Inventory of Supplementary Materials

Figure S1, related to Figure 1. Analysis of sDAP components required to recruits others done by siRNA.

Figure S2, related to Figure 3. Analysis of AKAP450 localization and Golgi Structure in C-Nap1 mutant cells.

Figure S3, related to Figure 6. Data demonstrating the dilution of Arl13b in clustered cilia of WT versus lack of dilution in cilia of C-Nap1$^{-/-}$; CEP128$^{-/-}$.

Figure S4, related to Figure 7. Accumulation of Smoothened and Smoothened GFP at surfaced cilia in various cell lines.

Table S1, related to Figure 1. Contains information regarding the exact lesions in the Genomic DNA of each sDAP protein mutant line.

Movie S1 and S2, related to Figure 4. Time-lapse of WT and C-Nap1$^{-/-}$; CEP128$^{-/-}$ mutant cilia expressing Arl13b shows behavior of each cilia.

Movie S3, related to Figure 4. Time-lapse depicting cilia and Golgi within C-Nap1$^{-/-}$; CEP128$^{-/-}$ mutant cells. Shows behavior and Golgi separation occur simultaneously.

Movie S4 and S5, related to Figure 4. Shows the deflection of cilia in response to fluid flow in WT and C-Nap1$^{-/-}$; CEP128$^{-/-}$ mutant cells.

Movie S6 and S7, related to Figure 5. Shows cilia that were later processed for electron microscopy.

