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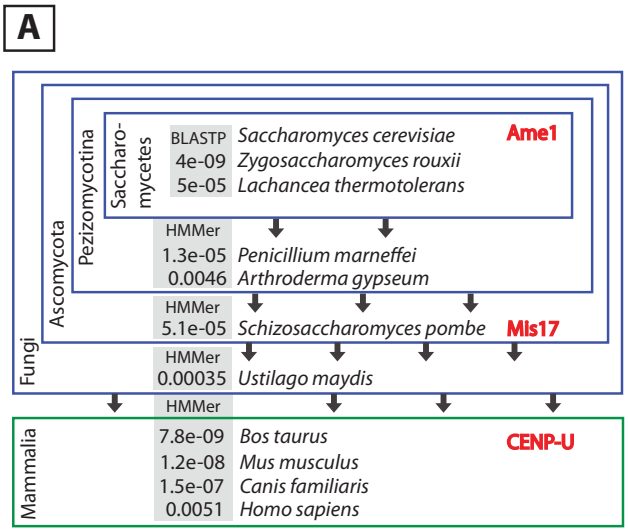

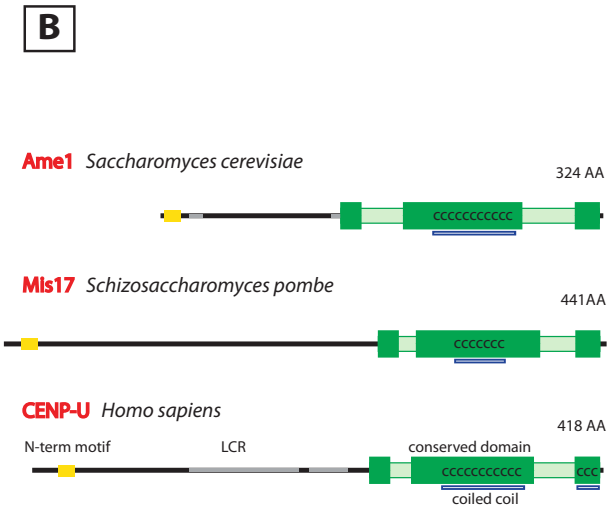

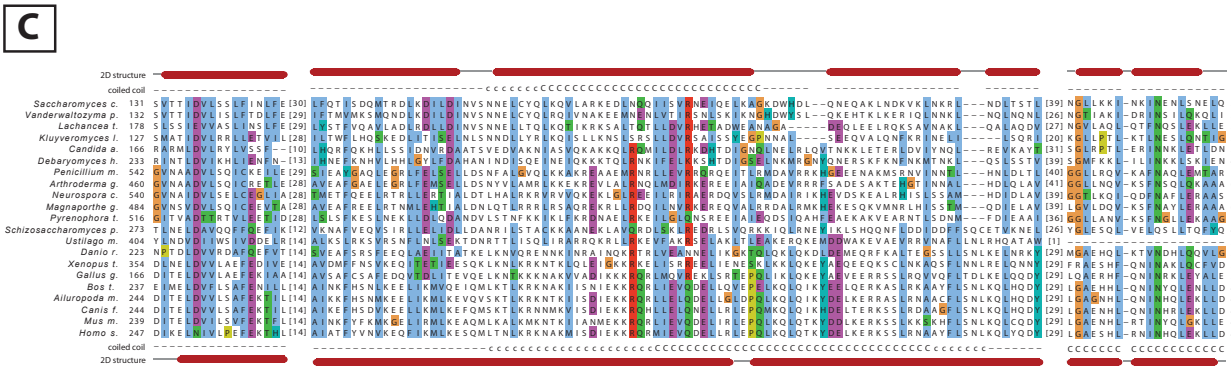

Figure S1 Ame1, Mis17 and CENP-U are homologous. (A) Summary of the search strategy to deduce phylogenetic relationships between *Saccharomyces cerevisiae* Ame1, *Schizosaccharomyces pombe* Mis17 and human CENP-U. A series of BLAST and Hidden Markov Model searches using sequences of the conserved domain were applied (see Methods). (B) All three proteins share a similar architecture: a short motif close to the N-terminus (*yellow*) and a conserved domain at the C-terminus (*green*). Regions within the conserved domain, that are not shown in the multiple alignment are indicated in *light green*. Polar regions of compositional bias (low complexity) are in *grey*. Coiled coil regions are marked with a *blue* bar. (C) Multiple alignment of the C-terminal conserved domain. Species and accession numbers are listed in Supplementary Table 2. The numbers of residues omitted in two long, mostly unaligned, polar regions are indicated in brackets. *S. cerevisiae* (above) and *H. sapiens* (below) coiled coil and secondary structure predictions are derived from the Jpred 3 server (alpha helical regions are in *red*). The alignment was calculated using MAFFT and graphically processed with Clustalx colors using Jalview.

