## **Supplemental Data**

## The Conserved KMN Network Constitutes

## the Core Microtubule-Binding

### Site of the Kinetochore

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#### Figure S1. Fractionation of MIS-12 Complex Subunits

Western blots showing the behavior of KNL-3, KBP-1, KBP-2 and MIS-12 (FLAGtagged) during purifications with a 6xHis tagged KNL-3 (see Figure 1F) or 6xHis tagged KNL-1 (see Figure 1H). All subunits co-fractionate with the tagged protein during the different steps of the purification, but MIS-12 is reduced relative to the other subunits in purifications with KNL-1-6xHis. All subunits are also detectable near the column void in the KNL-1/MIS-12 complex + NDC-80 complex (KM+N) mixture (see Fig. 2A-7).



## Gel Filtration of C. elegans Embryo Extract

#### Figure S2. Gel Filtration of Embryo Extracts

To characterize the biochemical properties of the endogenous KMN network, we performed gel filtration of *C. elegans* embryo extracts. Western blots showing the fractionation of the indicated subunits under low salt (50 mM) and high salt (750 mM) conditions. All tested subunits co-fractionated near the column void volume suggesting the existence of a large oligomeric assembly that exceeds the 372.1 kD combined molecular weights of the network constituents. At higher salt concentrations (750 mM), the pool near the column void is largely retained indicating that the oligomeric state is not a consequence of low ionic strength buffer conditions. Size standards are identical to Figure 2.

## Α



# Figure S3. Concentration-Dependent Changes in Binding Properties of the NDC-80 Complex and KNL-1/MIS-12 Complex

(*A*) The concentration of NDC-80 complex was varied from 50 nM to 5  $\mu$ M with the microtubule concentration held constant at 5  $\mu$ M. Loading of supernatant and pellet fractions was adjusted relative to the lowest tested concentration (50 nM) to allow comparison across the entire tested concentration range. (*B*) Graph depicting the

average percent bound complex over the input range from 2 different experiments compared to a theoretical prediction for a 4  $\mu$ M K<sub>D</sub> interaction. For simplicity, the theoretical curve is for a bi-molecular interaction and does not take into account the multiplicity of connected binding sites on the microtubule lattice. (*C*) & (*D*) Scatchard plots depicting the anomalous binding observed for the NDC-80 complex and KNL-1/MIS-12 complex. Both show an increase in the apparent binding affinity at higher input concentrations. This is in contrast to tau, which exhibits a reduction in apparent binding affinity at higher input concentrations (see Kar et al., 2003).

#### Ndc80 Family Proteins

**Aurora Consensus** 

[RK]-x-[ST]-[ILVM]