Supplementary Experimental Procedures

Figure S1

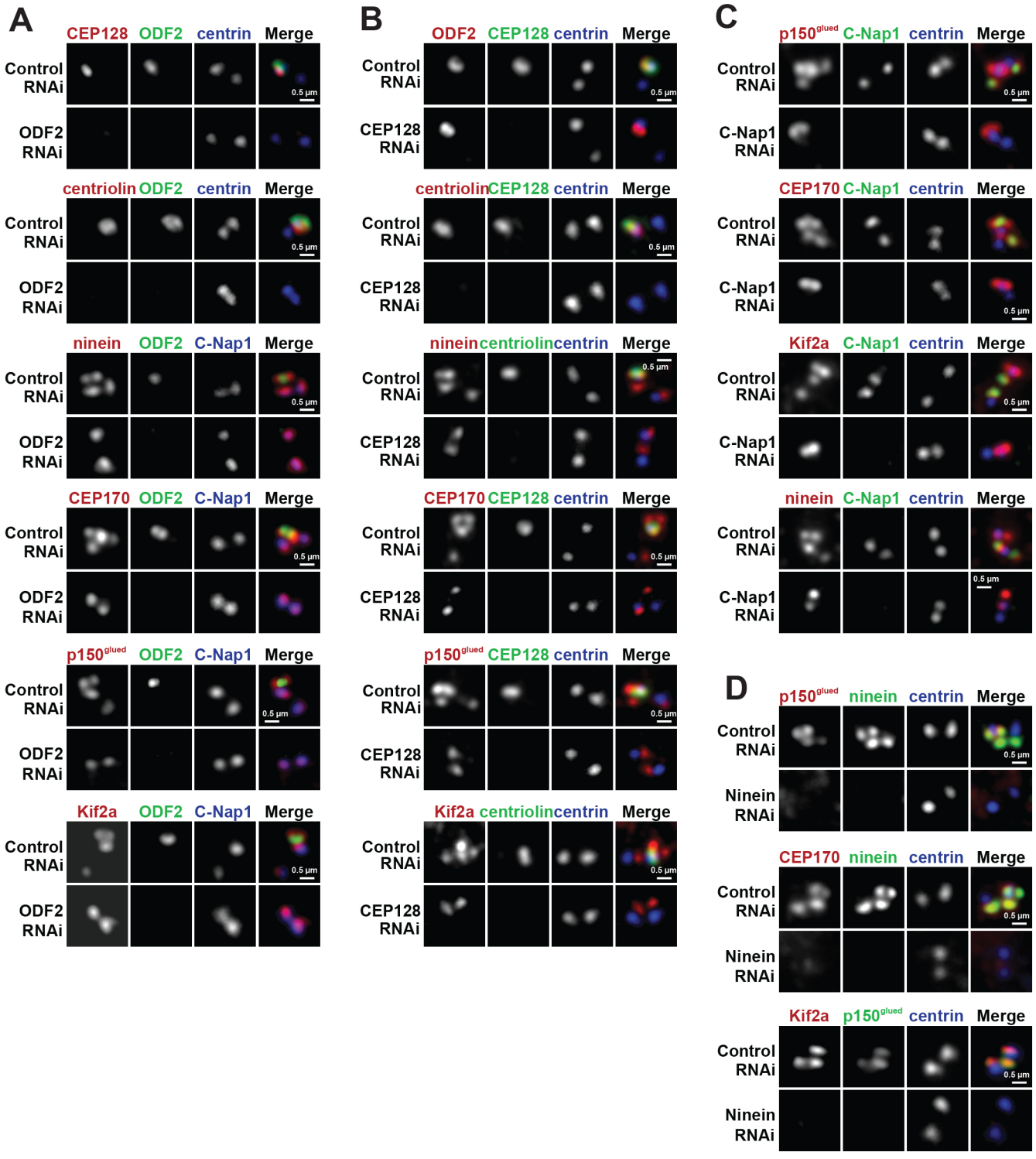


Figure S1, Related to Figure 1. Depletion of sDAP-associated proteins by RNAi phenocopies depletion by CRISPR.

(A through D) Hela cells transfected with siRNAs against the indicated centriolar proteins or with control siRNA for 60-80 hours. Cells were stained with indicated antibodies to mark the centrosomes (blue) and show the RNAi effects (green and red).

