Supplemental Data

The Conserved KMN Network Constitutes the Core Microtubule-Binding Site of the Kinetochore

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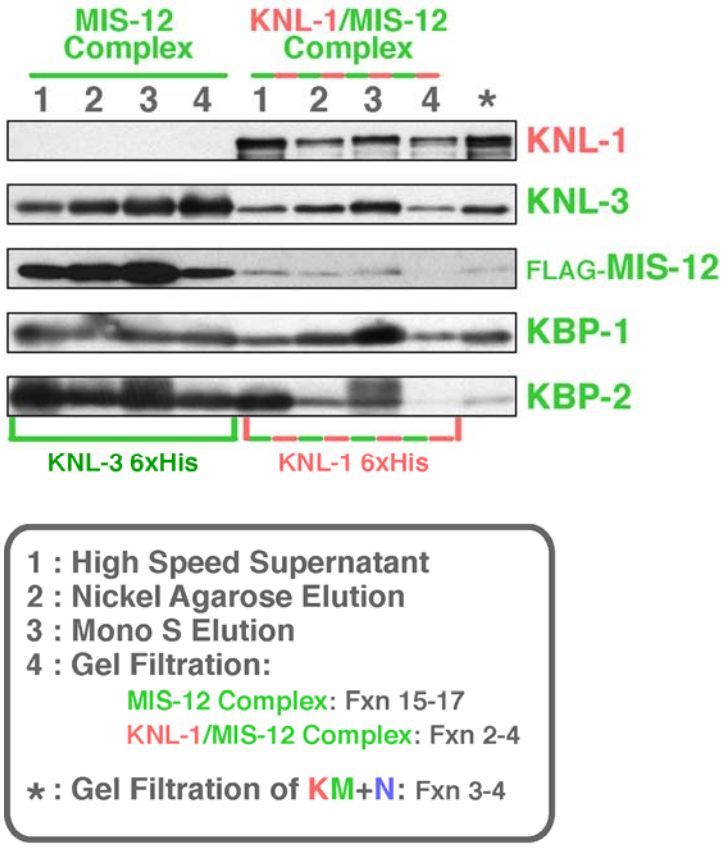

Figure S1. Fractionation of MIS-12 Complex Subunits
Western blots showing the behavior of KNL-3, KBP-1, KBP-2 and MIS-12 (FLAG-tagged) during purifications with a 6xHis tagged KNL-3 (see Figure 1F) or 6xHis tagged KNL-1 (see Figure 1H). All subunits co-fractionate with the tagged protein during the different 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).

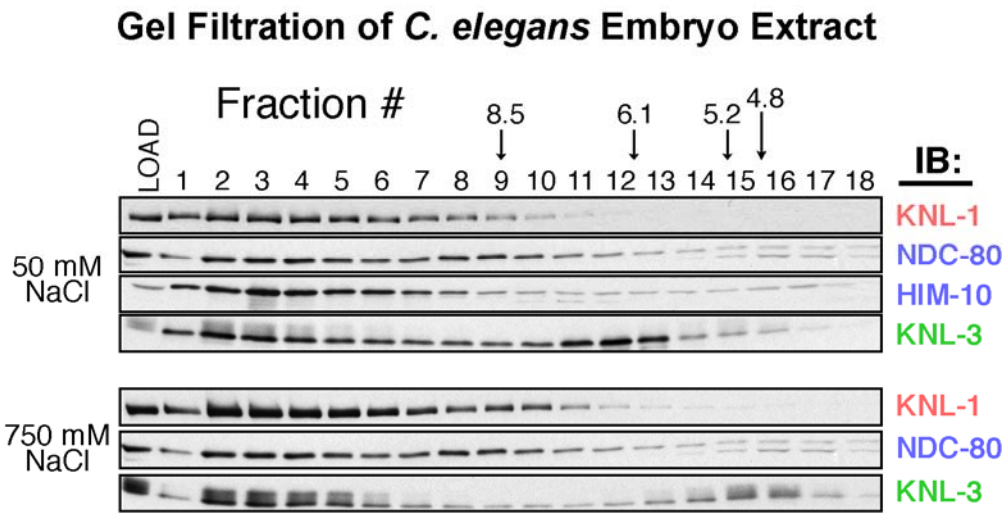

Figure S2. Gel Filtration of Embryo Extracts

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.

