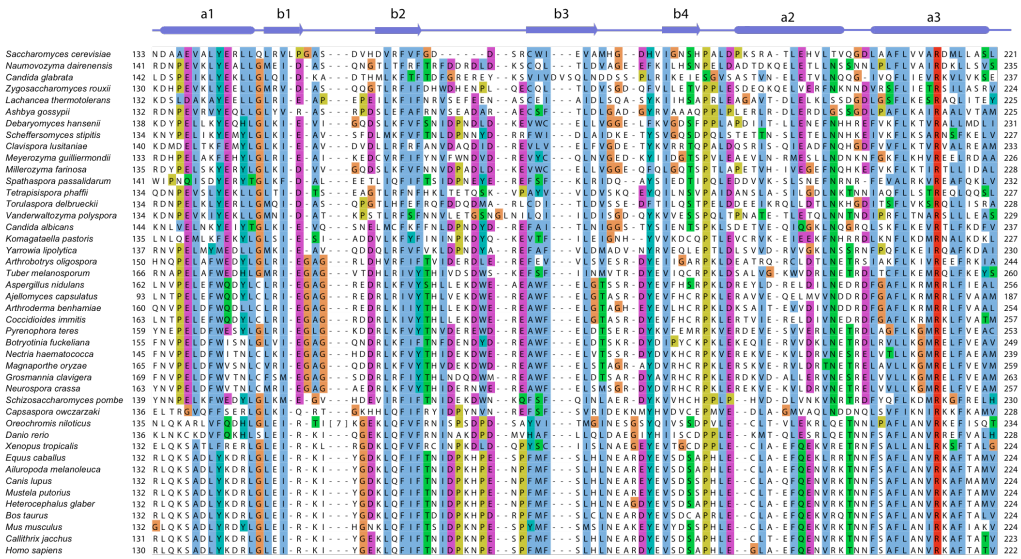


Figure S2 Malvezzi et al., 2012

Figure S2: Sequence alignment of Spc24 and Spc25 homologues Background coloring of the residues is based on the clustalx coloring scheme.

