Supplemental Materials

Molecular Biology of the Cell

Dean and Mitchell

Supplemental Figures 1-4

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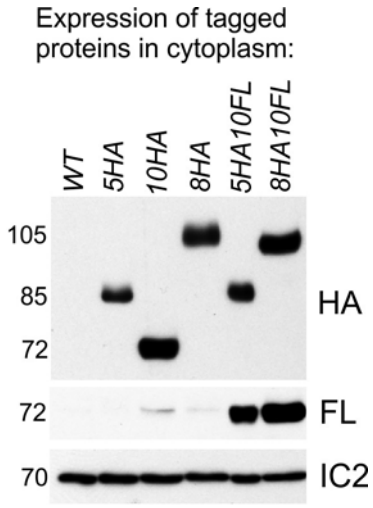
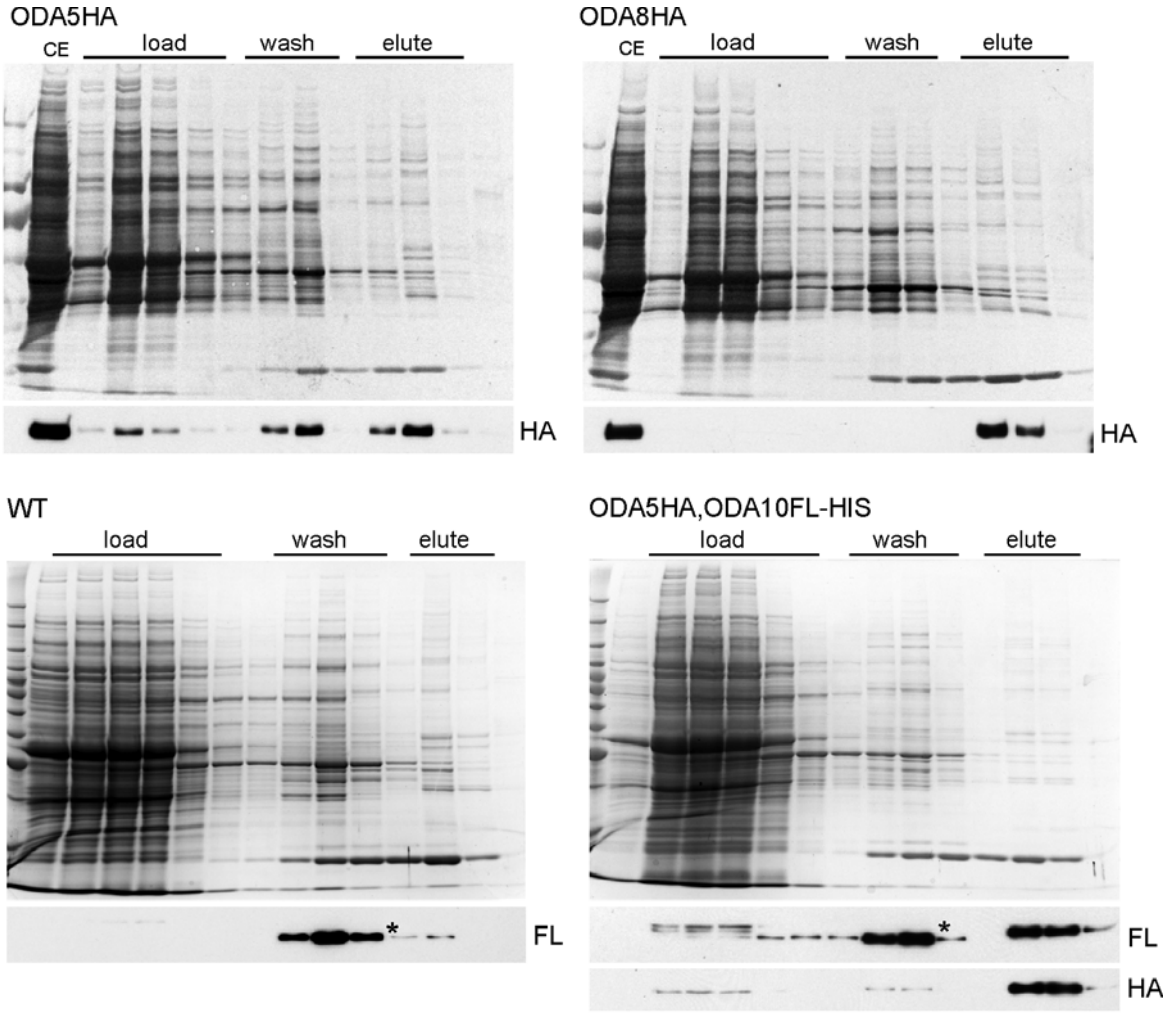
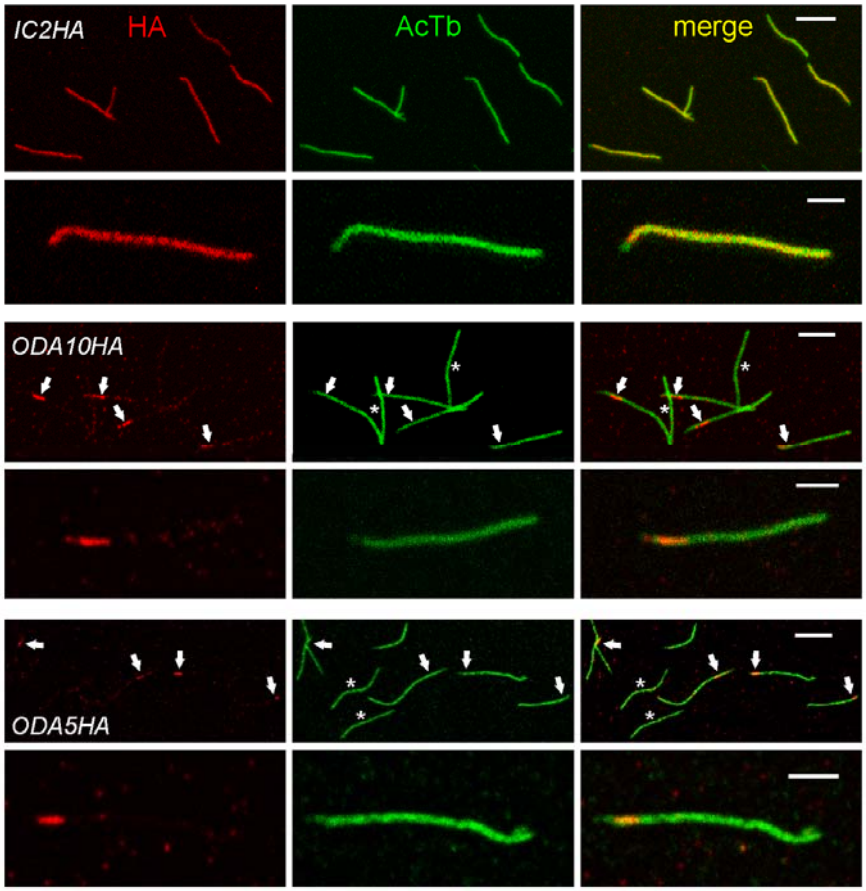