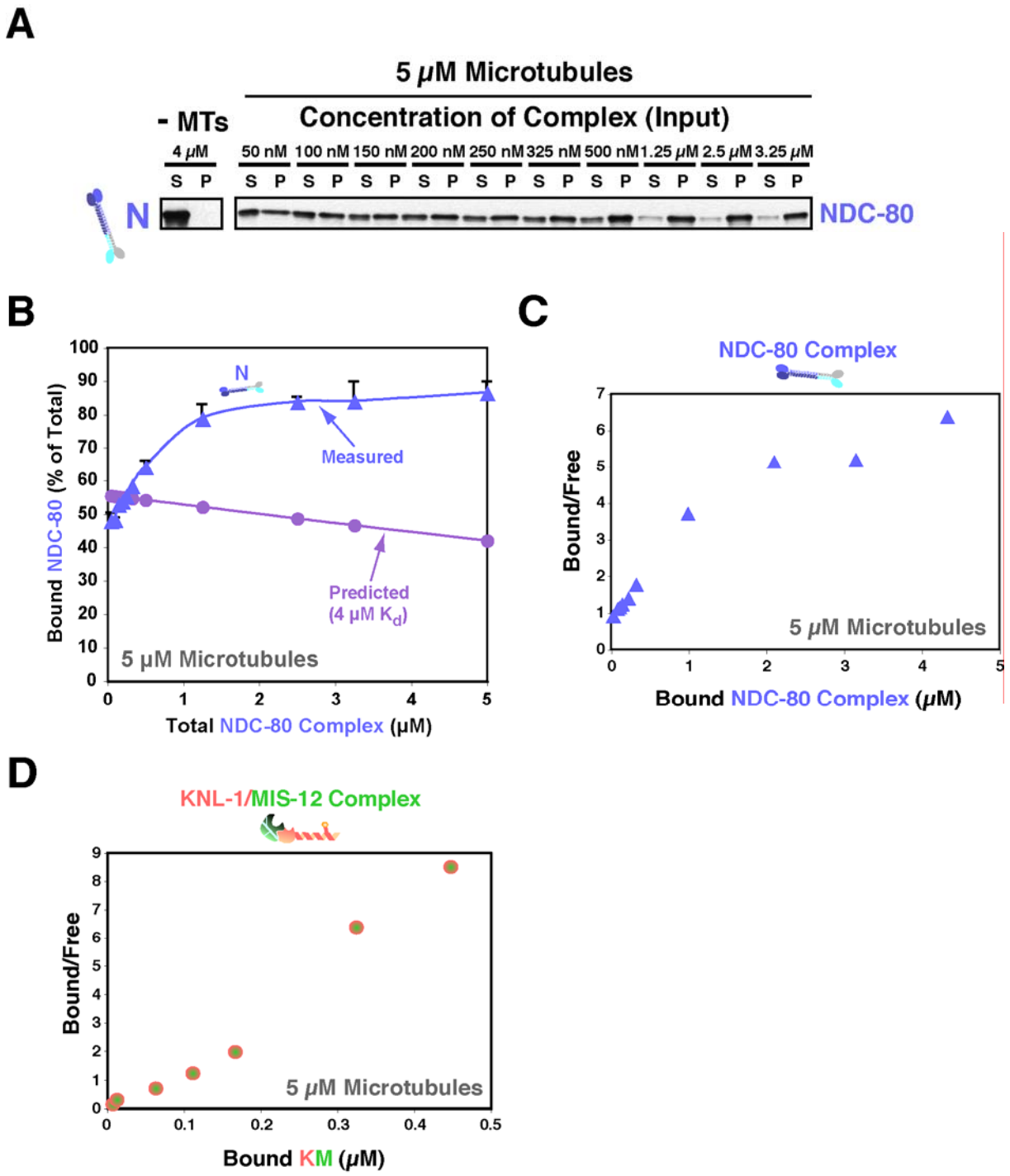

Figure S3. Concentration-Dependent Changes in Binding Properties of the NDC-80 Complex and KNL-1/MIS-12 Complex

(*A*) The concentration of NDC-80 complex was varied from 50 nM to 5 μM with the microtubule concentration held constant at 5 μM. 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 μM K_D 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).