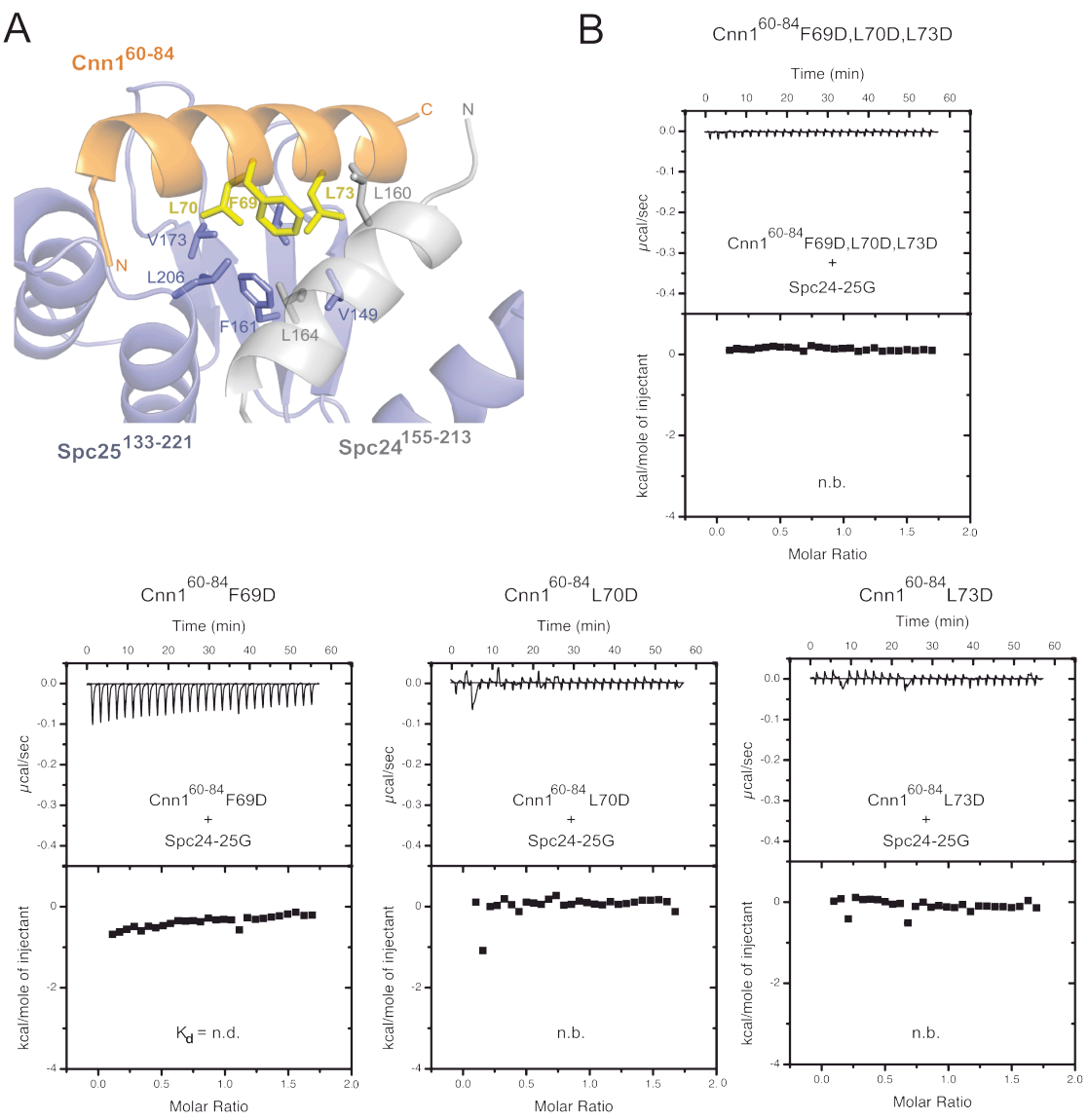


Figure S3 Malvezzi et al., 2012

Figure S3: Validation of the Spc24-25/Cnn1 interface by mutation of Cnn1

A: Role of the Cnn1$^{60-84}$ amino acids F69, L70 and L73 in Spc24$^{155-213}$-25$^{133-221}$ binding. The secondary structure of Spc24, Spc25 and Cnn1 is displayed in light grey, slate blue and orange, respectively (transparency 40%). The Cnn1 residues

F69, L70 and L73, mutated individually or in combination to aspartic acid for validating the Spc24$^{155-213}$-25$^{133-221}$/Cnn1$^{60-84}$ interface, are highlighted as yellow sticks. Spc24 and Spc25 residues that establish van der Waals contacts with F69, L70 or L73 are displayed as sticks. **B**: Isothermal titration calorimetry performed by titrating wild-type Spc24-25 globular domain (Spc24-25G) Cnn1$^{60-84}$ triple mutant (F69D, L70D, L73D) or each single mutant. n.b. = no binding. n.d. = not definable.

