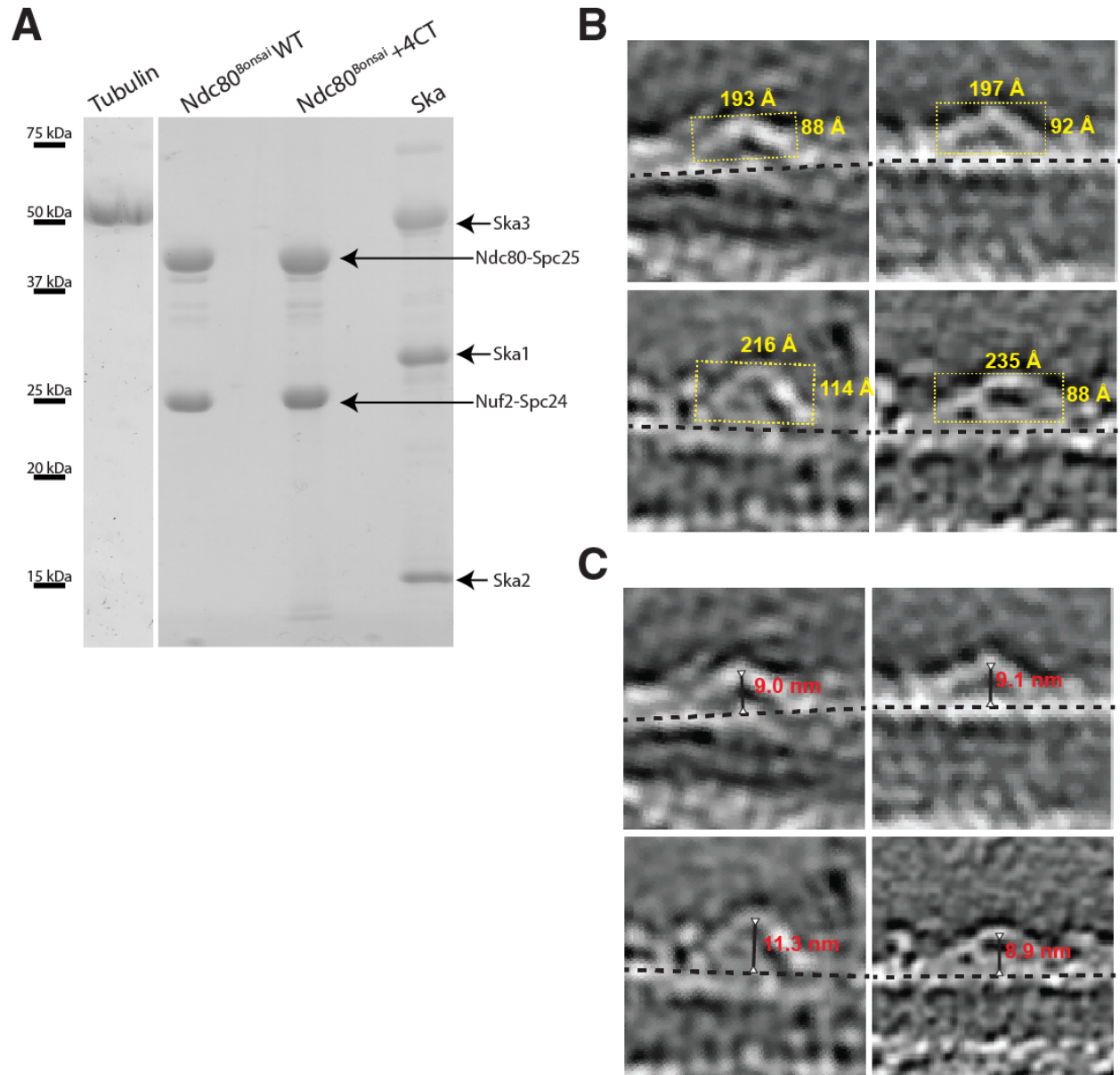# SUPPLEMENTAL FIGURES

Figure S1. Measurements of V-shape dimensions and distance from the tip of the V-shape to the microtubule, related to Figure 1.

(A) Coomassie stained gel showing purified bovine brain tubulin, recombinant Ndc80[Bonsai WT], Ndc80[Bonsai +4CT] and Ska complexes.

(B) Method employed to analyze dimensions of the V-shapes using a bounding box (yellow). Width and height of representative V-shapes from Figure 1A are shown.

