Supplemental Materials

Molecular Biology of the Cell

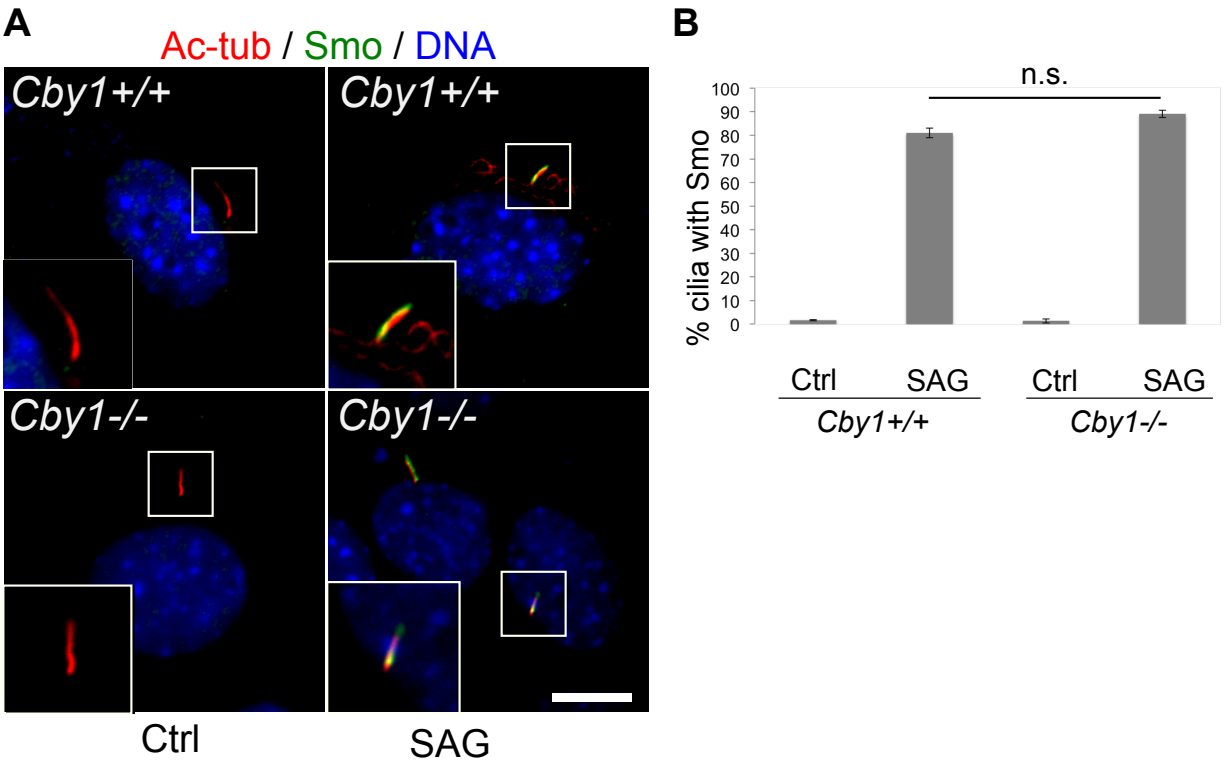
Lee et al.

Supplementary Figure 1. Smoothened is recruited to primary cilia upon SAG stimulation in both *Cby1*$^{+/+}$ or *Cby1*$^{-/-}$ MEFs (A) *Cby1*$^{+/+}$ or *Cby1*$^{-/-}$ MEFs were serum-starved for 24 h, treated with 200 nM SAG in serum starvation media for an additional 24 h, fixed and stained for acetylated α-tubulin (Ac-tub, red), Smoothened (Smo, green) and DNA (DAPI, blue). Insets are enlarged images of centrosomal/ciliary regions. Scale bar, 10 μm. (B) Quantification of the frequency of Smo-positive cilia in SAG-treated *Cby1*$^{+/+}$ or *Cby1*$^{-/-}$ MEFs. Results shown are the mean of three independent experiments ± SEM (>100 cells/experiment).