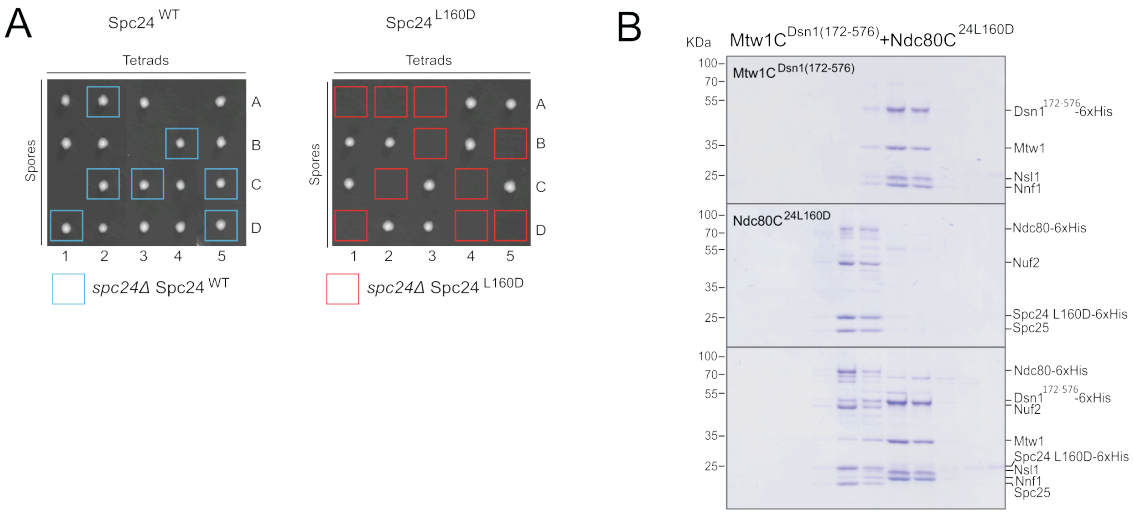


Figure S4 Malvezzi et al., 2012

Figure S4: The L160D mutation of Spc24 is lethal and disrupts interaction between Ndc80 and Mtw1 complexes *in vitro*.

A: Asci dissection of *Δspc24*/Spc24 Spc24^{WT} (left) and *Δspc24*/Spc24 Spc24^{L160D} (right). The viable *Δspc24* Spc24^{WT} spores are highlighted with a light blue square. The *Δspc24* Spc24^{L160D} spores, marked with a red rectangle, failed to be recovered, indicating that L160D is a lethal Spc24 mutation. **B**: Elution profiles and Coomassie-stained gels of analytical size-exclusion chromatography performed with 4 μM Mtw1$^{Dsn1(172-576)}$ complex (Mtw1C$^{Dsn1(172-576)}$), 4 μM Ndc80 complex including the Spc24^{L160D} mutant (Ndc80C^{24L160D}) and their combination. The lack of a shift to earlier elution volumes of both molecules indicates abolished complex formation.

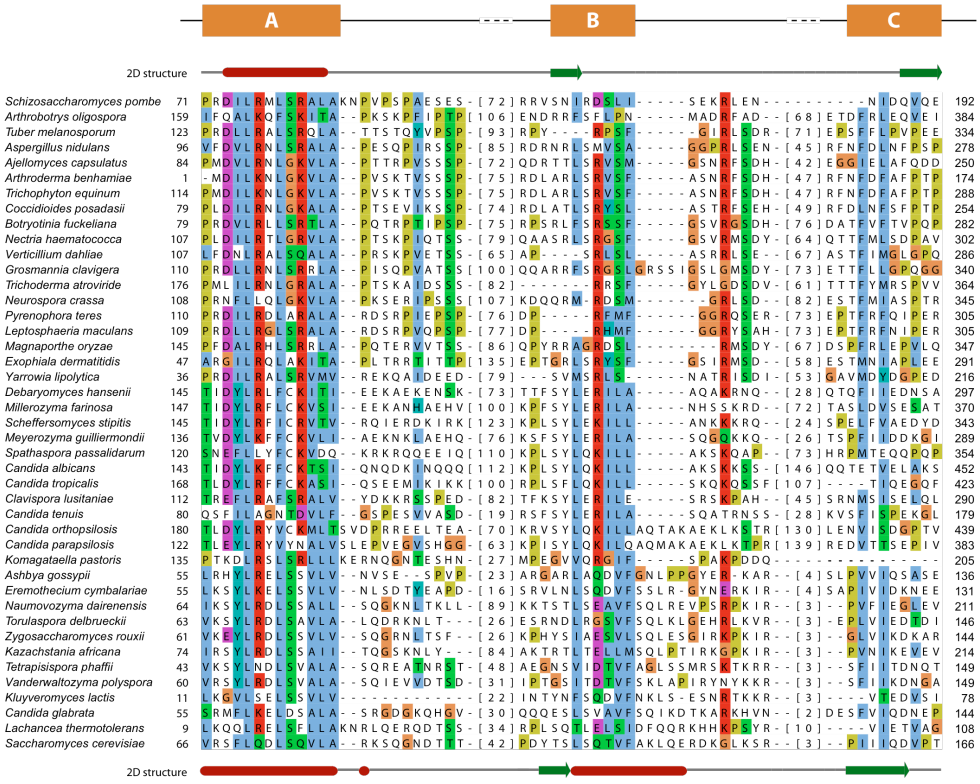
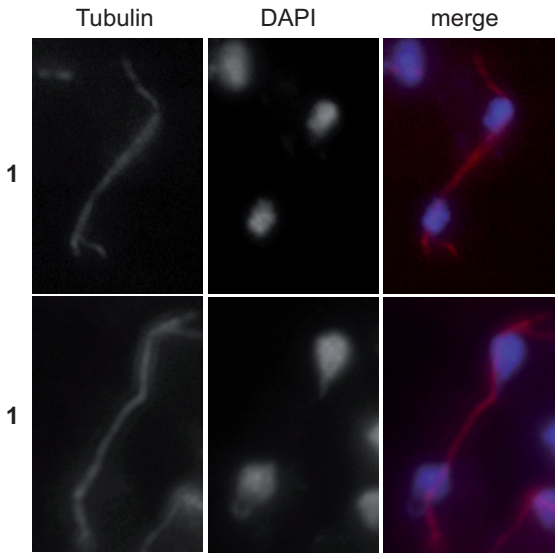