S. cerevisiae Ndc80 S. pombe Ndc80 H. sapiens HEC C. elegans NDC-80	1 MQSS 1 1 1	TSTDQH - MQDSS MKRS MFGD	VLHHM SYARR SVSSG RRKTG	D P H <mark>R</mark> F T Y S Q A P S G A G R L <mark>S</mark> G L N L N G	SQIPT/ SSNLR MQELRS RASIA	ATSSQL TTFGF GQDVNK ITPTKR	R R R N S T N G L G T S Q G L Y T P F T D Y T G	NQGLT RT <mark>SLA</mark> QTKEK STSVR	D M I N K S P Q R T L V P T K T	IARNTI NARQSI FGKLSI DARPSI	S G T G I P DP D G L S N S	T G G I N K S R V L T P K P	NKRTRS TMRPSL TSERKV QPRV	T V A G G T N G A P N T S L F G S L F N	T A L A L N D K S N 91 72 58 54
92 73 59 55	2 S R N S 3 R R S S 9 K R T S 5 T K N S	V S R L <mark>S</mark> I VR V S N A G H G S R N S V A P R C	N Q L G S S F I S Q I S Q L G I V K S L V	L Q Q H L S T P <mark>N</mark> I T S F S S S E K S L <mark>N</mark> G S K	N R D P R F I K D P R F I K D P R F I Y N	PLRDKN PLSDRR PLNDKA	F Q S A I C Y Q Q E C A F I Q Q C I	EEIYD TQVVN RQLCE -FLVE	Y L K K N K Y L L E S G F L T E N G Y E S S D A	F D I E T N F S Y A P S	NHPISIK QPLGLN AHNVSMK EQLIMK	FLKQPT NRFMPS SLQAPS PRG	QKGFII TREFAA VKDFLK KNDFIA	I F K WL Y L R I F K H L Y N K I F T F L Y G F C F E L I <mark>Y</mark> Q H	L DP G Y G F T K - 181 L DP N F R F G 157 L C P S Y E L P D - 144 L S K D Y E F P R H 123
182 158 145 124	2 - SIE 8 ARYE 5 TKFE 4 ERIE	NEIYQI EDVTTC EEVPRI EEVSQI	LKNLR LKALN FKDLG FKGLG	Y P F L E S Y P F L D S Y P F A Y P Y P	I N K S Q I S R S R I L S K S S L K N S Y Y	ISAV <mark>G</mark> G VAIGS MYTVGA (QPM <mark>G</mark> S	SN-WHK PHVWPA PHTWPH SHGYPH	F L G ML I L G ML I I V A A L I L D A L	HWMVR1 HWVVSL VWLIDC SWLIDI	N I K L D M	MC L N K V D	RSLING	N T Q E I T QC T E K A K I H T A M R I N S A V	ILSQPLKT VAMVYTVE KESSPLFD SEDTQNIL	L DE QDQR QE R 270 QNS L 225 DGQP WGE E TE 216 F GDF ME QGK A 195
271 226 217 196	1 YELN 6 DDHL 7 DGIN 6 QEKT	VE <mark>KLLI</mark> VDKVLF HNKLFL LNYAWN	DY FTE DYLVR DYTIK TSTFR	S <mark>Y</mark> K S F L T Y H L Y L C Y E <u>S F</u> M D <mark>Y</mark> T N D R	K L E DN DE S P - IS G A D S I K A A E N F	YE EES DEMNA SSSYW	PSMQEL EPEKEL ELQSKL DDTKHR	K L G F E K A T F N K D L F N L R K Y F	K F V H I I QQNQD L V D A F K L E QS NE F	NTDIAN YNQTEA ESLEAK EDMTKT	NLQTQND ALKSTNE (NRALNE FAASALE	NLYEKY ELINQI QIARLE MLNYEC	QEVMKI KSAEEL QEREKE DEIEAD	S QK I K T T R D S A I QV L E P N R L E S L R K G N E A S L K	E K WK A L K S <mark>D</mark> S 357 E R Y R T MQR D E 312 K L K A <mark>S</mark> L QG D V 307 E E I S R I R D D I 286
358 313 308 287	8 N <mark>K</mark> YE 3 VKFQ 8 QKYQ 7 R <mark>K</mark> AK	N <mark>Y</mark> V N A M S A MS G M A <mark>Y MS</mark> N L D <mark>Y</mark> L E Q N	1K QK S Q 1K S K ME E S H S A I L H V K Q	EWPG <mark>KL</mark> SRTNLM ILDQ <mark>KL</mark> HMEKEL	EKMKS KQLQV NGLNE AMVKS	CELKE NIEEKE IARVE QEEKI	E <mark>E I K A L</mark> S Q L Q L L L <mark>E</mark> C E T I S E N E K V	QSNIS KEKRD KQENT QKMVD	E <mark>L</mark> H K I L S L K Y Q V R L Q N I I D L K N K I	RKKGIS ENQDIS DNQKYS ELQKQI	TEQFEL ISEFEK VADIER HGLTGK	QNQ <mark>ER</mark> E MVSERE INHERN EVRQMN	K L TREL QLDRNL IE LQQTI ILDNNKD	D <mark>K I</mark> NIQSD NMIGSKIS NKLTKDLE KEVVLE <mark>I</mark> C	KLTSSIKSRK448 ELR
449 397 392 371	9 LEAE 7 2 1	G I F K S L K E V QK L DR L	L D T L R F D T D L WNE E L S K E T W	Q <mark>Y</mark> DS <mark>SI</mark> LIQASI KYARGK KLK-DE	QNLTRS DSLEK EAIETO DFFKEO	8 R <mark>S Q L G</mark> V Q K F N Q L A E Y H Q K S K F I	H N V N D S S L A Y R I K L A R K L H L A E Q I	S L K I N G I V P I K L I P K MK I L S	S E N L L A A I R S A G A E N S K G L N I	DRDFHE NNDFEL GYDFEI QMNLEP	GISYEC EINPEG KFNPEA PLRAPTN	L F P K G S P N Y I N L G A N C L V IE R D L K D	GINESI DLKNKV KYRAQV YWETLN	K K <mark>S</mark> I L K L N R P F I N E V R Y V P L K E L L K I WV P E I S	IDE I QE R I K T I 539 R S I T L E F H E E 480 NE T E E E I N K A 475 R Q L H Q R K L E L 451
540 481 476 452	0 EKDN 1 QNKS 6 LNKK 2 ETEQ	IT <mark>LE</mark> KD LKLQEH MG <mark>LE</mark> DT SRFSNK	U K N L K V D T V N L E Q L N A V T A E	HD <mark>INE</mark> K DLIAEL AMITES ER <mark>I</mark> QIQ	TQINE QDELR KR <mark>S</mark> VR SETLCE	CLELEL GIESRL FLKEEV EAKKNE	S E A N <mark>S</mark> K T S V L <mark>S</mark> E Q K L D D L A R E E R I	FELSK CNMLR YQQKI RRNER	QENERL ETASEE KEAEEE DSWKDA	L V A QR I K N A F D A D E K C A S R K H I E C	EIEKME AESDKLE SELESLE QRYEQLL	KKINDS RELQQL KHKHLL NEKEVL	N L L MK T K L S S H N E S T V N Q L K Q MK L	KISDAEEL SMLQLDQR GLSEAMNE DG-SLEKE	V T S T E L K L E E 630 I Q S I N I E A D Q 571 L D A V Q R E Y Q L 566 I E D E T A R MS A 541
631 572 567 542	1 LKVD 2 IAHA 7 VVQT 2 TGEE	L N R <mark>K R</mark> Y C ME Y K N T T E E <mark>R</mark> R H I Q K R S	<mark>K L</mark> HQQ IN I Y K E K V G N N Q L E A G	VIHVID VAFVLG LQRLLE IRQILD	T S K F F E     H F F MV A T H V L MV V E	CINIQS CLHVQD /GSVEK IAEIEN	S <mark>LE</mark> NSE S LE DLK H <mark>LE</mark> EQI K K I G F H	NELGN MDYQK AKVDR VQCAG	VIEELF ELDDLS EYEECM IEKAVL	N L E F E T R S E L AS E D L S E	NIKEIR	DKYEKK	A T L I K S	  S E E	691 624 642 590

#### Figure S4. Conservation of Aurora B Phosphorylation Sites in Ndc80

Alignment of Ndc80 homologues from fungi, humans and *C. elegans* showing identical residues shaded. Sites matching the predicted Aurora B phosphorylation consensus site (Cheeseman et al., 2002) are indicated in red. There is an enriched cluster of multiple potential Aurora B phosphorylation sites in the first 100 amino acids of each protein.