Figure S2

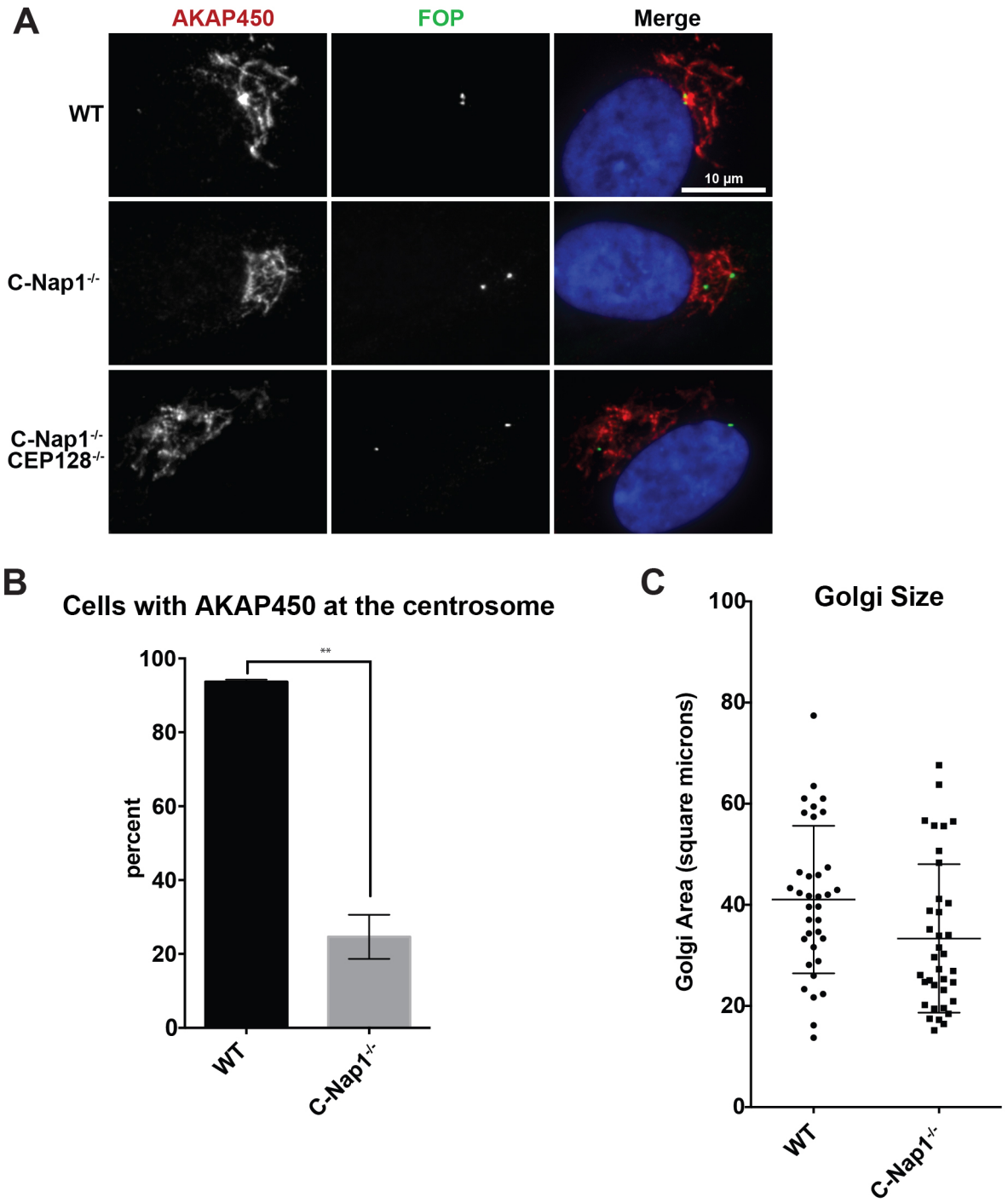


Figure S2, Related to Figure 3. AKAP450 localizes normally to the Golgi in *C-Nap1*$^{-/-}$*; CEP128*$^{-/-}$ double mutant cells

(A) AKAP450 depicted in red. RPE1 cells with indicated genotypes were stained with indicated antibodies. Note that the centrosomal localization of AKAP450 is lost in C-Nap1 knockout cells.

(B) Percentage of cells with AKAP450 at centrosomes quantified. In order to observe centrosomal AKAP450 distinctly from Golgi AKAP450, cells were treated with 5ug/ml of Brafeldin A for 2 hours prior to fixation staining and quantification. Error bars depict standard deviations. Significance was determined by unpaired two-tailed t-test with Welch's correction ($p<0.0025$).

(C) Scatterplot of Golgi Area based on GM130 staining in C-Nap1 mutant and Wild Type RPE1 cells, showing that Golgi area is similar.