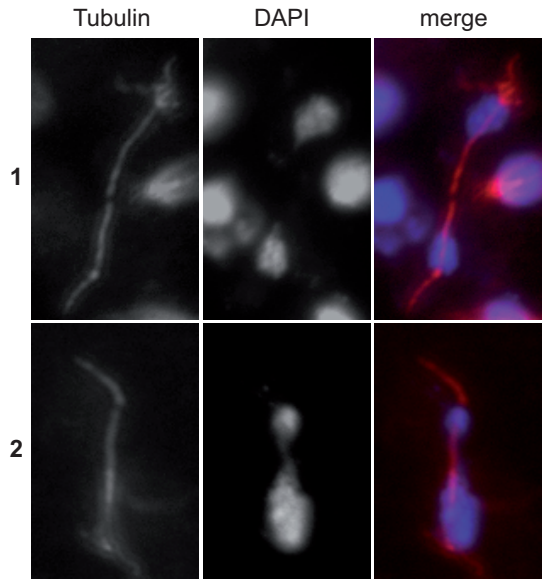
Figure S5 Malvezzi et al., 2012

Figure S5: Multiple sequence alignment of an N-terminal region in Cnn1 homologues

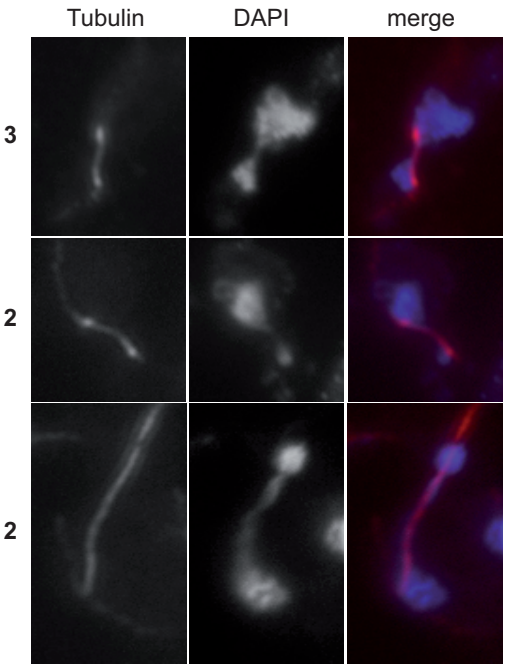
S. cerevisiae (above) and *S. pombe* (below) secondary structure predictions are derived from the Jpred 3 server (alpha helices in red, beta strands in green). Background coloring of the residues is based on the clustalx colouring scheme. The number of omitted residues is indicated in brackets.



