Inventory of Supplementary Material

Supplemental Figures

Supplemental Figure 1. Aurora B phosphorylates KNL-1 and the Mis12 complex. *(A)* Table summarizing the sequence coverage obtained for each protein of the KMN network by mass spectrometry. (*B*) Table summarizing the phosphorylated peptides identified by mass spectrometry following purification of GFP-Mis12^{LAP} from HeLa cells and in vitro phosphorylation of recombinant hMis12 complex, *C. elegans* MIS-12 complex, and KNL-1 by Aurora B. (*C*) Sequence alignments of Dsn1/KNL-3 and the N-terminus of KNL1 from human, chicken and *C. elegans* showing the sequence conservation of the regions containing the Aurora B phosphorylation sites. The sequences were aligned using CLUSTALW and formatted with ESPRIPT (Gouet et al., 1999). Sites identified as Aurora B phosphorylation sites across species are marked with a green triangle. Sites unique to *C. elegans* are indicated with a cyan star. Aurora B phosphorylates the N-terminus of KNL-1/hKNL1 and the hDsn1/KNL-3 subunit of the Mis12 complex. (*D*) Coomassie-stained gels and corresponding autoradiogram showing the protein levels and levels of phosphorylation respectively. In vitro Ipl1 kinase activity assay using human hKNL1 and hMis12 complex and *C. elegans* KNL-1 and MIS-12 complex and the corresponding non-phosphorylatable mutants.

Supplemental Figure 2. Analysis of KMN network assembly and kinetochore localization. Related to Figure 1. (*A*) Coomassie gel showing the KMN recombinant proteins after a two-step purification for the wild type, SA mutants, and SD mutants. KNL-1-6xHis was co-expressed with untagged MIS-12 complex and co-purified as described in (Cheeseman et al., 2006). (B) Immunofluorescence images acquired using antibodies against hDsn1 and phospho-Histone H3 (serine 10) in HeLa cells under control conditions or after 3 hr treatment with ZM447439. Scale bar, 10 μm.

Supplemental Figure 3. Analysis of KMN network microtubule binding activity. Related to Figure 2. (*A*) Western blot probed with antibodies against hKNL1 showing the pellet samples from microtubule binding assays with the N-terminus of human KNL1 (residues 1-86) for either the wild type or phosphomimetic (S24 and S60) mutant. (*B*) Western blots probed with the indicated antibodies to assess the microtubule binding properties of either the wild type KMN network, or KMN network with combinations of phosphomimetic mutants in KNL-1, KNL-3, and NDC-80. (*C*) Western blots probed with the indicated antibodies showing the microtubule binding properties of the KNL-1/MIS-12 complex (without the NDC-80 complex) in the wild type or KNL-1 and/or KNL-3 phosphomimetic states. (*D*) Co-sedimentation assays with wild type KMN network, and KMN network composed of NDC80$_{SA}$ complex and KNL-1$_{SA}$/MIS-12 complex$_{SA}$. (*E*) Graph showing the microtubule binding activity for 50 nM input protein of the $K_{SA}M_{SA}N_{SA}$ mutant and the wild type KMN network. The points were fitted with MATLAB using a modified Hill equation. Error bars represent the standard deviation.

Supplemental Figure 1

A

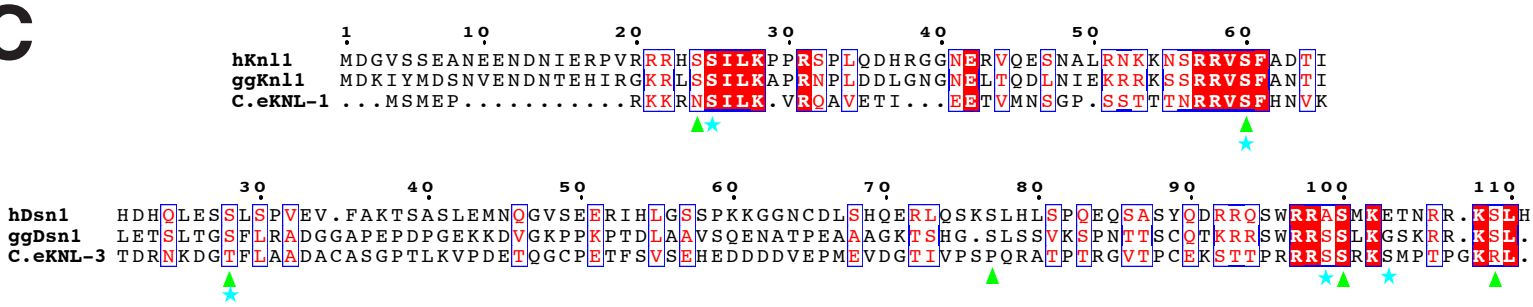
% Sequence Coverage

Protein	Endogenous KMN Network	Protein	Recombinant hMis12 Complex	Protein	Recombinant KNL-1/MIS-12 Complex
Dsn1	81.2	Dsn1	64.3	KNL-3	75.8
Mis12	58.0	Mis12	40.0	MIS-12	32.6
Nnf1	80.4	Nnf1	51.7	KBP-1	76.5
Nsl1	53.7	Nsl1	41.6	KBP-2	96.0
Nuf2	56.2			KNL-1	68.8
Ndc80	69.0				
Spc24	86.3				
Spc25	66.5				
hKNL1	38.2				