Figure S3

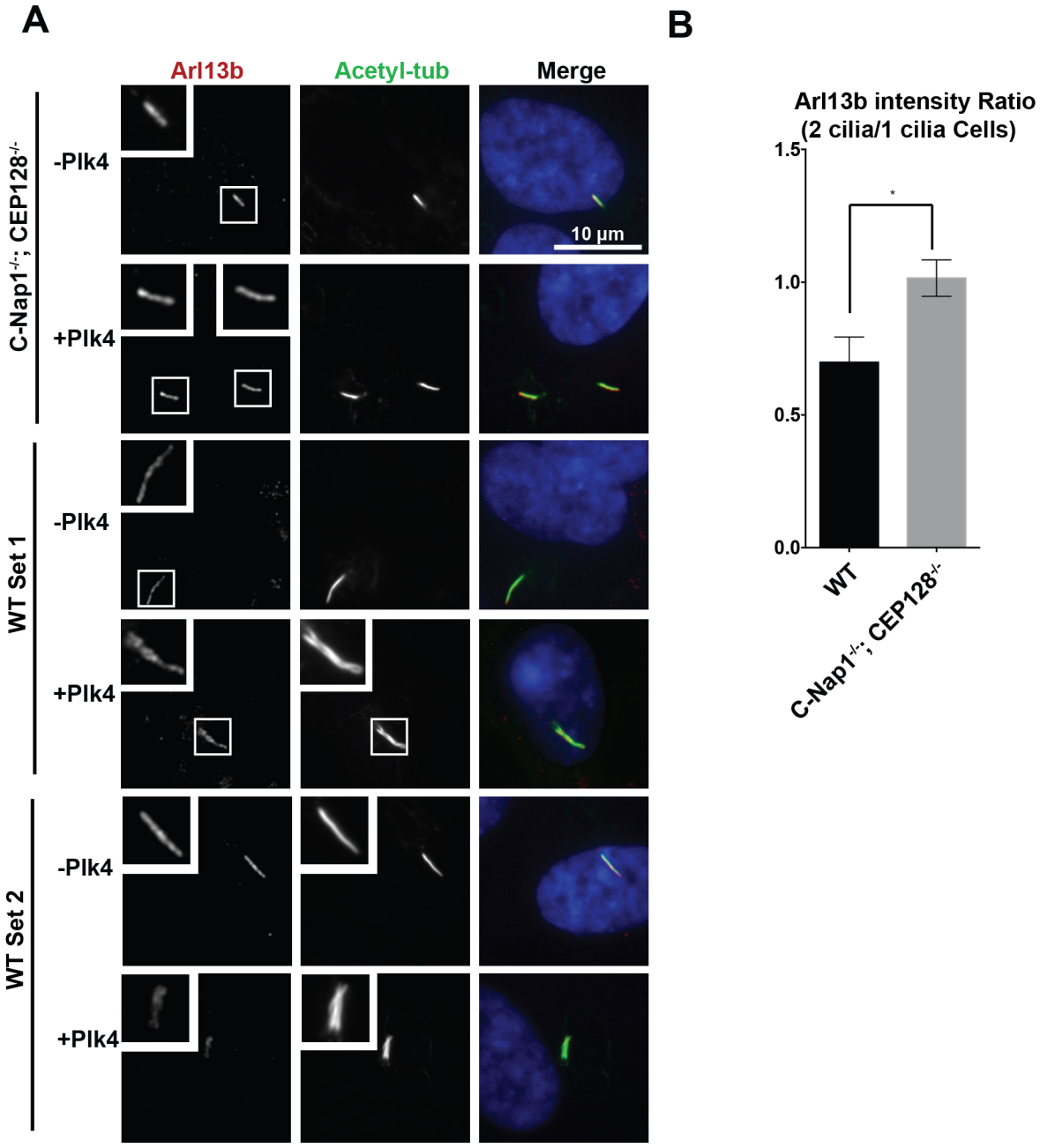
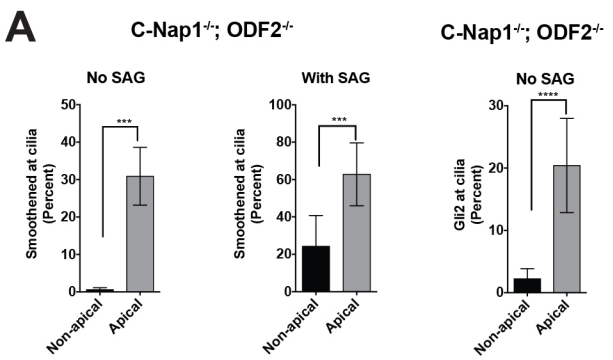
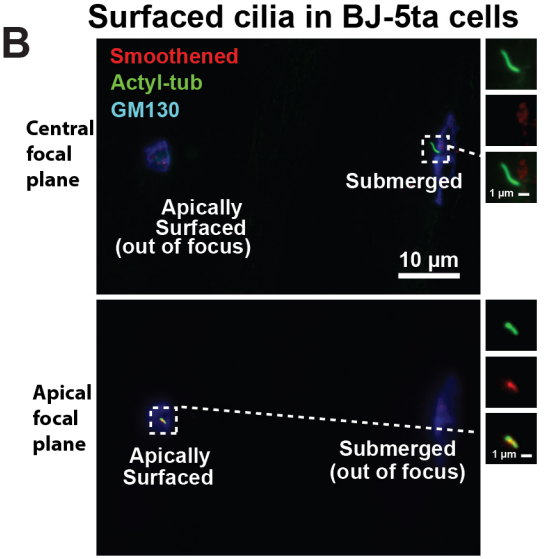
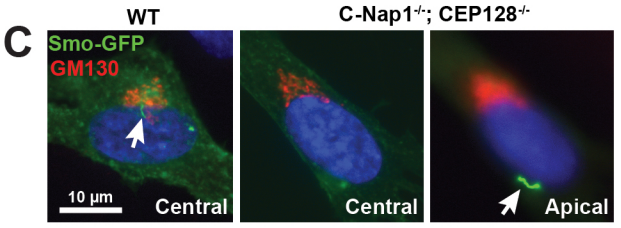


