

Supplemental Figures/Tables

Supplemental Table 1. Expansion of the cross-validated *Chlamydomonas* centriole proteome. Table of all POC and BUG proteins including version three gene identification numbers which are as specified in v3.0 of the *Chlamydomonas* genome sequence, available at the Joint Genomes Institute web site: <http://genome.jgi-psf.org/chlre2/chlre2.home>. Table also indicates protein name, human Refseq ID numbers, localizations of the protein to other proteomes of interest, GFP-localization to centrioles in human cells, and any associated human diseases. Bold Xs indicate that localization was done by another group, ♥ symbol indicates that this gene was found only in the version two *Chlamydomonas* genome and has not yet been annotated in the version three genome.

Supplemental Figure S1. POC C-terminal GFP fusion protein localizations in HeLa, U2OS or RPE-1 cells. Immunofluorescence of human cells expressing GFP fusion proteins: GFP alone, POC1A, POC2, POC3, POC4, POC6, POC7, POC8, POC9, POC11, POC17, POC20. In all cases, the GFP fusion proteins (green) co-localizes with gamma-tubulin (red, except POC8). All cells are U2OS except for the panels depicting POC1A and POC8, which were imaged in HeLa and RPE-1 cells respectively. Note the cilia in the RPE-1 cell staining of POC8 and the colocalization of the GFP-signal at the base of the acetylated-tubulin stain (red). POC8 and POC14 both had the same mutual best match human homolog as did POC9 and POC6, thus only one picture is represented for both of these sets of proteins. Scale bar, 10µm.

Supplemental Figure S2. BUG protein C-terminal GFP fusion protein localizations in U2OS cells. Immunofluorescence of human cells expressing GFP fusion proteins: DIP13, Rib43a, Hsp90, CCT3, BUG5, BUG7, BUG11, BUG22, BUG30, BUG32. In all cases, the GFP fusion proteins (green) colocalizes with gamma-tubulin (red). Scale bar, 10µm.

Supplemental Figure S3. *Chlamydomonas* POC1 antibody specificity.

Chlamydomonas POC1 peptide antibody recognizes one clear band in purified basal bodies. The molecular weight of this band is comparable to the predicted molecular weight of POC1, which is 54.4kD. No other bands were visible with this antibody. In addition to biochemical specificity as judged by Western blotting, we also analyzed immunochemical staining specificity using peptide blocking controls. *Chlamydomonas* cells were stained with POC1 antibody (red) that was previously incubated with either a control peptide that is not within the POC1 protein sequence or with the peptide that was made to produce the POC1 antibody. Merged images were stained with a nuclear stain (blue, DAPI). ImmunoEM images show a pair of basal bodies with POC1 localizing to the triplet microtubule barrel. The images indicate that POC1 antibody staining is completely abolished when the antibody was preincubated with the POC1 peptide, while the control peptide had no effect on staining, thus supporting specificity of staining. Scale bar, 250nm.

Supplemental Figure S4. Quantification of POC1 localization within centrioles from *Chlamydomonas*. (A) POC1 is highly concentrated in the basal bodies of *Chlamydomonas* but is still present in flagellar axonemes but at a reduced amount. (B) Quantification of gold-conjugated particles representing POC1 localization. Particles were quantified as being either in the distal or proximal half and either in the inner or outer walls of the triplet microtubules. The percent of all gold particles that localized to the lumen, sites of attachment, or the cartwheel structure were also quantified.