WT



Nuf2-13myc Nnf1-3HA



Nuf2-13myc Nnf1-3HA *Δcnn1* Cnn1 16A

1: equal, wild-type segregation
2: unequal segregation
3: “cut” phenotype: unequal segregation with short spindle and cut DNA

Figure S6: The phospho-inhibiting Cnn1 16A mutant shows a variety of chromosome segregation defects.

Immunofluorescence microscopy performed on large budded cells of the indicated strains grown at the restrictive temperature (37°C). DNA is stained with DAPI and microtubules visualized using anti-tubulin antibody. The different phenotypic categories are indicated with a number and described below the panel.

Supplementary Table 1

Yeast strains

Strain	Genotype	Source
SWY572	Mat a ade2-1, his3Δ200, ura3-52, leu2-3,11::Cnn1(T3A, T21A, T42A, S177A, S192) ΔHF-TetR::LEU2	This study
SWY573	Mat a ade2-1, his3Δ200, ura3-52, leu2-3,11::Cnn1(delta 105-270) ΔHF-TetR::LEU2	This study
SWY574	Mat a ade2-1, his3Δ200, ura3-52, leu2-3,11::Cnn1(delta 193-270) ΔHF-TetR::LEU2	This study
SWY579	Mat a ade2-1, his3Δ200, ura3-52, leu2-3,11::Cnn1(T3D, T21D, T42D, S74D, S177D, S192D) ΔHF-TetR::LEU2	This study
SWY601	Mat alpha, ura3-52, lys2-801, ade2-1, Cnn1-6xFlag::KanMX, Nuf2-13xmyc::TRP1, cdc15-2::LEU	This study
SWY605	Mat a ade2-1, his3Δ200, ura3-52, leu2-3,11::Cnn1ΔHF-TetR (S74D)::LEU2	This study
SWY606	Mat a ade2-1, his3Δ200, ura3-52, leu2-3,11::Cnn1ΔHF-TetR (delta175-270)::LEU2	This study
SWY607	Mat a ade2-1, his3Δ200, ura3-52, leu2-3,11::Cnn1ΔHF-TetR (delta 130-166)::LEU2	This study

SWY624	Mat a ade2-1, his3Δ200, ura3-52, leu2-3,11::Cnn1ΔHF-TetR (delta 91-125)::LEU2	This study
SWY627	Mat a, cdc15-2, leu2::PDS1-myc18::LEU2, ura3::pGAL-MPS1-myc::URA3, Cnn1-6xFlag::KanMX	This study
SWY631	Mat a, GAL-1xmyc-MPS1::URA3, Cnn1-6xFlag::KanMX	This study
SWY637	MATa, leu2-3,112::Cnn1-5A-6xFlag::LEU2, ura3-52, ade2-1, cnn1Δ::HIS3	This study
SWY638	MATa, leu2-3,112::Cnn1-11A-6xFlag::LEU2, ura3-52, ade2-1, cnn1Δ::HIS3	This study
SWY630	Mat a, lys2-801, ura3-52, cnn1Δ::HIS3, leu2-3,112::Cnn1-WT-6xFlag::LEU2	This study
SWY544	Mat a ade2-1, his3Δ200, ura3-52, leu2-3,11::Cnn1ΔHF-TetR::LEU2	Schleiffer et al., 2012
SWY545	Mat a ade2-1, his3Δ200, ura3-52, leu2-3,11::Cnn1ΔHFΔ65-79-TetR::LEU2	Schleiffer et al., 2012
SWY643	Mat a, lys2-801, ura3-52, leu2-3,112::Cnn1-16A-6xFlag::LEU2, Nuf2-13xmyc::TRP, Nnf1-3xHA::KanMX	This study
SWY648	Mat a, ura3-52, ade2-1, Nuf2-13xmyc::TRP1, Nnf1-3xHA::KanMX, cnn1Δ::HIS3	This study
SWY650	Mat a, lys2-801, ura3-52, leu2-3,112::Cnn1-WT-6xFlag::LEU2, Nuf2-13xmyc::TRP, Nnf1-3xHA::KanMX	This study
SWY344	Mat alpha, leu2-3,112, his3Δ200, dsn1Δ::HIS3, ura3-52, (pRS316-Dsn1-WT-URA3)	Hornung et al., 2011
VFY85	Mat a/alpha, Ade2/ade2-1, his3Δ200/his3Δ200, ura3-52/ura3-52, Lys2/lys2-801, leu2-3,112/Spc24Δ::LEU2	This study
FMY13	Mat a/alpha, Ade2/ade2-1, leu2-3,112/leu2-3,112, ura3-52/ura3-52, Lys2/lys2-801, his3Δ200/Spc25Δ::HIS3	This study