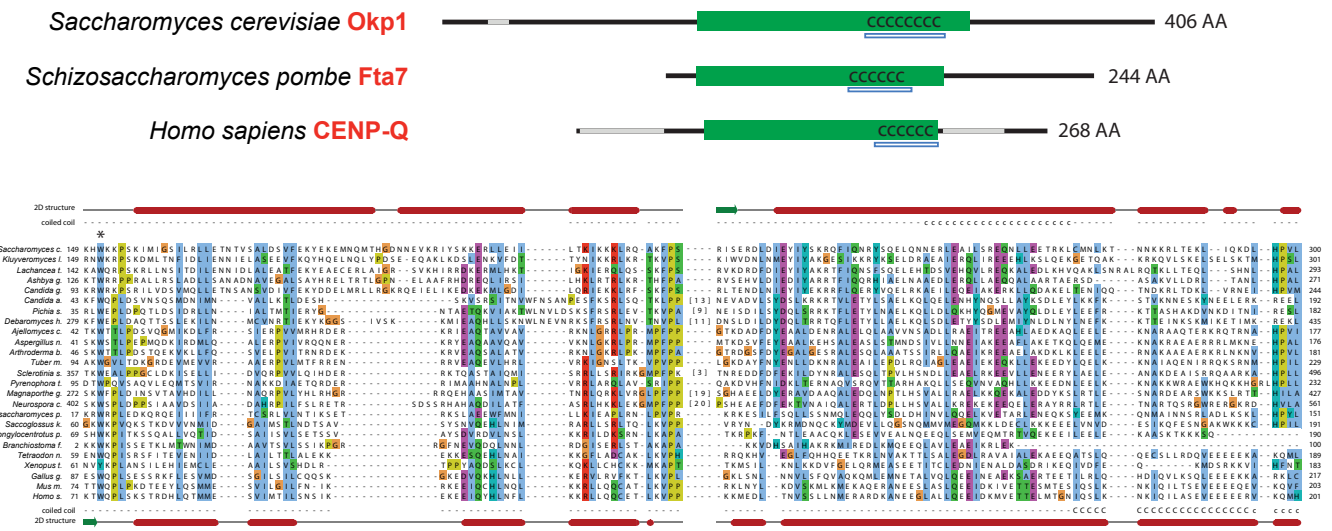

Figure S2 Multiple alignment of the Okp1/Fta7/CENP-Q conserved domain. A highly conserved tryptophan at the beginning of the conserved domain is marked with an asterisk. Scheme as in Supplementary Figure 1.

Saccharomyces cerevisiae **Mcm22**
Schizosaccharomyces pombe **Sim4**
Homo sapiens **CENP-K**