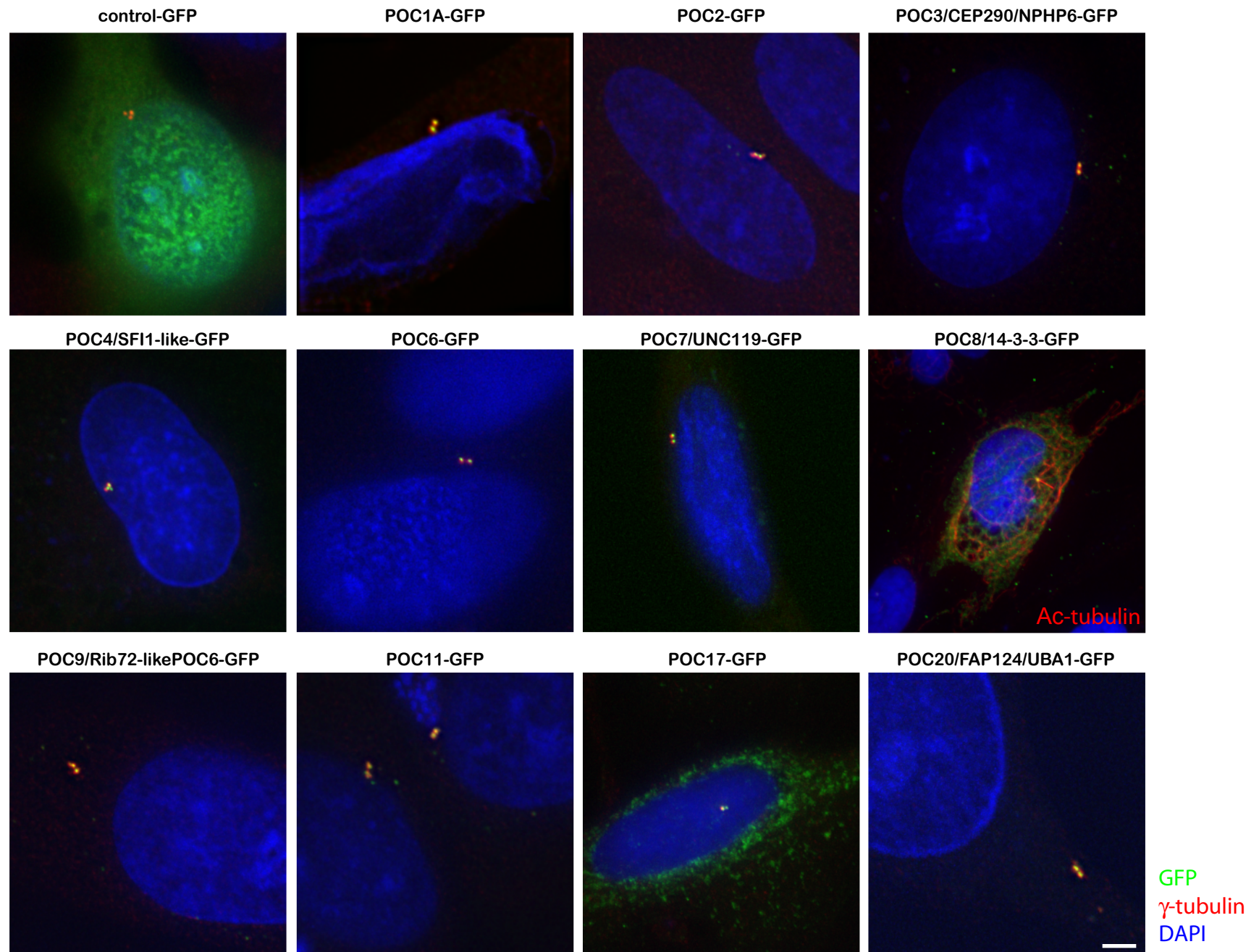
Supplemental Figure S5. POC1 is present at the basal bodies of human primary cilia. Transient transfection of POC1B-GFP into human RPE-1 cells demonstrate that POC1 localizes specifically to basal bodies (POC1B-GFP, green) and does not extend in the ciliary axoneme (actylated-tubulin, red).

Supplemental Figure S6. Elongated centriole-like structures stain with both centriole and pericentriolar material markers. A stably expressing U2OS POC1B-GFP line shows a large percent of cells with elongated centriole-like structures after S-phase arrest.