(C) Representative measurements of the distance from the tip of the V-shape to the microtubule lattice are shown (black solid line).
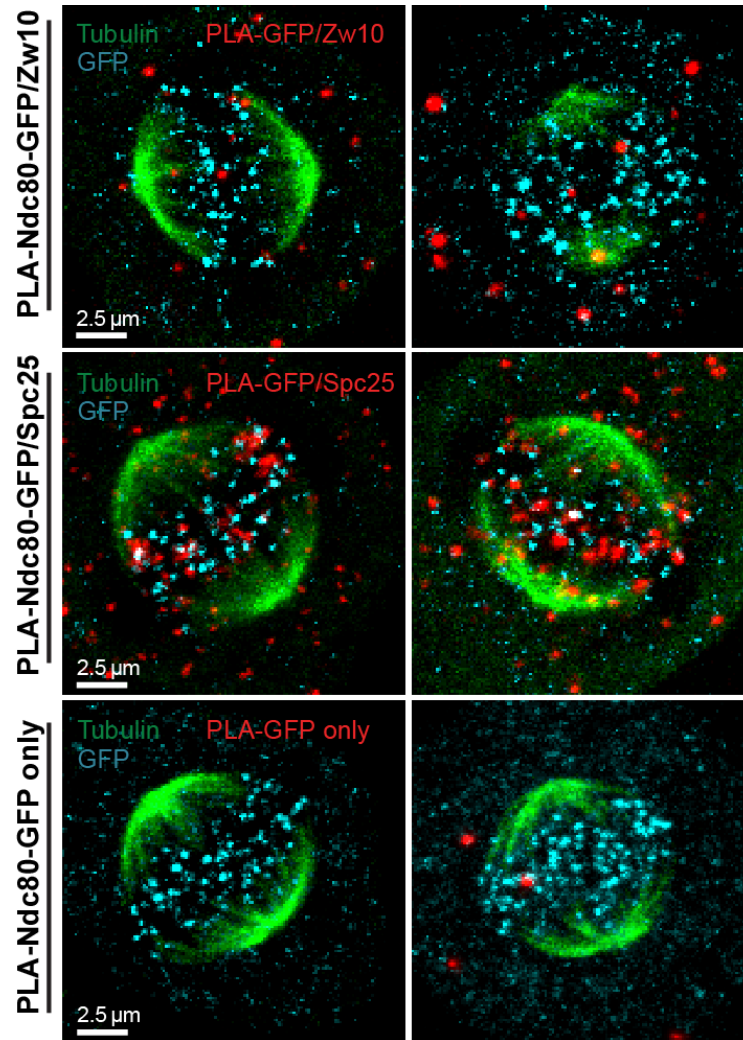
Figure S2. Additional controls for the proximity ligation assay, related to Figure 2.