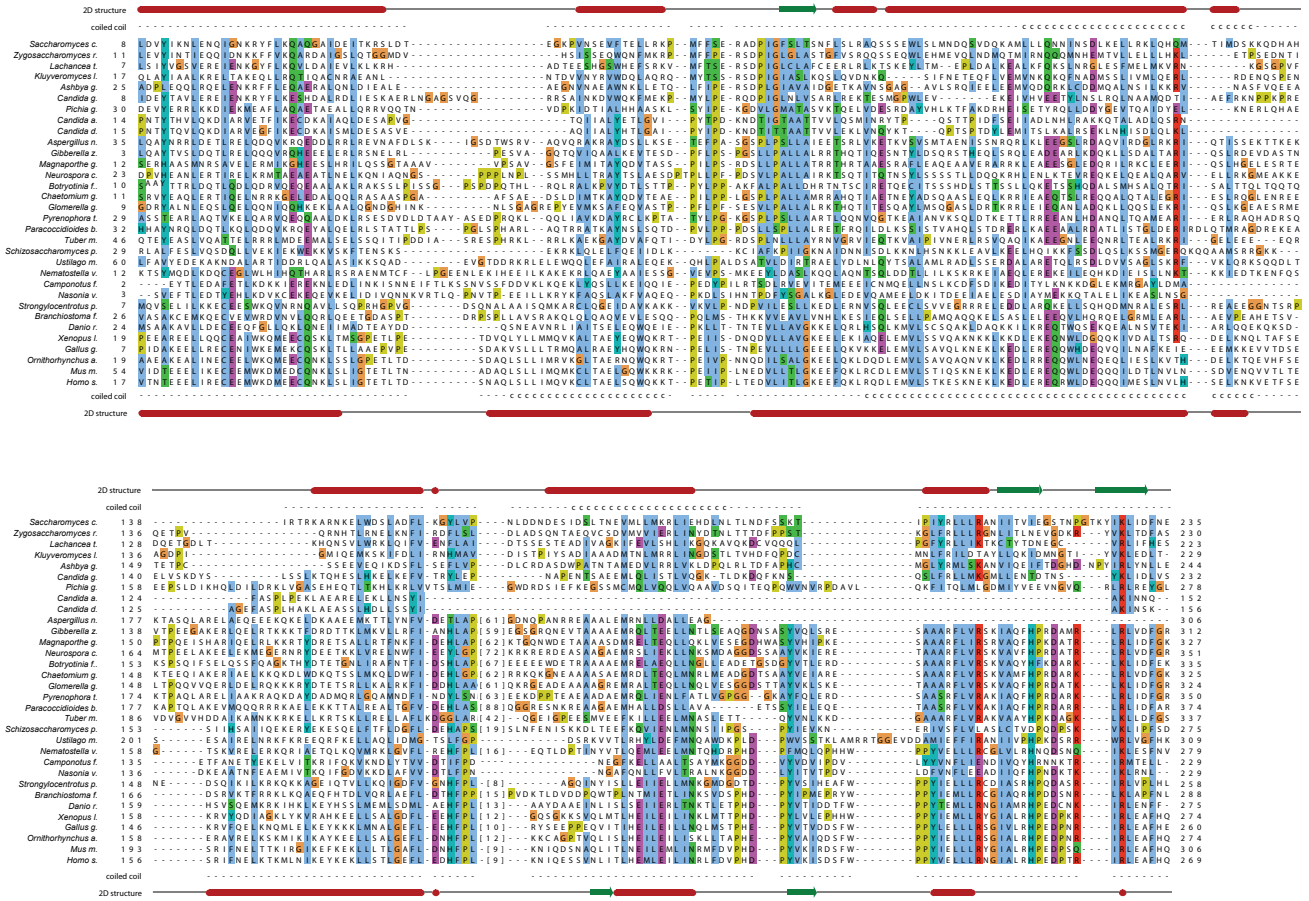

Figure S3 Multiple alignment of the Mcm22/Sim4/CENP-K conserved domain. Scheme as in Supplementary Figure 1.

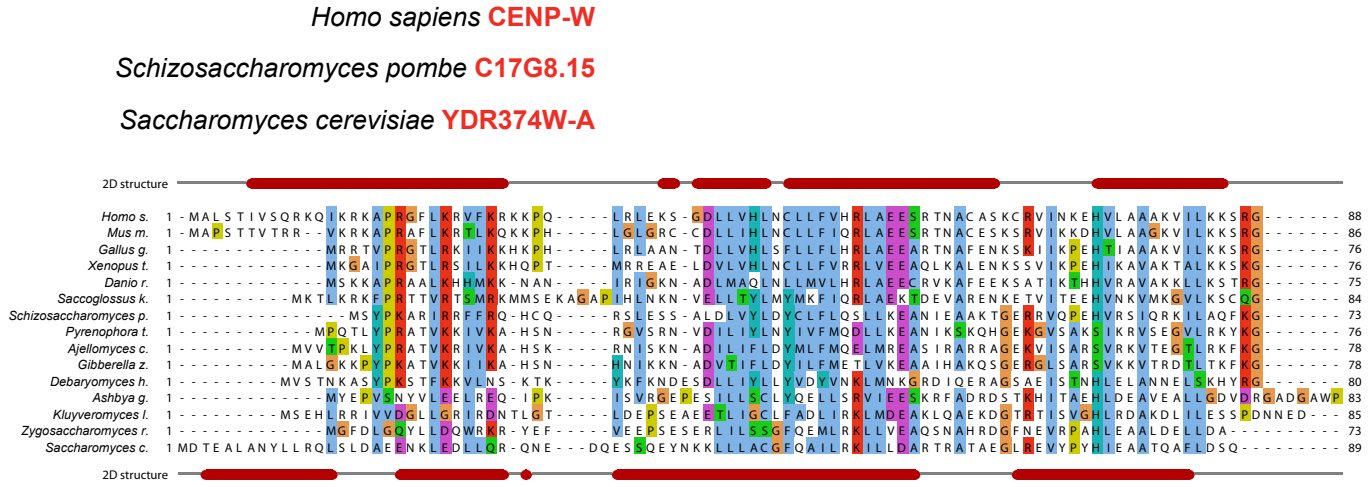

Figure S4 Multiple alignment of the CENP-W conserved domain. Scheme as in Supplementary Figure 1.