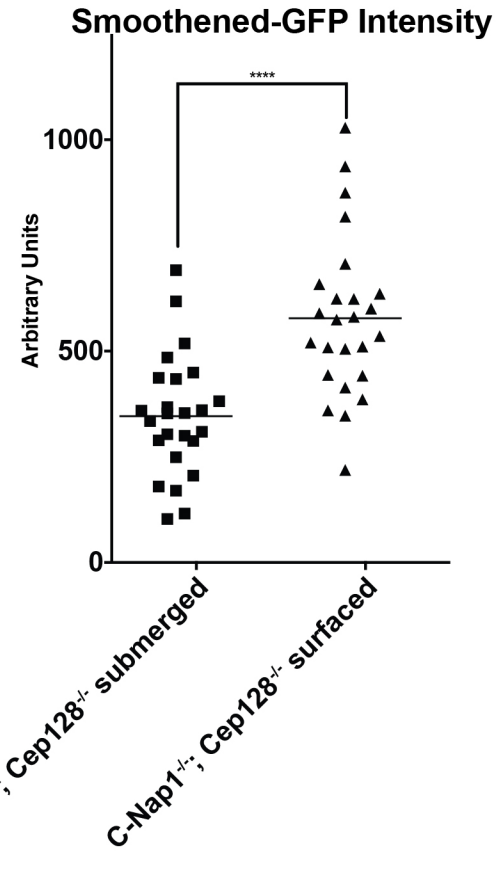
Figure S3, Related to Figure 6. Dilution of Arl13b in cells with two clustered cilia.
(A) Multiple cilia formed in wild type RPE1 cells were clustered and diluted of Arl13b while multiple cilia in *CEP128; C-Nap1* double knockout cells were distantly separated with undiluted Arl13b. Two sets of images for wild type cells are shown. Multiple cilia formation in the same cell was induced by overexpression of the PLK4 kinase, followed by 48 hours of serum starvation. Cells were stained with indicated antibodies.
(B) Quantification of Arl13b intensity. Mean value of Arl13b intensity in regions surrounding cilia was taken. Plot displays a ratio of Arl13b intensity per cilia in bi-ciliated (clustered or separated) cells over single ciliated cells. The bar lengths are an average of three independent experiments (n=10-20 cilia). Error bars represent standard deviation among the three. Significance was determined by unpaired two-tailed t-test with Welch's correction ($p<0.05$).

Figure S4


A
C-Nap1-/-; ODF2-/-
C-Nap1-/-; ODF2-/-
No SAG
With SAG
No SAG
Smoothened at cilia (Percent)
Smoothened at cilia (Percent)
Gli2 at cilia (Percent)
***
***
****
Non-apical
Apical
Non-apical
Apical
Non-apical
Apical


B
Surfaced cilia in BJ-5ta cells
Smoothened
Actyl-tub
GM130
Central focal plane
Apically Surfaced (out of focus)
Submerged
10 µm
1 µm
Apical focal plane
Apically Surfaced
Submerged (out of focus)
1 µm


C
WT
C-Nap1-/-; CEP128-/-
Smo-GFP
GM130
10 µm
Central
Central
Apical


Smoothened-GFP Intensity
****
Arbitrary Units
1000
500
0
C-Nap1-/-; Cep128-/- submerged
C-Nap1-/-; Cep128-/- surfaced

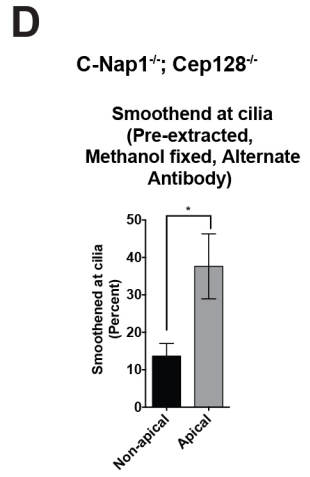

D
C-Nap1-/-; Cep128-/-
Smoothend at cilia (Pre-extracted, Methanol fixed, Alternate Antibody)
*
Smoothened at cilia (Percent)
Non-apical
Apical

Figure S4, Related to Figure 7, Accumulation of Smoothened at surfaced cilia is robustly seen in multiple cell lines and staining conditions

(A) Quantification of the ciliary accumulation of Smoothened for C-Nap1$^{-/-}$; ODF2$^{-/-}$ and cilia position in the presence or absence of SAG. Mean and standard deviation are depicted. 20-60 cilia were scored for each of the three repeats. Significance was determined by unpaired two-tailed t-test with Welch's correction ($p<0.001$).

(B) Two *BJ-5ta* cells in the same field, one carrying apically surfaced cilia and the other submerged cilia, were stained with Smoothened, cilia, and Golgi antibodies as indicated. At the central focal plane, submerged cilia, the Golgi and nucleus were in focus. At the apical focal plane, only the surfaced cilium was in focus.

(C) WT and C-Nap1$^{-/-}$; Cep128$^{-/-}$ cells expressing Smoothened-GFP are shown. Plot depicts the Smooothened-GFP intensity for 25 cells of each location. Lines depict means. Significance was determined by unpaired two-tailed t-test with Welch's correction ($p<0.0001$).

