The structure of purified kinetochores reveals multiple microtubule attachment sites

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1. Supplementary Figures and Movies

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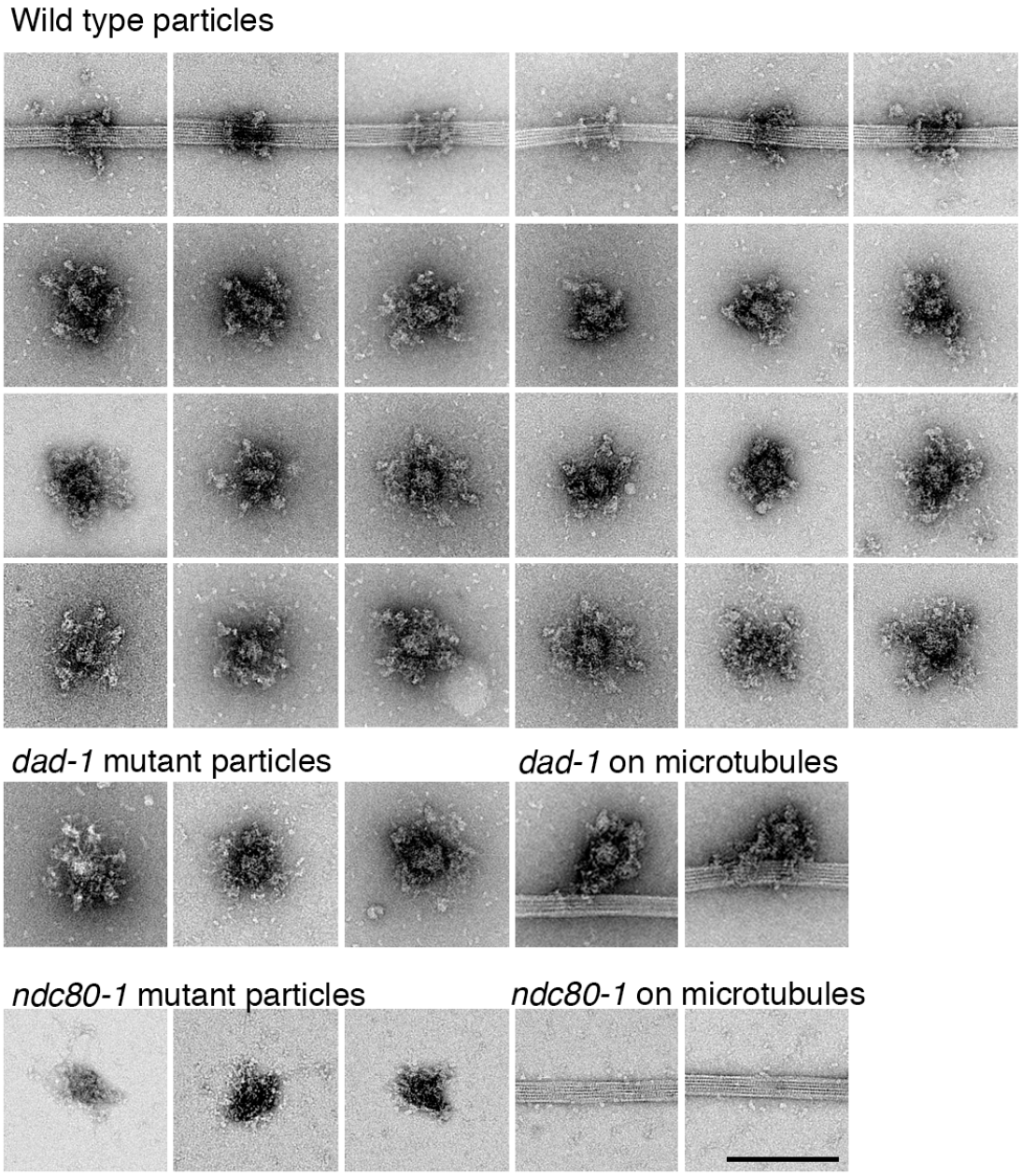
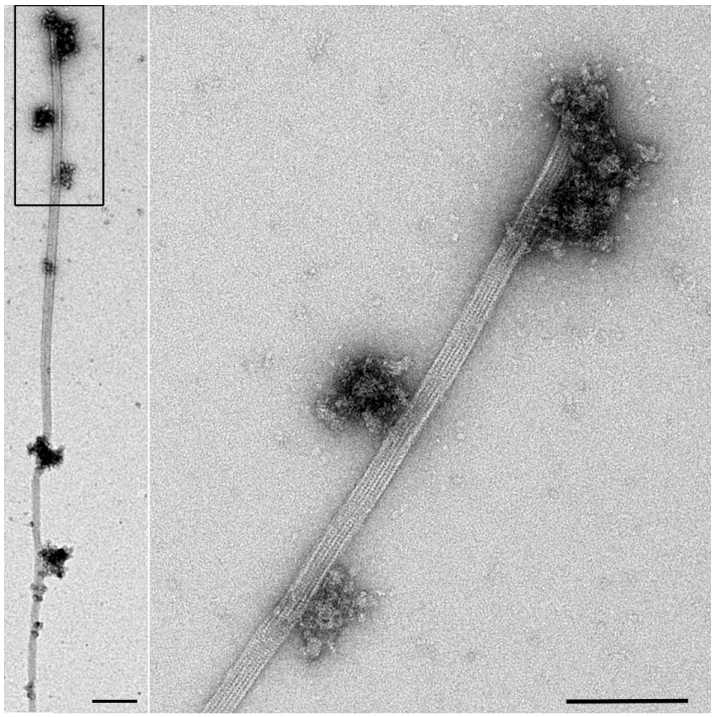
1. Supplementary Figures and Movies