The C-terminal GFP fusion the Ndc80 protein is close enough to Spc25, but not to Zw10, to generate a PLA signal. Representative images of Proximity Ligation Assay (red) in cells expressing Ndc80-GFP (C-term), with additional immunostaining of GFP (cyan) and tubulin (green). Top – PLA Zw10/GFP middle – PLA Spc25/GFP, bottom – control PLA-GFP only. Green and Cyan channel were scaled independently between the images.
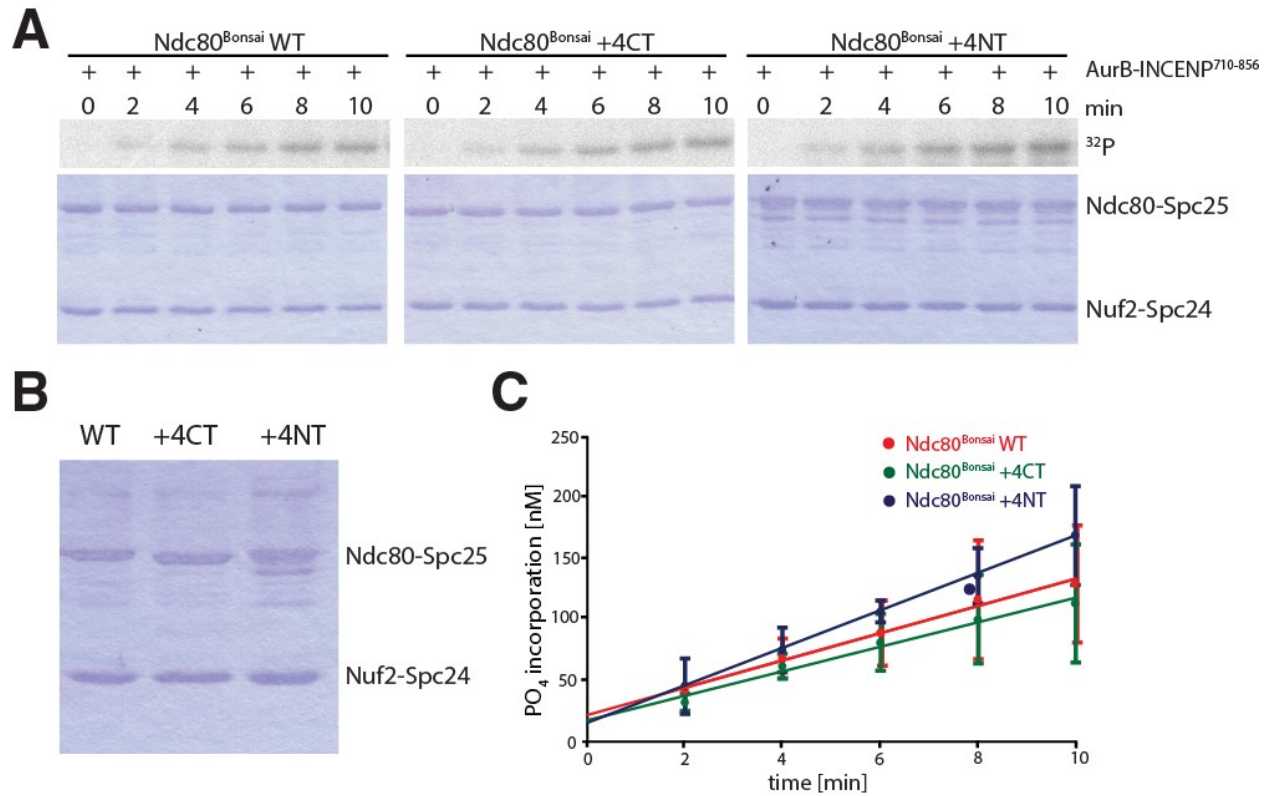
Figure S3. Ndc80 tail mutants are phosphorylated in vitro by Aurora B at similar rates as the wild-type protein, related to Figure 3.

(A) Phosphorylation of Ndc80[Bonsai] tail mutants by Aurora B-INCENP[710-856]. **Top:** Representative images showing incorporation of ^{32}P to Ndc80 in presence of Aurora B. **Bottom:** Coomassie stained gels representing Ndc80[Bonsai] samples taken from different time-points during phosphorylation reaction.

(B) Representative Coomassie stained gel showing similar amounts of Ndc80[Bonsai] tail mutants were used for the kinase assay.