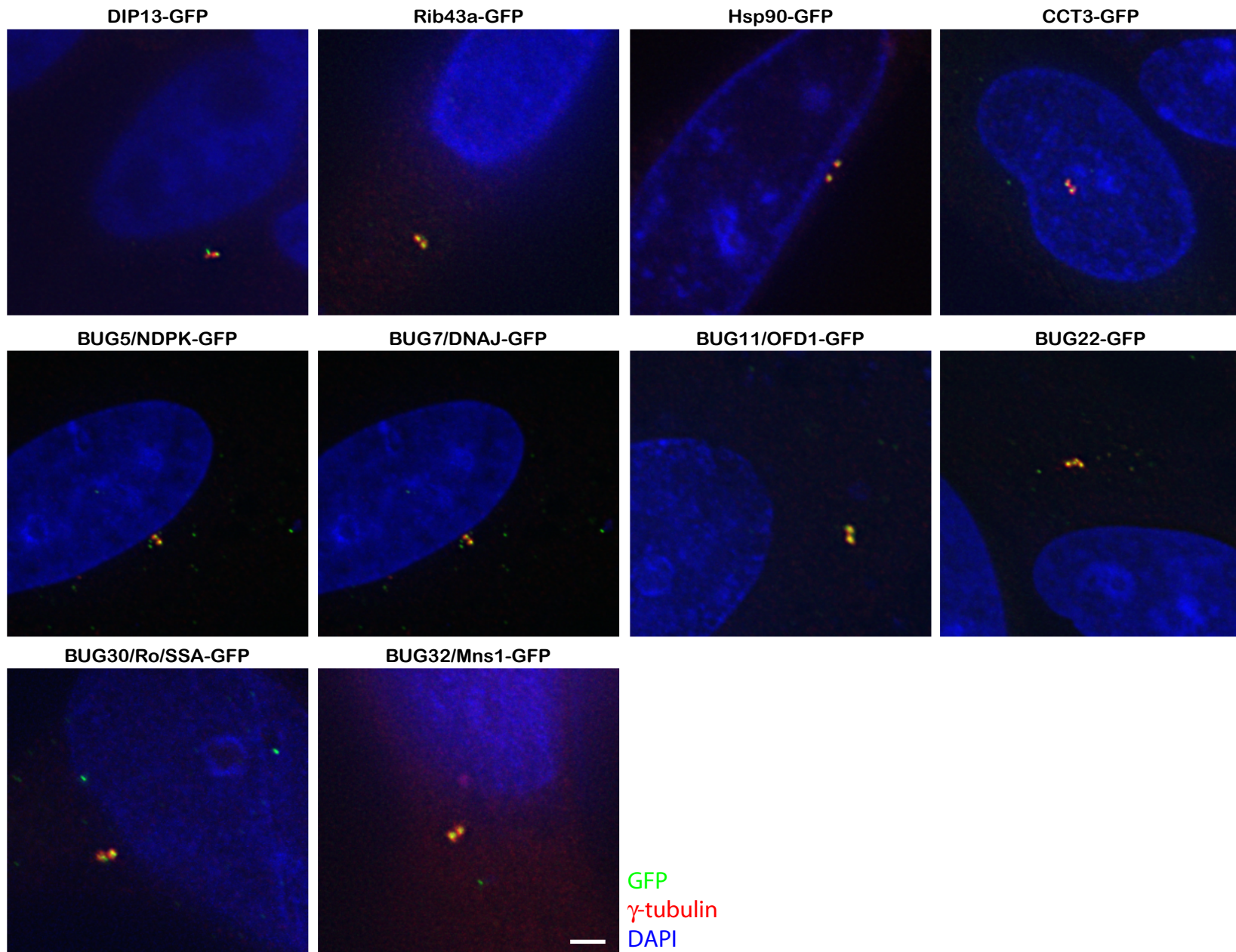
Keller et al., 2008

These structures stain with Centrin 2, acetylated-tubulin, polyglutamylated-tubulin, and gamma-tubulin. ODF2, which specifically stains the distal appendages of the mother centriole, fails to colocalize with the elongated centriole-like structures. Scale bar, 5 μ m.

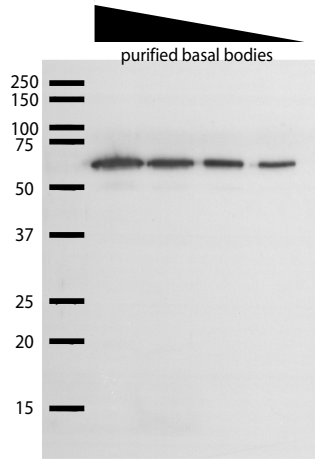
Supplemental Figure 1. POC protein C-terminal GFP fusion protein localizations in HeLa, U2OS, or RPE-1 cells



Supplemental Figure 2. BUG protein C-terminal GFP fusion protein localizations in U2OS cells