Supplementary Figure 1. Additional images of Dsn1-Flag material purified from WT, *dad1-1* and *ndc80-1* yeast strains. Representative negative stain images of Dsn1-His-Flag eluates from WT (SBY8253), *dad1-1* (SBY8460) and *ndc80-1* (SBY8362) strains. Scale bar 200 nm.



Supplementary Figure 2: Kinetochores are enriched on microtubules. An image of a microtubule after incubation with purified kinetochore particles. The kinetochores are enriched on the microtubule relative to the rest of the grid despite being diluted by the incubation with the microtubules. Scale bar 200 nm.

Supplementary Table 1. Average kinetochore particle measurements.

	end to end (nm)	central hub (nm)	globular domains (nm)[a]	Number of globular domains
Average	126	37	21	N/A
standard deviation	13	3	2	N/A
Numbers of data points	88	72	97	79
max	150	44	28	7
min	100	30	17	5

[a]The globular domains generally showed varied morphologies rendering accurate quantification impossible. The diameters provided here represent the average that was calculated from 97 selected globular domains that showed the most uniform globular structure.

Supplementary Table 2. Average measurements of small particles laterally bound to microtubules.

	Diameter of ring (nm)	Length of rod (nm)	Kink position relative to ring (nm)
Average	50	56	25
standard deviation	3	4	2
numbers of data points	99	128	67
max	58	65	30
min	45	48	20

Supplementary Table 3. Quantification of WT and mutant particles bound to microtubules.

	Large particles with ring	Large particles without ring	Total microtubule length (μm) analyzed	Total particles/ μm microtubule length
WT	55	355	493.1 (n=200)	0.68
Ndc80-1	0	3	585.5 (n=200)	0.005
Dad1-1	0	175	231.6 (n=100)	0.76

Supplementary Table 4. Yeast strains used in this study. All strains are isogenic with the W303 background.

Strain	Genotype
SBY3	*MAT**a** ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 LYS2 can1-100 bar1Δ*
SBY7441	*MAT**a** ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 lys2 can1-100 bar1Δ Dsn1-3Flag::KAN*
SBY7653	*MAT**a** ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 LYS2 ALK1-Flag::TRP1 can1-100 bar1Δ*
SBY8253	*MAT**a** ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 can1-100 LYS2 bar1Δ DSN1-6His-3Flag::URA3*
SBY8362	*MAT**a** ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 can1-100 LYS2 bar1Δ DSN1-6His-3Flag::URA3 ndc80-1*
SBY8460	*MAT**a** ura3-1 leu2,3-112 his3-11 trp1-1::256lacO::TRP1 ade2-1 can1-100 LYS2 bar1Δ DSN1-6His-3Flag:URA3 dad1-1::KAN*
SBY9047	*MAT**a** ura3-1 leu2,3-112 his3-11 trp1-1::256lacO::TRP1 ade2-1 Dsn1-3Flag::KAN ndc80::NAT:Ndc80-6His:TRP1 can1-100 LYS2 bar1Δ*

Supplementary Movies: Three-dimensional tomographic reconstructions of two representative kinetochore complexes bound to microtubules. For each particle we present a sliced view. Kinetochore complexes in pink, microtubule in yellow.