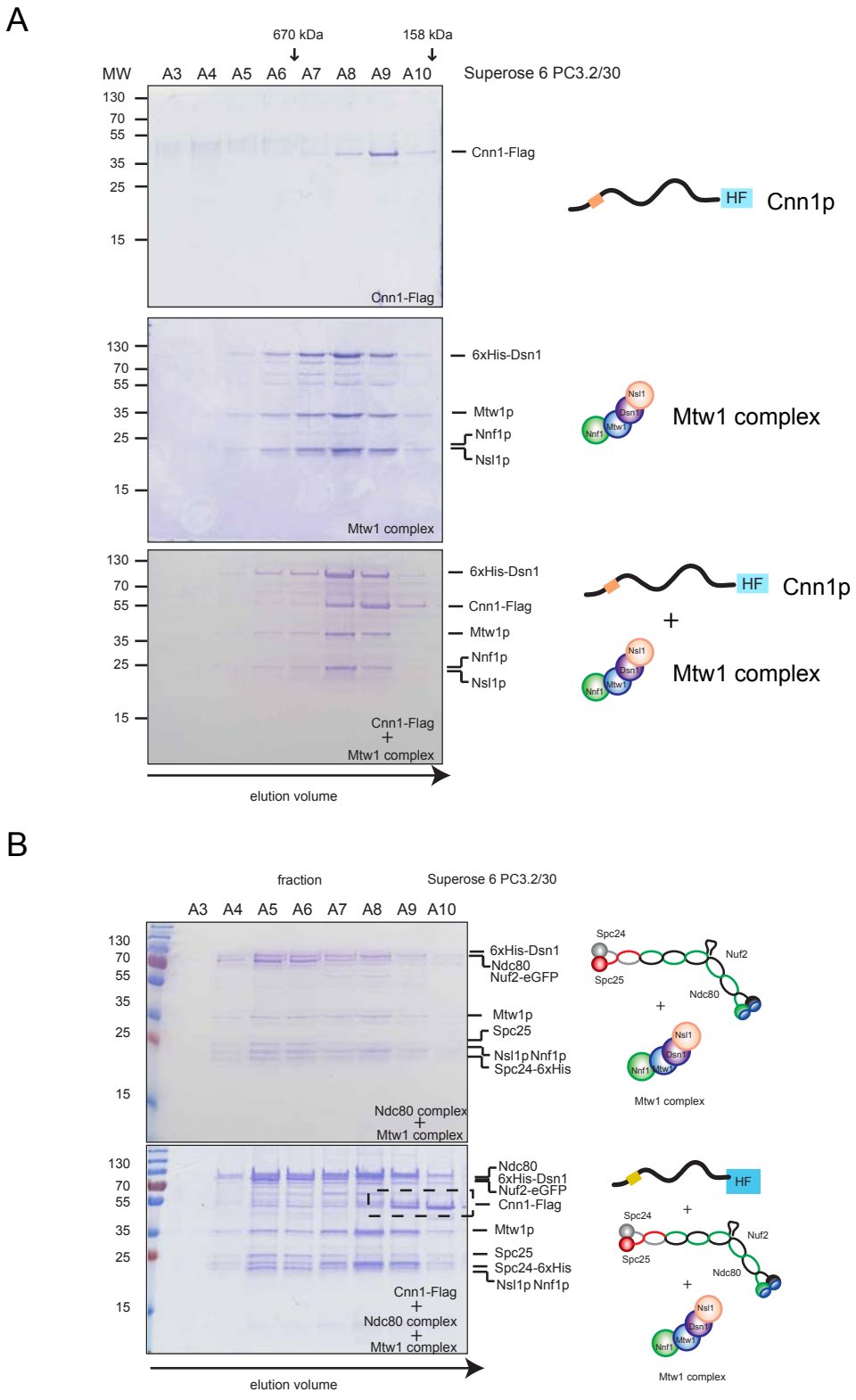

Figure S5 Cnn1 and the Mtw1 complex are competing binding partners for the Ndc80 complex. A. Size exclusion chromatography of Cnn1-Flag alone (upper panel), recombinant Mtw1 complex alone (middle panel), or in combination (lower panel). Note that the elution position is unchanged in the combined sample. B. Size exclusion chromatography of Ndc80 complex and Mtw1 complex in the absence (top panel) or presence (lower panel) of Cnn1-Flag. Note that Cnn1-Flag fails to efficiently co-elute with the Ndc80-Mtw1 complex assembly.