Supplementary Table 2: Organisms and accession numbers:

Spc24	
Saccharomyces cerevisiae	NP_013835.1
Naumovozyma dairenensis	XP_003671689.1
Candida glabrata	XP_448382.1
Zygosaccharomyces rouxii	XP_002498858.1
Lachancea thermotolerans	XP_002552913.1
Ashbya gossypii	NP_985750.1
Debaryomyces hansenii	XP_457153.1
Scheffersomyces stipitis	XP_001383793.1
Clavispora lusitaniae	XP_002617657.1
Meyerozyma guilliermondii	XP_001485425.1
Millerozyma farinosa	CCE72763.1
Spathaspora passalidarum	EGW33243.1
Tetrapisispora phaffii	XP_003686554.1
Torulaspora delbrueckii	XP_003678746.1
Vanderwaltozyma polyspora	XP_001647470.1
Candida albicans	XP_722533.1
Komagataella pastoris	XP_002490616.1
Yarrowia lipolytica	XP_503439.1
Arthrobotrys oligospora	EGX46547.1
Tuber melanosporum	XP_002836028.1
Aspergillus nidulans	XP_663828.1
Ajellomyces capsulatus	XP_001538360.1
Arthroderma benhamiae	XP_003016504.1
Coccidioides immitis	XP_001245621.1
Pyrenophora teres	XP_003295680.1
Botryotinia fuckeliana	XP_001548924.1
Nectria haematococca	XP_003040061.1
Magnaporthe oryzae	XP_369388.1
Grosmannia clavigera	EFX03083.1
Neurospora crassa	Q7S8M2.1
Schizosaccharomyces pombe	NP_596128.1
Capsaspora owczarzaki	EFW45374.1
Oreochromis niloticus	XP_003443129.1
Danio rerio	NP_001019611.1
Xenopus tropicalis	NP_989057.1
Equus caballus	XP_001490260.1
Ailuropoda melanoleuca	XP_002921402.1
Canis lupus	XP_853911.1
Mustela putorius	AES07274.1
Heterocephalus glaber	EHB12947.1
Bos taurus	NP_001068859.1