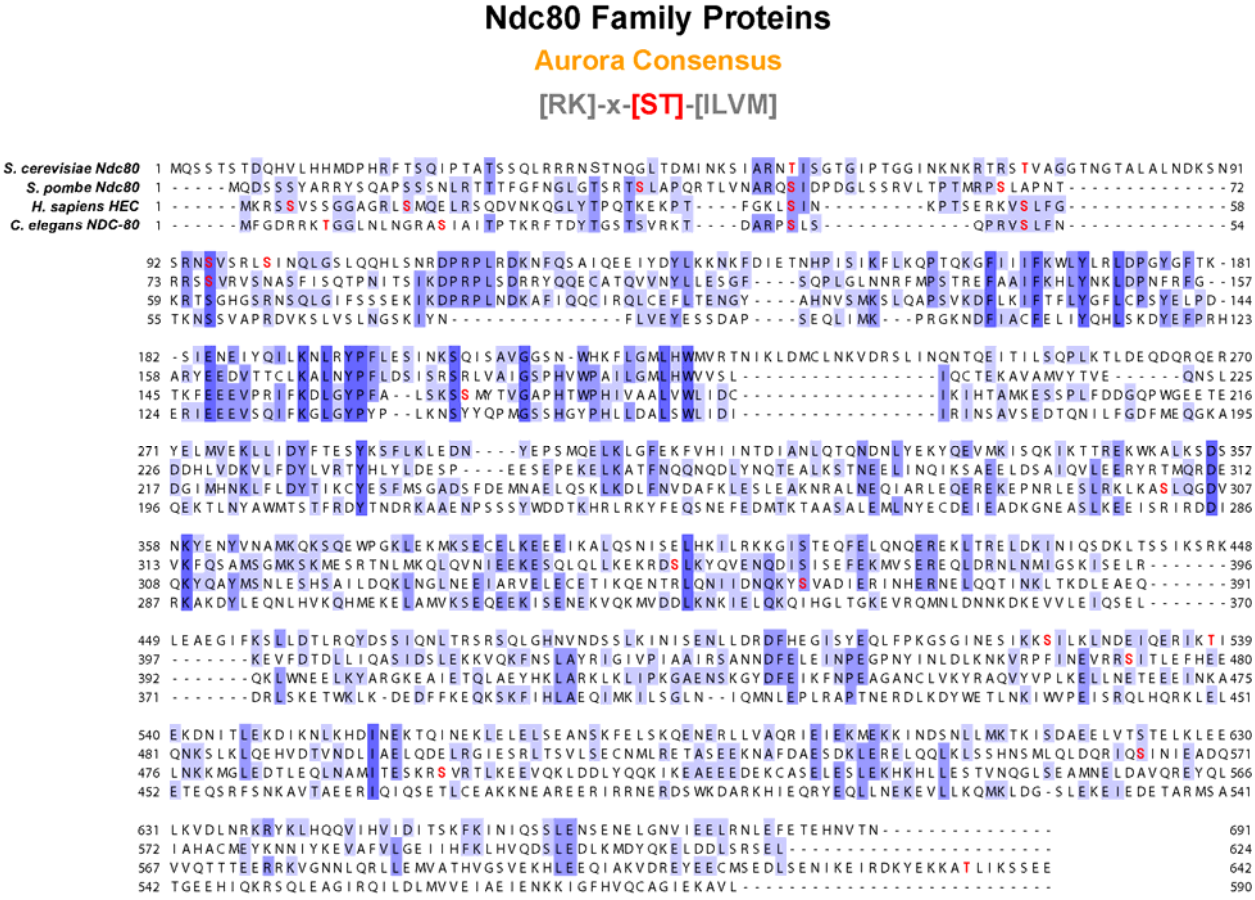

Figure S4. Conservation of Aurora B Phosphorylation Sites in Ndc80
Alignment of Ndc80 homologues from fungi, humans and *C. elegans* showing identical residues shaded. Sites matching the predicted Aurora B phosphorylation consensus site (Cheeseman et al., 2002) are indicated in red. There is an enriched cluster of multiple potential Aurora B phosphorylation sites in the first 100 amino acids of each protein.