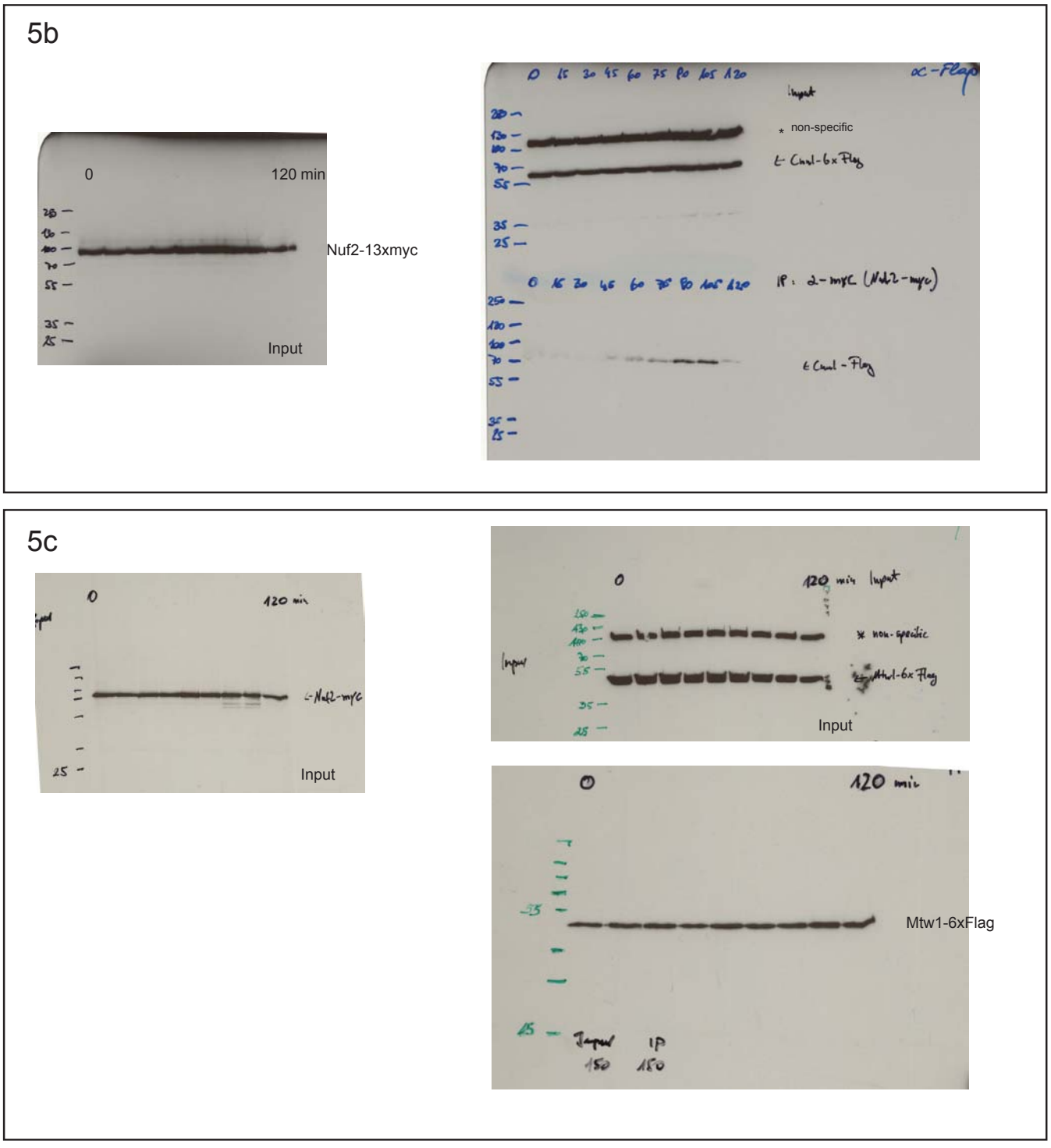

Figure S6 Uncropped Western blots corresponding to Figure 5b,c.