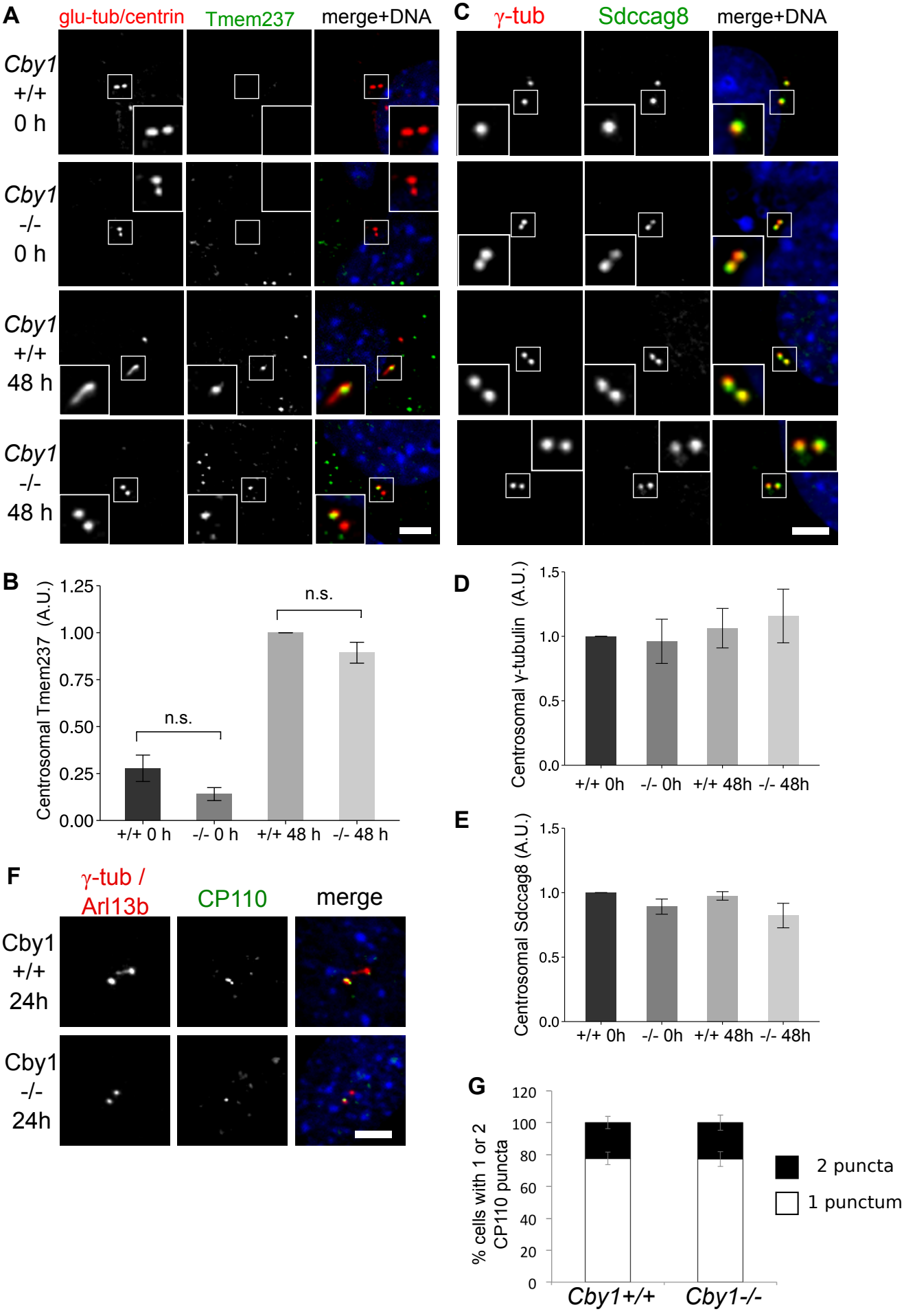
Supplementary Figure 2. Centrosomal localization of Tmem237, Sdccag8 and CP110 is unaffected by loss of Cby1. (A) *Cby1*$^{+/+}$ or *Cby1*$^{-/-}$ MEFs were fixed after 0 h or 48 h serum starvation, and stained for polyglutamylated tubulin, centrin (Glu-tub/centrin red), Tmem237 (green) or DNA (DAPI, blue). Scale bar, 5 μm. (B) Centrosomal levels of Tmem237 were quantified and normalized to *Cby1*$^{+/+}$ 48 h serum starvation condition. Results shown are the mean of three independent experiments ± SEM (>100 centrosomes/experiment). (C) *Cby1*$^{+/+}$ or *Cby1*$^{-/-}$ MEFs were fixed after 0 h or 48 h serum starvation, and stained for γ-tubulin (γ-tub, red), Sdccag8 (green) or DNA (DAPI, blue). Scale bar, 5 μm. (D) Centrosomal levels of γ-tubulin were quantified and normalized to *Cby1*$^{+/+}$ 0 h serum starvation condition. Results shown are the mean of three independent experiments ± SEM (>100 centrosomes/experiment). (E) Centrosomal levels of Sdccag8 were quantified as fluorescence intensity within the region defined by γ-tubulin labeling and normalized to *Cby1*$^{+/+}$ 0 h serum starvation condition. Results shown are the mean of three independent experiments ± SEM (>100 centrosomes/experiment). (F) *Cby1*$^{+/+}$ or *Cby1*$^{-/-}$ MEFs were fixed after 24 h serum starvation, and stained for γ-tubulin,