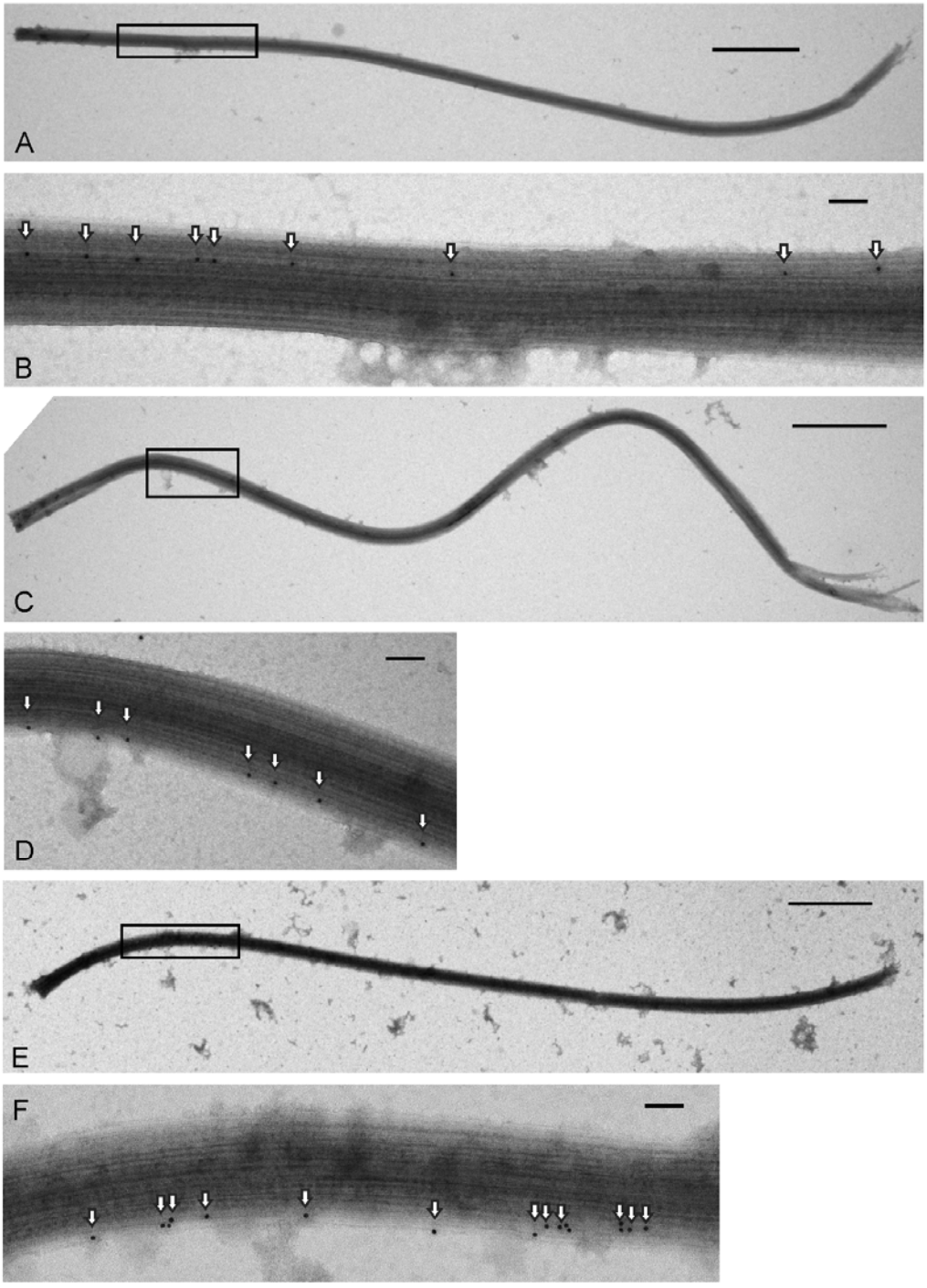
Supplemental Figure S1. Expression of tagged proteins. Blots of cell extracts demonstrate the level of expression of transgenes encoding HA-tagged ODA5, ODA10, and ODA8 (upper panel), and Flag-His tagged ODA10 (middle panel) relative to endogenous IC2 (bottom panel). Note that ODA5HA expression levels are lower than ODA10HA expression levels, and that in the *ODA5HA,ODA10FL* strain the lower level of ODA5HA results in a lower level of ODA10FL as well. Faint bands in the*10HA* and *8HA* lanes of the anti-Flag panel show the presence of an unrelated endogenous protein, which cross-reacts with the anti-Flag M2 antibody (see Supplemental Fig. S2). All transgenes are expressed in the background of strains that are null for the endogenous copy of the gene encoding the tagged protein.