Mus musculus	NP_080558.1
Callithrix jacchus	XP_002761796.1
Homo sapiens	NP_872319.1
Spc25	
Saccharomyces cerevisiae	NP_010934.1
Naumovozyma dairenensis	XP_003667454.1
Candida glabrata	XP_449857.1
Zygosaccharomyces rouxii	XP_002497939.1
Lachancea thermotolerans	XP_002554302.1
Ashbya gossypii	NP_986227.1
Debaryomyces hansenii	XP_460184.2
Scheffersomyces stipitis	XP_001385929.2
Clavispora lusitaniae	XP_002614624.1
Meyerozyma guilliermondii	XP_001483109.1
Millerozyma farinosa	CCE79629.1
Spathaspora passalidarum	EGW34088.1
Tetrapisispora phaffii	XP_003684562.1
Torulaspora delbrueckii	XP_003680731.1
Vanderwaltozyma polyspora	XP_001646965.1
Candida albicans	XP_712168.1
Komagataella pastoris	XP_002494316.1
Yarrowia lipolytica	XP_500108.1
Arthrobotrys oligospora	EGX44726.1
Tuber melanosporum	XP_002835120.1
Aspergillus nidulans	XP_658996.1
Ajellomyces capsulatus	XP_001544957.1
Arthroderma benhamiae	XP_003011093.1
Coccidioides immitis	XP_001243298.1
Pyrenophora teres	XP_003295304.1
Botryotinia fuckeliana	XP_001553139.1
Nectria haematococca	XP_003049634.1
Magnaporthe oryzae	XP_362871.1
Grosmannia clavigera	EFW99016.1
Neurospora crassa	XP_001728160.1
Schizosaccharomyces pombe	NP_588208.1
Capsaspora owczarzaki	EFW43520.1
Oreochromis niloticus	XP_003447378.1
Danio rerio	NP_001116529.1
Xenopus tropicalis	NP_001005041.1
Equus caballus	XP_001497531.1
Ailuropoda melanoleuca	XP_002916555.1
Canis lupus	XP_545510.3
Mustela putorius	AES07275.1
Heterocephalus glaber	EHB17922.1

Bos taurus	NP_001029402.1
Mus musculus	NP_001186052.1
Callithrix jacchus	XP_002749343.1
Homo sapiens	NP_065726.1
Cnn1	
Schizosaccharomyces pombe	NP_595115.1
Arthrobotrys oligospora	EGX48959.1
Tuber melanosporum	XP_002836693.1
Aspergillus nidulans	XP_662356.1
Ajellomyces capsulatus	XP_001544140.1
Arthroderma benhamiae	XP_003014739.1
Trichophyton equinum	EGE09556.1
Coccidioides posadasii	XP_003066562.1
Botryotinia fuckeliana	XP_001545878.1
Nectria haematococca	XP_003053257.1
Verticillium dahliae	EGY20957.1
Grosmannia clavigera	EFW98428.1
Trichoderma atroviride	EHK42916.1
Neurospora crassa	XP_964520.1
Pyrenophora teres	XP_003299018.1
Leptosphaeria maculans	CBX94982.1
Magnaporthe oryzae	XP_366656.1
Exophiala dermatitidis	EHY54348.1
Yarrowia lipolytica	XP_500233.1
Debaryomyces hansenii	XP_461484.2
Millerozyma farinosa	CCE85478.1
Scheffersomyces stipitis	XP_001385381.2
Meyerozyma guilliermondii	XP_001485403.1
Spathaspora passalidarum	EGW35176.1
Candida albicans	XP_717589.1
Candida tropicalis	XP_002549682.1
Clavispora lusitaniae	XP_002614395.1
Candida tenuis	EGV60430.1
Candida orthopsilosis	CCG21148.1
Candida parapsilosis	CCE43188.1
Komagataella pastoris	XP_002493147.1
Ashbya gossypii	NP_985803.2
Eremothecium cymbalariae	XP_003645120.1
Naumovozyma dairenensis	XP_003668899.1
Torulaspora delbrueckii	XP_003681422.1
Zygosaccharomyces rouxii	XP_002499334.1
Kazachstania africana	CCF60088.1
Tetrapisispora phaffii	XP_003688342.1
Vanderwaltozyma polyspora	XP_001643240.1