Arl13b (γ-tub/Arl13b, red), CP110 (green) or DNA (DAPI, blue). Scale bar, 5 μm. (G) The number of centrosomal CP110 puncta per cell was scored for each condition. Results shown are the mean of three independent experiments ± SEM (>100 cells/experiment).

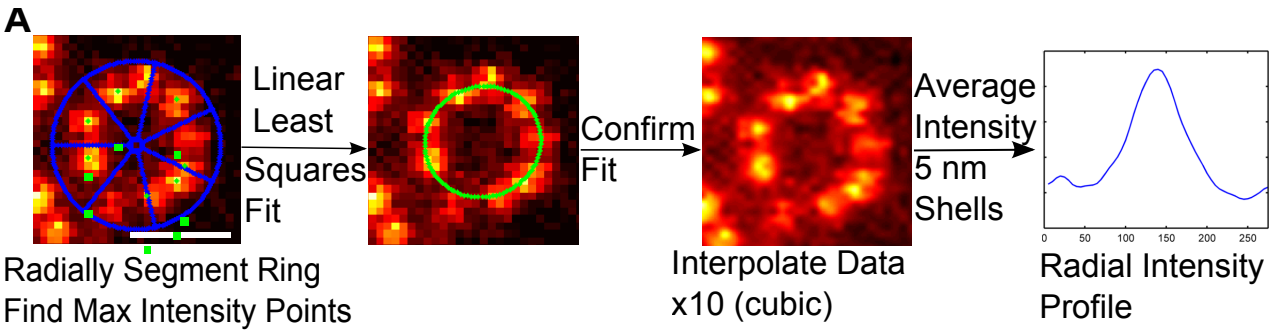
Supplementary Figure 3. STED microscopy determination of centriolar labeling radii for Tmem237, Ahi1, Cby1, Ofd1, and Sdccag8. (A) An outline of the MATLAB program used to determine radii of labeling. Individual centriole rings were isolated using an annulus (far left, blue), and the maximum intensity points from 7 radially equal sections (green pixels) were used to fit an ellipse (middle left, green). The raw data were interpolated 10x (middle right), and then the average pixel intensity in 5 nm wide annuli was used to determine the radial intensity profile (far right). Scale bar, 250 nm. (B) The ellipse fits (in green) for each of the STED images shown in Figure 4D. Scale bar, 250 nm. (C) Upper line graph shows averaged radial intensity profiles for each of the proteins. The bottom histograms show the distribution of single ring radii for each protein. Results shown are mean ± SD of N centrioles from greater than 8 cells for each protein. (D) Comparison of the average of the major and minor axes from the ellipse fit (x-axis) with the radii from the radial intensity profile (y-axis). R = 0.88.