Supplemental Figure S2. Fractionation of cytoplasmic extracts on Ni-NTA columns. CE, total cell extract; load, proteins that did not bind (5 mM imidizole); wash, proteins eluted with 13 mM imidizole; elute, proteins eluted with 52 mM imidizole. Top panels, elution of ODA5HA and ODA8HA when ODA10 is not tagged with 6xHIS. Bottom panels, untagged wild type extracts and double-tagged ODA5HAODA10FL extracts, showing the elution of a non-specific Flag-positive band (*) in the wash, and co-elution of ODA5HA and ODA10FL at 52 mM imidizole. The upper portion of each panel is an 8% acrylamide gel (Coomassie blue stain), lower portions are immunoblots probed with the indicated antibodies. Anti-Flag blots (FL) only show bands in the region of 60-100 kDa; additional bands representing untagged endogenous proteins appear at other molecular weights.



Supplemental Figure S3. Distribution of ODA10HA and ODA5HA on isolated axonemes. IF images of isolated axonemes showing the distribution of HA (red), acetylated tubulin (green) and merged images. Top panels, *oda6,IC2HA* axonemes demonstrate the specificity of anti-HA staining of ODAs, which extend along the entire axoneme except the distal tip. Middle panels, *oda10,ODA10HA* axonemes show that HA staining is visible on most axonemes (arrows) but not all (asterisks) and is limited to a region near one end of the axoneme. Bottom panels, *oda5,ODA5HA* axonemes show a similar distribution of label to that seen in *oda10,ODA10HA* axonemes. Scale bars = 5 μm in low-magnification images, 2 μm in high magnification images.



Supplemental Figure S4. Distribution ODA5HA on wild type axonemes and ODA10HA on *oda9* axonemes. Negative stain TEM images of immunogold labeled axonemes demonstrate the distribution of ODA5HA along a single doublet microtubule in an *oda5,ODA5HA* strain (A-D), and the similar distribution of ODA10HA in the absence of ODAs in an *oda9,oda10,ODA10HA* strain (E-F). Boxed regions near the proximal ends of axonemes in A, C and E are shown enlarged in B, D and F, where gold particles are highlighted with arrows. Scale bar = 1 μm in A, C and E and 100 nm in B, D and F.