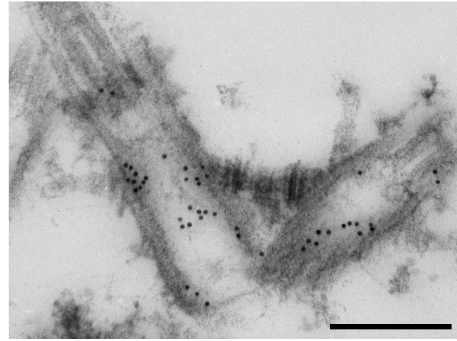
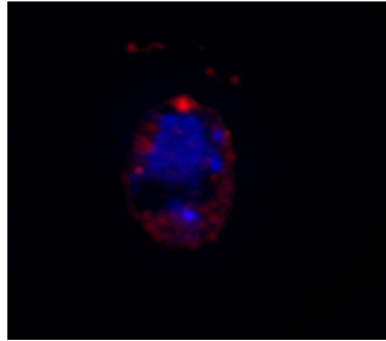
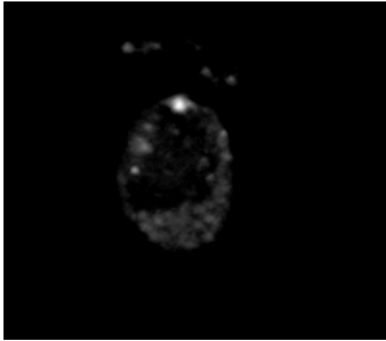
Supplemental Figure 3. *Chlamydomonas* POC1 Antibody Specificity



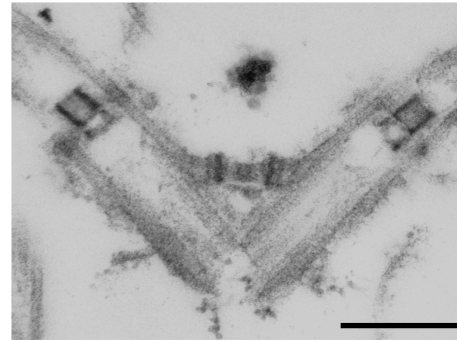
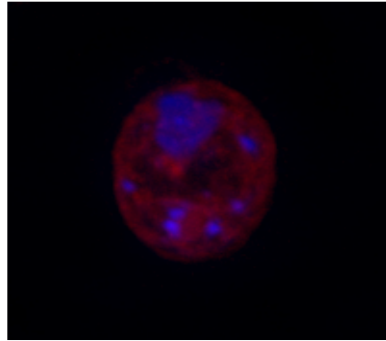
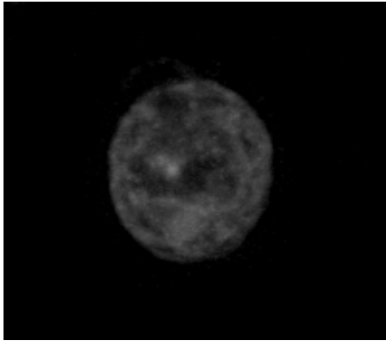
POC1