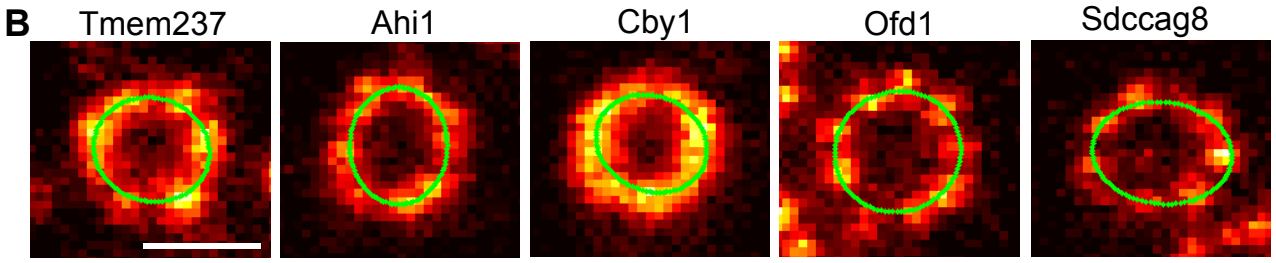


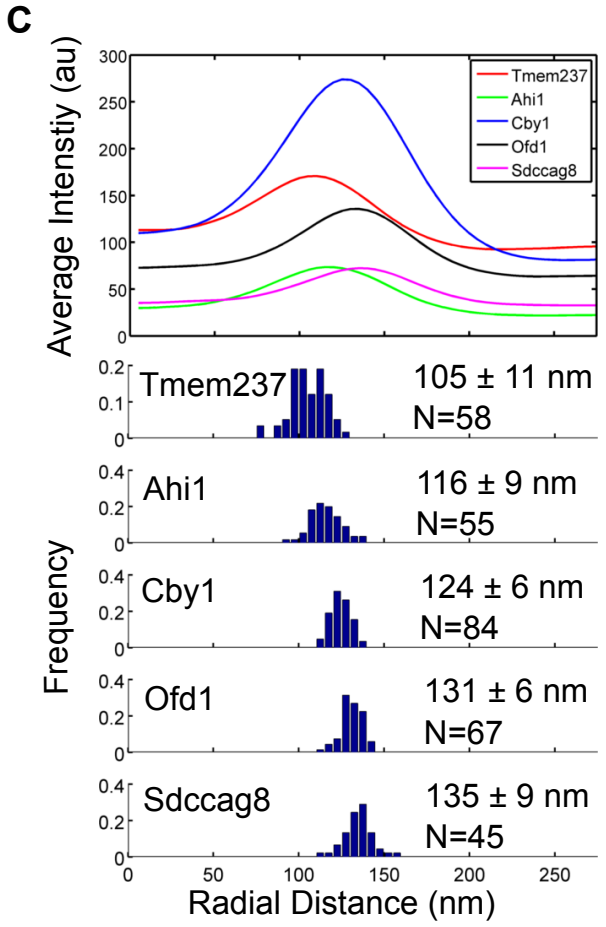
Supplementary Figure 1. Smoothened is recruited to primary cilia upon SAG stimulation in both *Cby1*$^{+/+}$ or *Cby1*$^{-/-}$ MEFs (A) *Cby1*$^{+/+}$ or *Cby1*$^{-/-}$ MEFs were serum-starved for 24 h, treated with 200 nM SAG in serum starvation media for an additional 24 h, fixed and stained for acetylated α-tubulin (Ac-tub, red), Smoothened (Smo, green) and DNA (DAPI, blue). Insets are enlarged images of centrosomal/ciliary regions. Scale bar, 10 μm. (B) Quantification of the frequency of Smo-positive cilia in SAG-treated *Cby1*$^{+/+}$ or *Cby1*$^{-/-}$ MEFs. Results shown are the mean of three independent experiments ± SEM (>100 cells/experiment).