B

Organism	Peptide	Site	In vitro	In vivo	# of peptides
Dsn1/KNL-3					
Human	K.THDHQLESS*LSPVEVFAK.T	S28	+	+	16
Human	K.S*LHLSPQEQSASYQDR.R	S76	+	+	9
Human	RRAS*MKETNRRKSLHPIHQ	S100	+	-	western blot
Human	R.RKS*LHPIHQGITELSR.S	S109	+	+	49
KNL-1/Blinkin					
C. elegans	K.KRNS*ILKVRQAVETIEETVMNSGPSSTTTNR.R	S11	+	-	2
Human	R.HS*SILKPPRS*PLQDLR.G	S25, S33		+	2
Human	R.RVS*FADTIK.V	S60		+	1

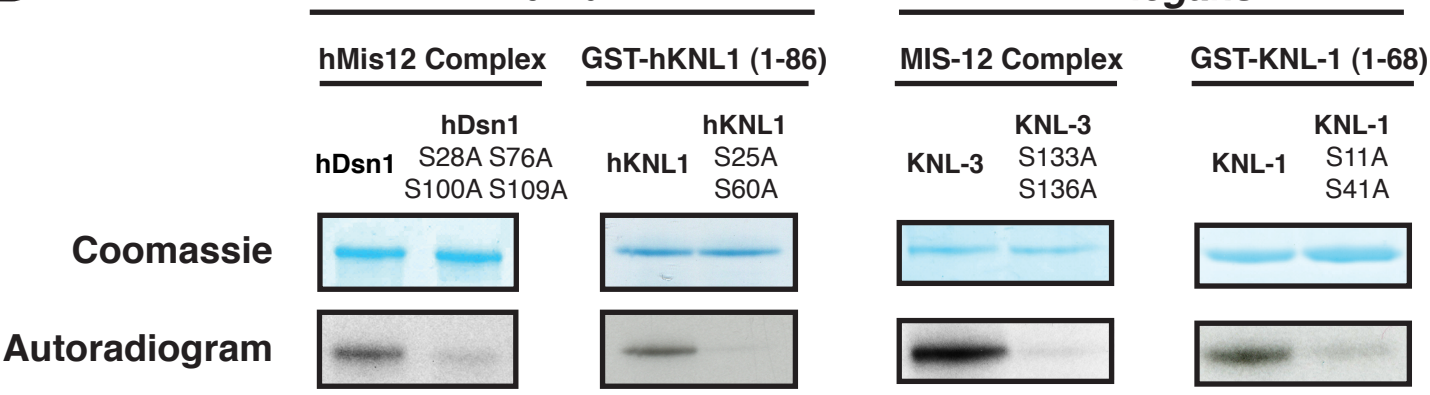
C



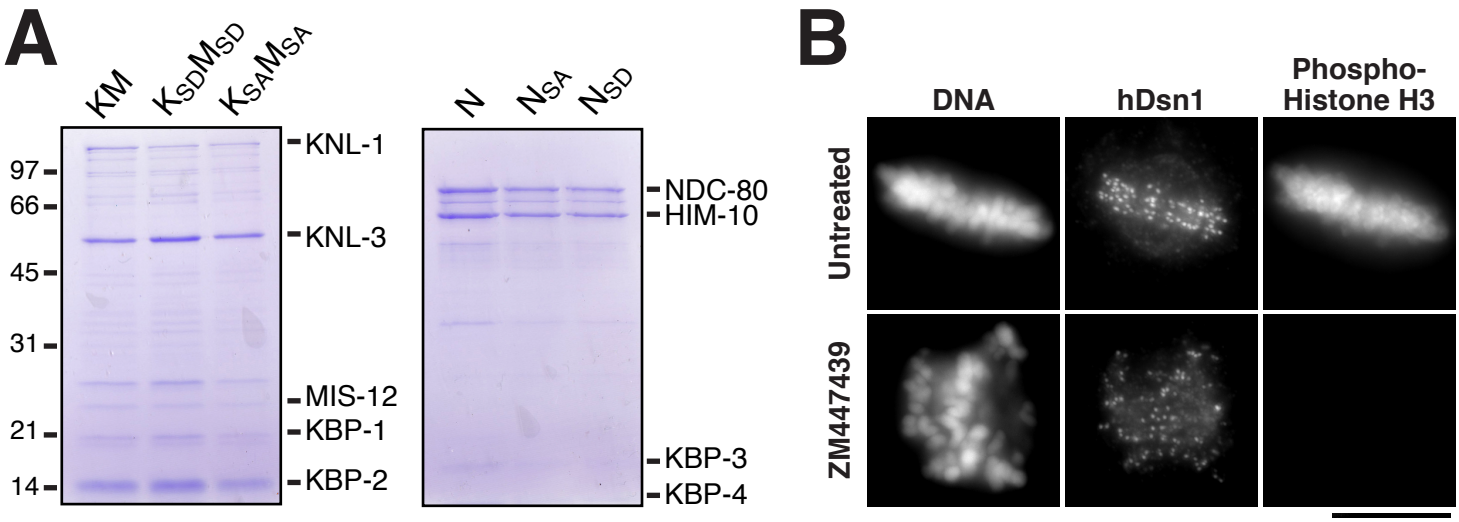
▲ Conserved Aurora B phosphorylation sites found in human, chicken

★ Aurora B phosphorylation sites found in *C. elegans*

D



Supplemental Figure 2



Supplemental Figure 3