Kluyveromyces lactis	XP_451039.1
Candida glabrata	XP_445262.1
Lachancea thermotolerans	XP_002555079.1
Saccharomyces cerevisiae	NP_116704.1
Dsn1	
Saccharomyces cerevisiae	EGA58243.1
Schizosaccharomyces pombe	NP_595459.1
Ustilago maydis	XP_760485.1
Batrachochytrium dendrobatidis	EGF78698.1
Kluyveromyces lactis	XP_453754.1
Ajellomyces dermatitidis	XP_002623137.1
Coprinopsis cinerea	XP_001838507.2
Coccidioides immitis	XP_001246294.1
Gibberella zeae	XP_390317.1
Aspergillus fumigatus	XP_001481632.1
Danio rerio	XP_001923648.1
Xenopus tropicalis	XP_002940613.1
Pyrenophora teres	XP_003299634.1
Neurospora crassa	XP_961430.1
Gallus gallus	NP_001038125.1
Homo sapiens	NP_001138787.1
Capsaspora owczarzaki	EFW43264.1
Candida glabrata	XP_449189.1
Monosiga brevicollis	XP_001744789.1
Phytophthora infestans	XP_002900073.1
Salpingoeca sp.	EGD83324.1

Supplementary Experimental procedures

Details of protein expression and purification

All versions of His-tagged Spc24-25 full length and globular domain were cloned in cassette 1 and 2 of the pETduett-1 vector (Novagen) and co-expressed in *E. coli* strain Bl21-CodonPlus(DE3)-RIL (Agilent Technologies) ON at 18°C after induction at OD_{600} 0.6-0.7 with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG). N-terminal 6XHis-tagged Cnn1ΔHFD and Cnn1ΔHFD$^{\Delta BC}$ were cloned in cassette 1 of the pETduett-1 vector and expressed in *E. coli* strain Rosetta2(DE3)pLysS for 3h at 37°C after induction with 0.5 mM IPTG. Mtw1$^{Dsn1(172-576)}$ complex, Mtw1$^{Dsn1(172-547)}$ complex, Ndc80 complex-subunits Nuf2 and Ndc80 were cloned and expressed as described in Schleiffer et al., 2012, Lampert et al., 2010. For the purification of all versions of Spc24-25 full length and globular domain, binding and washing to Ni-NTA agarose beads (Qiagen) was performed in 30 mM HEPES (pH 7.0), 300 mM NaCl, 30 mM imidazole and 1mM dithiothreitol (DTT). After elution with 250 mM imidazole, the complexes were subjected to Ndc80 complex-reconstitution with Ndc80-Nuf2 (see below) or loaded on Superdex 200 HiLoad 16/60 (GE Healthcare) column equilibrated in 10 mM Bis-Tris propane (pH7.6), 250 mM NaCl and 10 mM DTT for crystallization experiments or 20 mM Hepes (pH 7.5) and 120 mM NaCl for all other purposes. 6xHis-Ndc80-Nuf2 complex was bound to and washed on Ni-NTA agarose beads with 30 mM HEPES (pH7.5), 500 mM NaCl and 30 mM imidazole, eluted with 30 mM HEPES (pH7.5), 150 mM NaCl and 250 mM imidazole, subjected to Ndc80 complex-reconstitution *in vitro* with full length

Spc24-25 and loaded on Superdex 200 HiLoad 16/60 column equilibrated in 20 mM Hepes (pH 7.5) and 120 mM NaCl. Ni-NTA purification of 6xHis-Cnn1ΔHFD or 6xHis-Cnn1ΔHFD$^{\Delta BC}$ was performed in 25 mM HEPES (pH7.5), 150 mM NaCl, 30 mM imidazole and 5% glycerol, the protein eluted with 250 mM imidazole and subsequently loaded on Superdex 200 HiLoad 16/60 column equilibrated in 20 mM Hepes (pH 7.5), 120 mM NaCl and 5% glycerol. The purification of Mtw1$^{Dsn1(172-576)}$ and Mtw1$^{Dsn1(172-547)}$ complexes was performed as described in Hornung et al., 2011, with the following modification: the anion-exchange chromatography was performed applying a first linear gradient of 10 bed volumes from 80 mM NaCl to 240 mM NaCl and a second elution step at 500 mM NaCl in 30 mM Tris-HCl (pH 8.5) and 5% glycerol.