(D) Quantification of the ciliary accumulation of Smoothened for C-Nap1$^{-/-}$; Cep128$^{-/-}$. Cells were triton pre-extracted, fixed with -20C methanol and then stained with a different smoothened antibody from that used elsewhere. Mean and standard deviation are depicted. 20-60 cilia were scored for each of the three repeats. Significance was determined by unpaired two-tailed t-test with Welch's correction ($p<0.05$).

Movie Legends

Movies S1, S2, Related to Figure 4
WT (movie S1) or *C-Nap1*$^{-/-}$*; CEP128*$^{-/-}$ mutant (movie S2) RPE1 cells expressing GFP-tagged Arl13b were serum-starved for 36hr, and imaged by time-lapse fluorescence microscopy with 2-min interval. Images in the movie were maximum intensity projections of a z-stack.

Movies S3, Related to Figure 4
C-Nap1$^{-/-}$*; CEP128*$^{-/-}$ mutant RPE1 cells expressing both Arl13b-GFP and GalT-GFP were serum-starved for 36hr, and imaged by time-lapse fluorescence microscopy with 2-min interval. Images in the movie were maximum intensity projections of a z-stack.

Movies S4, S5, Related to Figure 4
Cilia mobility assay. WT (movie SS4) or *C-Nap1*$^{-/-}$*; CEP128*$^{-/-}$ mutant (movie 5) cells growing Arl13b-GFP labeled cilia were imaged by time-lapse fluorescence microscopy for 10 sec during which fluid flow was activated in the last 5 sec. The time interval is 200 milliseconds. Images in the movie were maximum intensity projections of a z-stack.

Movies S6, S7, Related to Figure 5
LM/EM analyses. *C-Nap1*$^{-/-}$*; CEP128*$^{-/-}$ double mutant cells carrying Arl13b-GFP labeled cilia were imaged by time-lapse fluorescence microscopy in the presence of fluid flow. Cells carrying flow-sensitive or insensitive cilia were marked and processed for serial sectioning transmission electron microscopy (STEM).