POC1 + DAPI

ImmunoEM



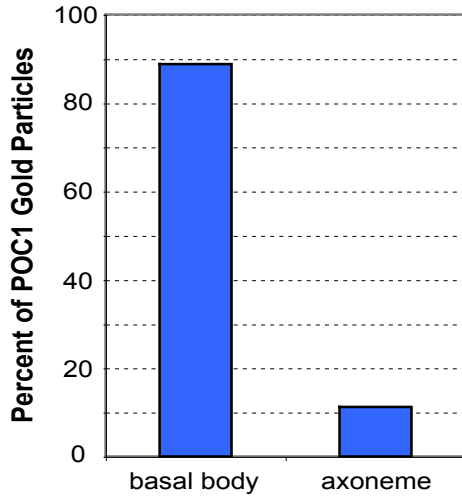
+control peptide



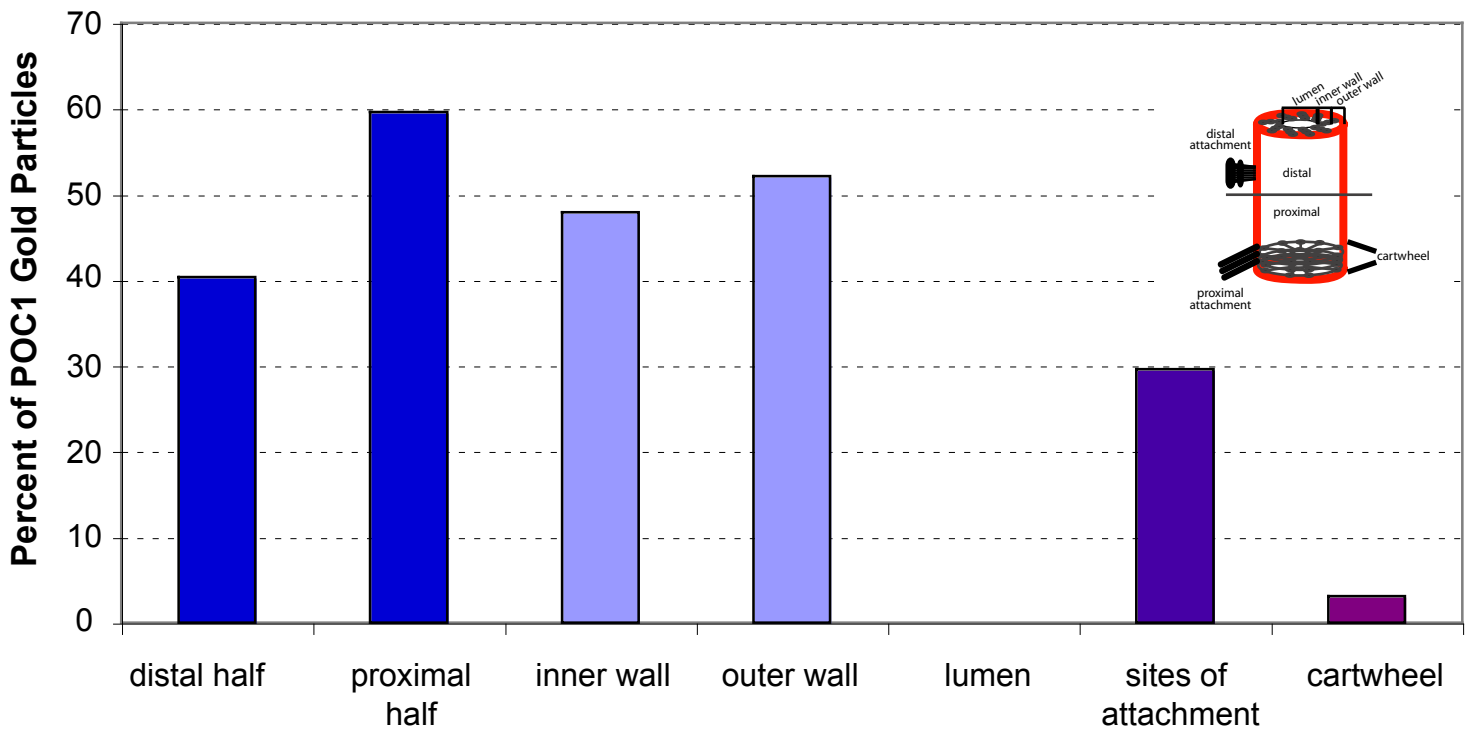
+RAG peptide

Supplemental Figure 4. Quantification of POC1 localization within centrioles and flagellar axonemes

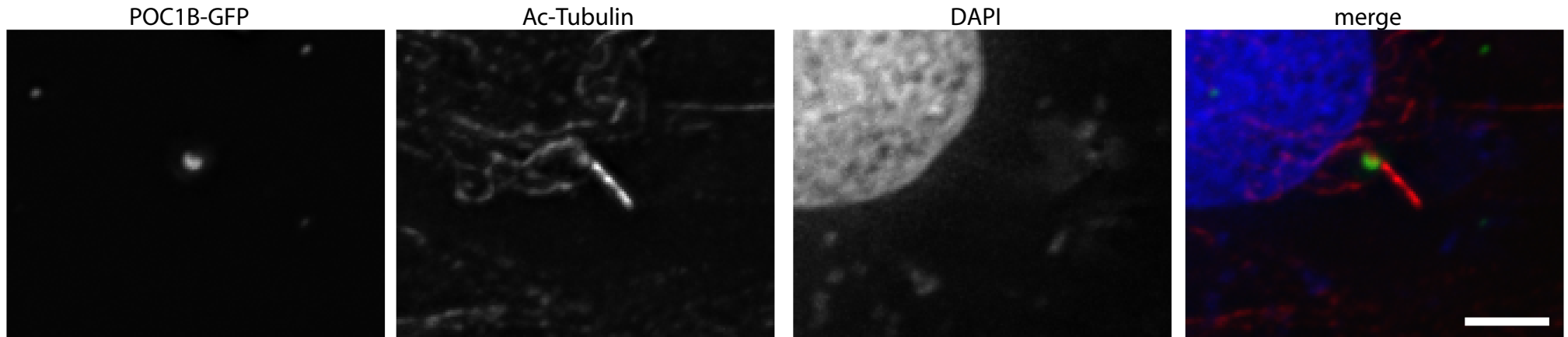
A



B



Supplemental Figure 5. POC1 localizes to Basal Bodies but not to Primary Cilia



Supplemental Figure 6. Elongated Centriole-like Structures stain with both centriole and pericentriolar material markers