(C) Graph representing quantification of time dependent incorporation of [PO$_4$] to Ndc80Bonsai tail mutants by Aurora B (mean ± SD, N = 2).
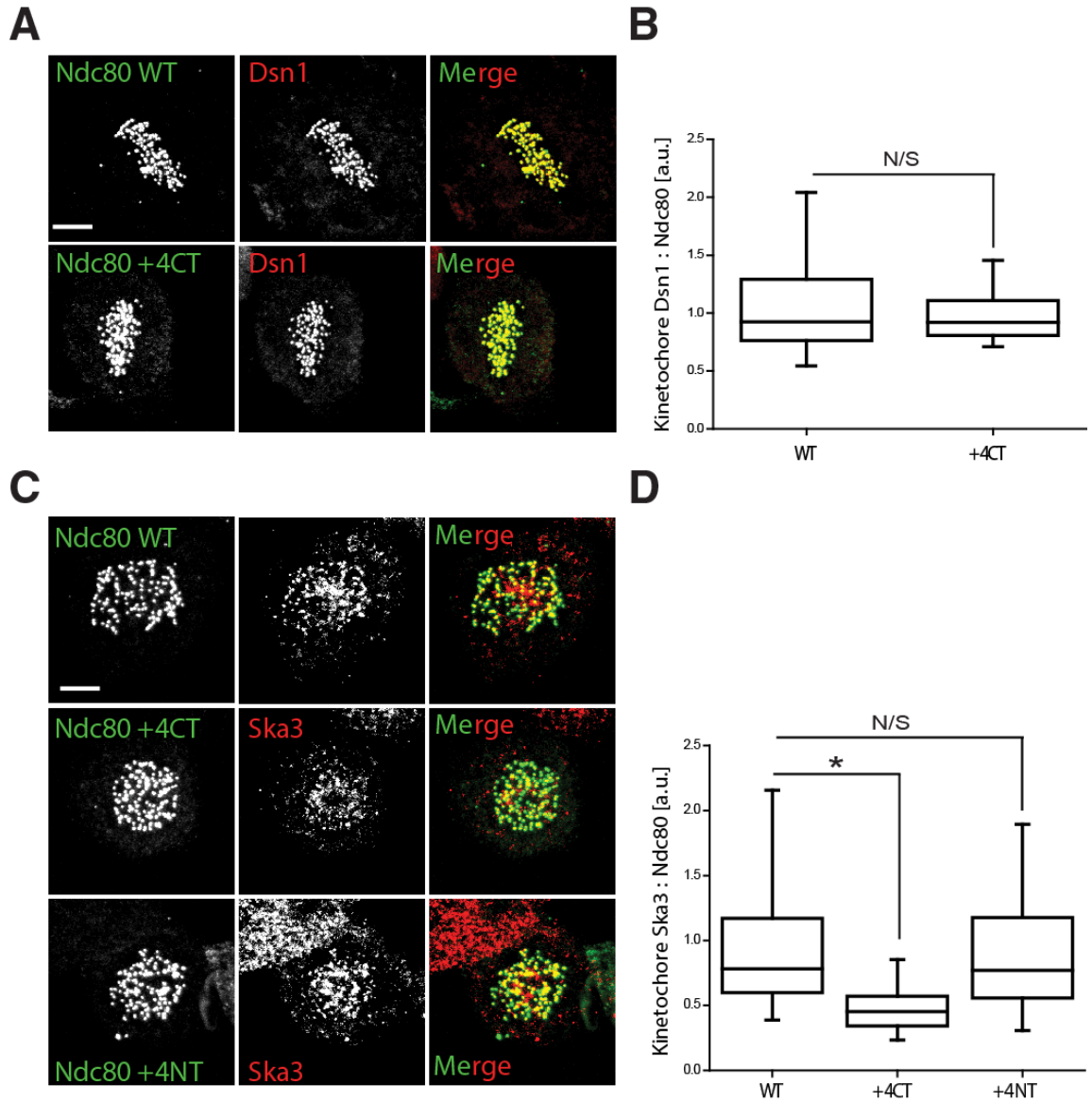
Figure S4. The recruitment of Ska, but not Dsn1, to kinetochores requires charge in 40-60aa region of Ndc80 tail, related to Figure 3.

(A) Immunofluorescence staining of Ndc80^{WT}, Ndc80^{+4CT} and Ndc80^{+4NT} stable cell lines after depletion of endogenous Ndc80 shows reduced levels of Ska3 on prometaphase kinetochores of Ndc80^{+4CT} stable cell lines, but not on the prometaphase kinetochores of Ndc80^{+4NT} stable cell lines. Scale bar = 5 µm.

(B) Box and whisker plots representing the quantification of Dsn1 staining intensities on kinetochores represented in **(A)** (N > 100 kinetochores from at least 4 cells, Whiskers – 5-95% percentile). a.u. = arbitrary units. N/S - p = 0.84 (Unpaired t-test with Welch's correction).

(C) Immunofluorescence staining of Ndc80^{WT} and Ndc80^{+4CT} stable cell lines after depletion of endogenous Ndc80 does not show significantly changed kinetochore levels of Dsn1 cells expressing Ndc80^{+4CT}. Scale bar = 5 µm.