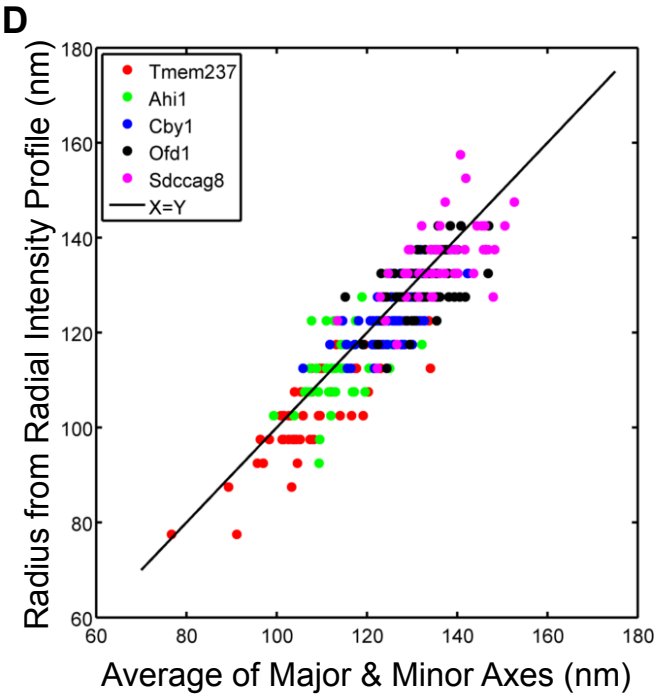

A
glu-tub/centrin
Tmem237
merge+DNA
Cby1 +/+ 0 h
Cby1 -/- 0 h
Cby1 +/+ 48 h
Cby1 -/- 48 h
C
γ-tub
Sdccag8
merge+DNA
B
Centrosomal Tmem237 (A.U.)
1.25
1.00
0.75
0.50
0.25
0.00
n.s.
n.s.
+/+ 0 h
-/- 0 h
+/+ 48 h
-/- 48 h
D
Centrosomal γ-tubulin (A.U.)
1.5
1.0
0.5
0.0
+/+ 0h
-/- 0h
+/+ 48h
-/- 48h
E
Centrosomal Sdccag8 (A.U.)
1.5
1.0
0.5
0.0
+/+ 0h
-/- 0h
+/+ 48h
-/- 48h
F
γ-tub / Arl13b
CP110
merge
Cby1 +/+ 24h
Cby1 -/- 24h
G
% cells with 1 or 2 CP110 puncta
120
100
80
60
40
20
0
2 puncta
1 punctum
Cby1+/+
Cby1-/-

Supplementary Figure 2. Centrosomal localization of Tmem237, Sdccag8 and CP110 is unaffected by loss of Cby1. (A) *Cby1*$^{+/+}$ or *Cby1*$^{-/-}$ MEFs were fixed after 0 h or 48 h serum starvation, and stained for polyglutamylated tubulin, centrin (Glu-tub/centrin red), Tmem237 (green) or DNA (DAPI, blue). Scale bar, 5 μm. (B) Centrosomal levels of Tmem237 were quantified and normalized to *Cby1*$^{+/+}$ 48 h serum starvation condition. Results shown are the mean of three independent experiments ± SEM (>100 centrosomes/experiment). (C) *Cby1*$^{+/+}$ or *Cby1*$^{-/-}$ MEFs were fixed after 0 h or 48 h serum starvation, and stained for γ-tubulin (γ-tub, red), Sdccag8 (green) or DNA (DAPI, blue). Scale bar, 5 μm. (D) Centrosomal levels of γ-tubulin were quantified and normalized to *Cby1*$^{+/+}$ 0 h serum starvation condition. Results shown are the mean of three independent experiments ± SEM (>100 centrosomes/experiment). (E) Centrosomal levels of Sdccag8 were quantified as fluorescence intensity within the region defined by γ-tubulin labeling and normalized to *Cby1*$^{+/+}$ 0 h serum starvation condition. Results shown are the mean of three independent experiments ± SEM (>100 centrosomes/experiment). (F) *Cby1*$^{+/+}$ or *Cby1*$^{-/-}$ MEFs were fixed after 24 h serum starvation, and stained for γ-tubulin, Arl13b (γ-tub/Arl13b, red), CP110 (green) or DNA (DAPI, blue). Scale bar, 5 μm. (G) The number of centrosomal CP110 puncta per cell was scored for each condition. Results shown are the mean of three independent experiments ± SEM (>100 cells/experiment).


A
Linear
Least
Squares
Fit
Confirm
Fit
Average
Intensity
5 nm
Shells
Radially Segment Ring
Find Max Intensity Points
Interpolate Data
x10 (cubic)
Radial Intensity
Profile


B
Tmem237
Ahi1
Cby1
Ofd1
Sdccag8


C
Average Intenstiy (au)
Tmem237
Ahi1
Cby1
Ofd1
Sdccag8
Frequency
105 ± 11 nm
N=58
116 ± 9 nm
N=55
124 ± 6 nm
N=84
131 ± 6 nm
N=67
135 ± 9 nm
N=45
Radial Distance (nm)


D
Tmem237
Ahi1
Cby1
Ofd1
Sdccag8
X=Y
Radius from Radial Intensity Profile (nm)
Average of Major & Minor Axes (nm)

Supplementary Figure 3. STED microscopy determination of centriolar labeling radii for Tmem237, Ahi1, Cby1, Ofd1, and Sdccag8. (A) An outline of the MATLAB program used to determine radii of labeling. Individual centriole rings were isolated using an annulus (far left, blue), and the maximum intensity points from 7 radially equal sections (green pixels) were used to fit an ellipse (middle left, green). The raw data were interpolated 10x (middle right), and then the average pixel intensity in 5 nm wide annuli was used to determine the radial intensity profile (far right). Scale bar, 250 nm. (B) The ellipse fits (in green) for each of the STED images shown in Figure 4D. Scale bar, 250 nm. (C) Upper line graph shows averaged radial intensity profiles for each of the proteins. The bottom histograms show the distribution of single ring radii for each protein. Results shown are mean ± SD of N centrioles from greater than 8 cells for each protein. (D) Comparison of the average of the major and minor axes from the ellipse fit (x-axis) with the radii from the radial intensity profile (y-axis). $R = 0.88$.