Table S1

Cell line #	Genotype	Genetic Lesion(s) introduced to one allele	Genetic Lesion(s) introduced to the second allele	Lesion Description and other comments	Expected protein resulting from lession
1	*ODF2-/-*	GAGTGTCCGGGTGAAAAACCAAGG	GAGTGTCCGGGTGAAAAACCAAGG	Small insertions. Result frameshift	Expect 170AA and 166AA long protein fragments (Missense after 145AA). Out of 893 full length ODF2
2	*ODF2-/-*	GAGGGAACAGCACT~~GC~~AAAGAGG	GAGGGAACAGCA~~CTGC~~GTAAAGAGG	Small deletion and insertion. Result frameshift	Expect 342AA protein fragment (Missense after 339). Out of 893 full length ODF2
3	*Cep128-/-* (3 gRNAs)	lesion 1 (exon 3): GCTGCCAGATCAACGC~~AC~~AGGG lesion 2 (exon 14): ~~CCTTCAGA~~TCTCAGAGCTGACTC	lesion 1(exon 3): GCTGCCAGATCAACGCAACAGGG lesion 2 (exon 13 and 14): AACTTCAGCG <large deletion 38.2 KB> ATCTCAGAGCTG	At the first gRNA target site, there are small indels that result in a frameshift of both alleles. At the other two gRNA target sites, there is one small and one large deletion. The large deletion eliminates everything between two gRNA target sites.	Expect 68AA and 69AA protein fragments (Missense after 25) out of 1094 AA full length Cep128.
4	*CNTRL-/-* (CNTRL was targetted by 2 gRNAs)	lesion 1 (exon 14): ACATTATGTTTTGAAAAAA<196 bp deletion extending into intron>ACATTATGTTTTGAAAAAA lesion 2 (exon 15): CTCAGTGCCTATGAAGCT~~GAGCTA~~GAGGCTCGGCTAAACCT	lesion 1 (exon 14 and 15): ATAGCAGCAAATGAAGC<25KB large deletion>CTATATTTGTAGGTA	In one allele one there is a large deletion (196bp) that results in a frameshift and small in frame deletion at downstream site. On the second allele a very large deletion includes and goes beyond the cut sites of both gRNAs.	614 AA and 616AA protein fragments expected (missense after 603 and 610) out of 2326 AA full length Centriolin
5	*Nin-/-*	GCT~~CAGCCCAAATATGTTAGAGGTGGGAAGCG~~TTACGGACG AA	GCTCAGCCCAAATATGT~~T~~GCAGAGG	Small deletion and insertion. Result frameshift	Expect 111AA and 121AA protein fragments compared to 2090AA full length Ninein
6	*C-Nap1-/-*	GGAGACCACTGGGAT~~ACTACAGACCCAGC~~TCCAGGAGGCT	GGAGACCACTGGGAT~~ACTACAGACCCAGC~~TCCAGGAGGCT	Small deletion in both alleles. Result frameshift	984AA protein fragment expected out of 2442AA full length C-Nap1. (Missnse after 968)
7	*C-Nap1-/-; ODF2-/-*	GAGGGAACAGCACTGCAAAAGAGG	~~GAGGGAACAGCACTGCAAA~~GAGG	Small deletion and insertion. Result frameshift. ODF2 mutation was created in cell line #6	343AA and 342AA trunction proteins expected (Missense after 339). Out of 893 full length ODF2
8	*C-Nap1-/-; Cep128-/-* (Cep128 was targetted by 3 gRNAs)	lesion 1 (exon 3): GCTGCCAGATCAACGCA~~G~~AGGG lesion 2 (exon 13 and 14): AACTTCAGCG <large deletion 38.2 KB> ATCTCAGAGCTG	lesion 1 (exon 3): GCTGCCAGATCAACGCAACAGGG lesion 2 (exon 13 and 14): AACTTCAGCG <large deletion 38.2 KB> ATCTCAGAGCTG	At one gRNA target site, there are small 1 bp indels that result in a frameshift of both alleles. Downstream of the first site, there is a very large deletion of the sequences between the other two gRNAs target sites. Cep128 mutation was created in cell line #6	34AA and 69 AA protein fragments expected. (Missense after 25) out of 1094 AA full length Cep128.
9	*C-Nap1-/-; CNTRL-/-* (CNTRL was targetted by 2 gRNAs)	lesion 1 (exon 14): GTGTTATCAGTGGGTTGCAAGA~~AT~~ACCTGGGGACCA lesion 2 (exon 15): GTGCCTATGAAGCTGAGC <266bp insertion> TAGAGGCTCGGCTAAAC	lesion 1 (exon 14 and 15): GATTTAGAAGGTGTTATC TGT <large 10KB deletion> AGGCTCGGCTAAACCTAA	In one allele there is a small deletion that produces a frameshift at one site and a large insertion at the second site. On the second allele, there is a very large deletion of sequences in between the two gRNA target sites. Centriolin mutation was created in cell line #6	622 and 627 AA protein fragments expected out of 2326 AA full length Centriolin. Missense after 618
10	*Cep128 -/-; C-Nap1 */-* 'Restoration'	GGAGACCACTGG~~G~~AT~~ACTACAGACCCAGC~~TCCAGGAGGCT	GTGGGATATGATTTATTTATATAT <large deletion 379 bp> TCCAGGAGGCTCAACGGGAG	A small 1bp deletion in one allele that had previously been altered (14bp deletion) in creation of the *C-Nap1-/-*. Results in an open reading frame. Other allele has large deletion that does not. C-Nap1 'Restoration' was done in cell line #8.	Expect protein similar to full length C-Nap1 except 6 internal amino acids out of the 2442 AA protein are missing and one Phenylalanine residue is inserted in their place.

Table S1, Related to Figure 1

Sequence analyses of the mutation in each CRISPR knockout cell line generated in this study. Deleted nucleotide residues are depicted in red. Inserted nucleotides are depicted in green.

Supplementary Experimental Procedures

siRNA for knockdown

RNAi was performed using RNAiMax transfection reagent and siRNAs obtained from Life Technologies. Cells were fixed and stained 3 days after transfection. The control siRNA used was the Silencer® Select Negative Control No. 1 (Life Technologies). The antisense sequences of siRNA used to target Cep128 are 5'-UCACGUAUGAAAAUCUUGGAC-3' and 5'-UAACCUUCGAGAUAGCUCCAA-3'.
Those for ODF2 are 5'-UUUACAAGAUCUGUUACCCGG-3' and 5'-UUGGUUUUCACCCGGACACUC-3'. Those for ninein are 5'-AUACUCCUCACUGCGUUGCGU-3' and 5'-UUUGACCUCAUCGUAACUCUU-3'. That for Cep250 was 5'-UGACAUAUGGGCUUGCUCCAG-3'.