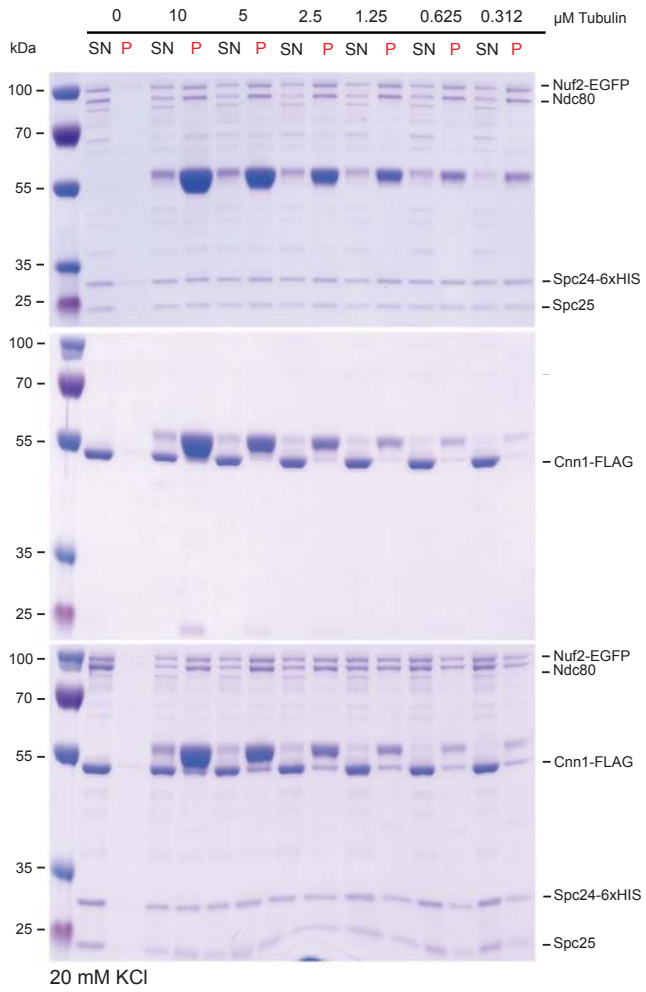

Figure S7 Microtubule cosedimentation experiment using Ndc80 complex alone (upper panel), Cnn1-Flag (middle panel) or a combination of both (lower panel) with different concentrations of taxol-stabilized microtubules. Note that Cnn1-Flag fails to cosediment with microtubules on its own, but is recruited into the pellet fraction in the presence of the Ndc80 complex.

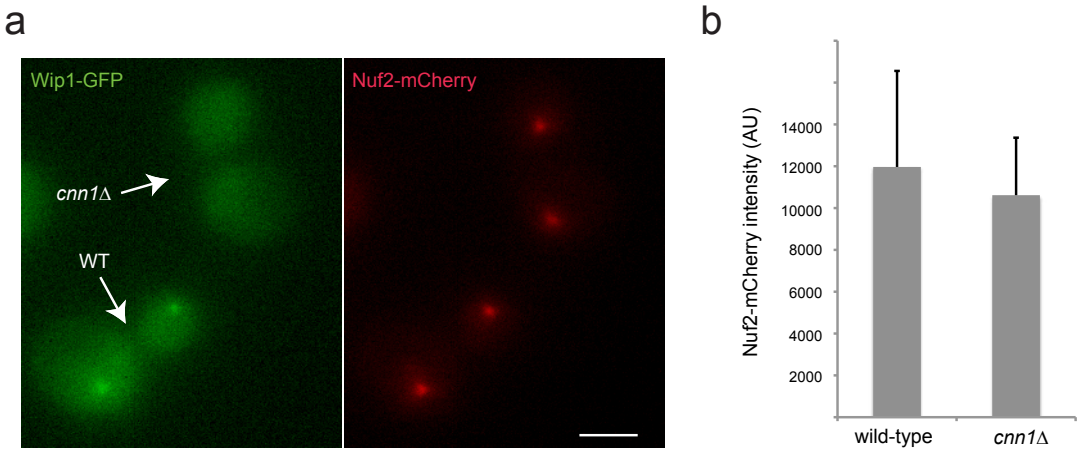

Figure S8 Wild-type and *cnn1* deletion cells (identified by the lack of Wip1 signal at kinetochores) were mixed, imaged on the same microscopy slide, and the Nuf2-mCherry signal in large budded anaphase cells was quantified. Anaphase kinetochore clusters in ten cells were analyzed and error bars represent standard error of the mean.