(D) Box and whisker plots representing the quantification of Ska staining intensities on kinetochores represented in **(C)** (N > 100 kinetochores from at least 4 cells, Whiskers – 5-95% percentile). a.u. = arbitrary units. * - p < 0.0001, N/S – p = 0.1114 (Unpaired t-test with Welch's correction).
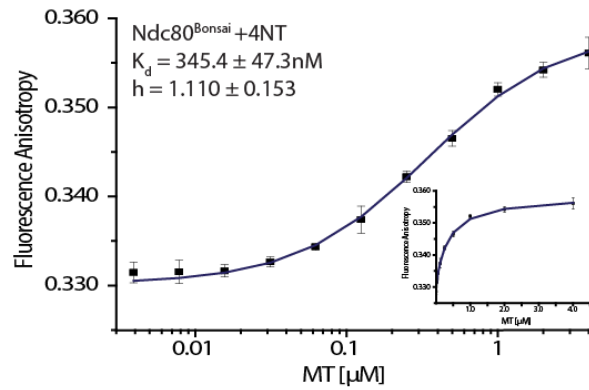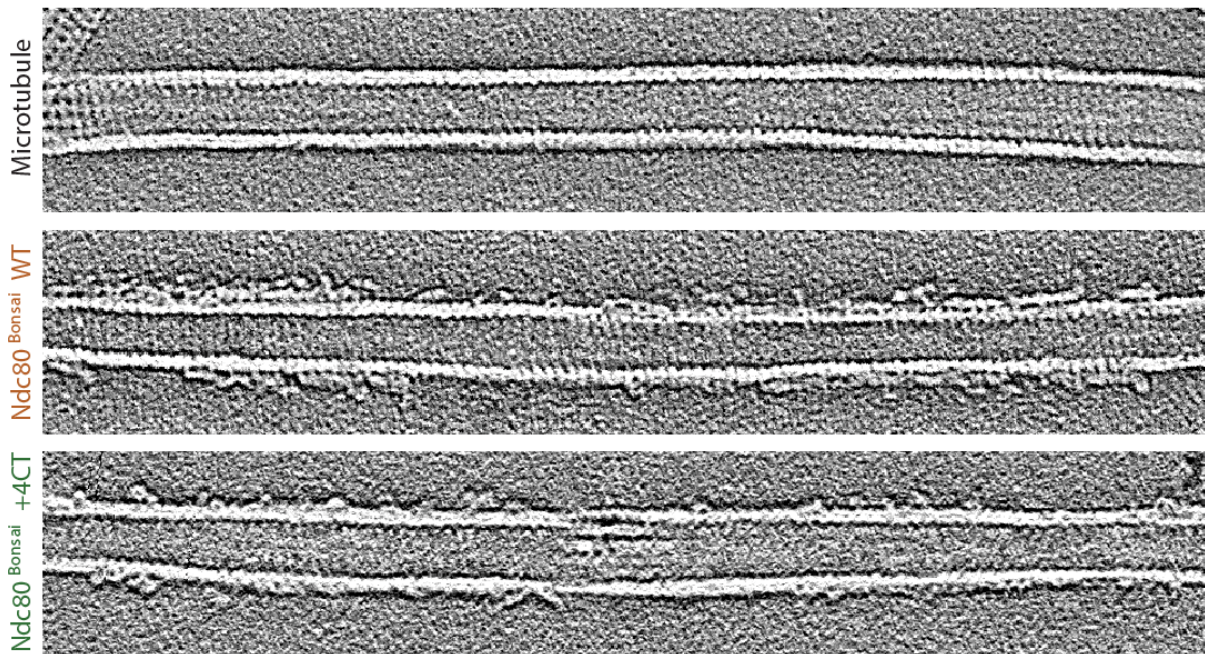
A

B

Figure S5. Ndc80[+4CT] tail mutant is deficient in clustering on microtubules, related to Figure 5.

(A) Fluorescence anisotropy measurements of fluorescently labeled Ndc80[Bonsai] +4NT (blue) incubated with increasing concentrations of taxol-stabilized microtubules, plotted on $\log_{10}$ scale. Hill equation was used for fitment of the data. Small graphs represent the data in the linear scale. Error bars = SD.

(B) Representative projections (same as in **Figure 5**) of 5 consecutive Z-sections (1.107 nm) of the tomographic reconstructions show Ndc80[Bonsai] +4CT form smaller clusters than Ndc80[Bonsai] WT. Black lines to indicate positions of Ndc80 molecules are omitted for clarity.

SUPPLEMENTAL MOVIES

Movie S1. Serial slices of tomographic projections of microtubules coated with Ndc80^{Bonsai} WT, related to Figure 1.

Movie displays series of slices (thickness: 0.4427 nm) of the tomographic reconstructions shown in Figure 1A. Scale bar = 10 nm.

Movies S2 and S3. Serial slices of tomographic reconstruction of microtubules coated with Ndc80^{Bonsai} WT and Ska complex showing representative V-shapes, related to Figure 1.

Movie displays series of slices (thickness: 0.4427 nm) of the tomographic reconstructions shown in Figure 1A. Representative V-shape is indicated by white arrow. Scale bar = 10 nm.

Movie S4. Movie showing structure of Ska core complex (PDB: 4AJ5) fitted to representative V-shape, related to Figure 1

Structure of Ska core complex was fitted to EM map of tomographic reconstruction. Thickness of visible map: 2.6568 nm (12 consecutive z-slices). Visualization was done in Chimera 1.11.2.