Pitaval et al., https://doi.org/10.1083/jcb.201610039

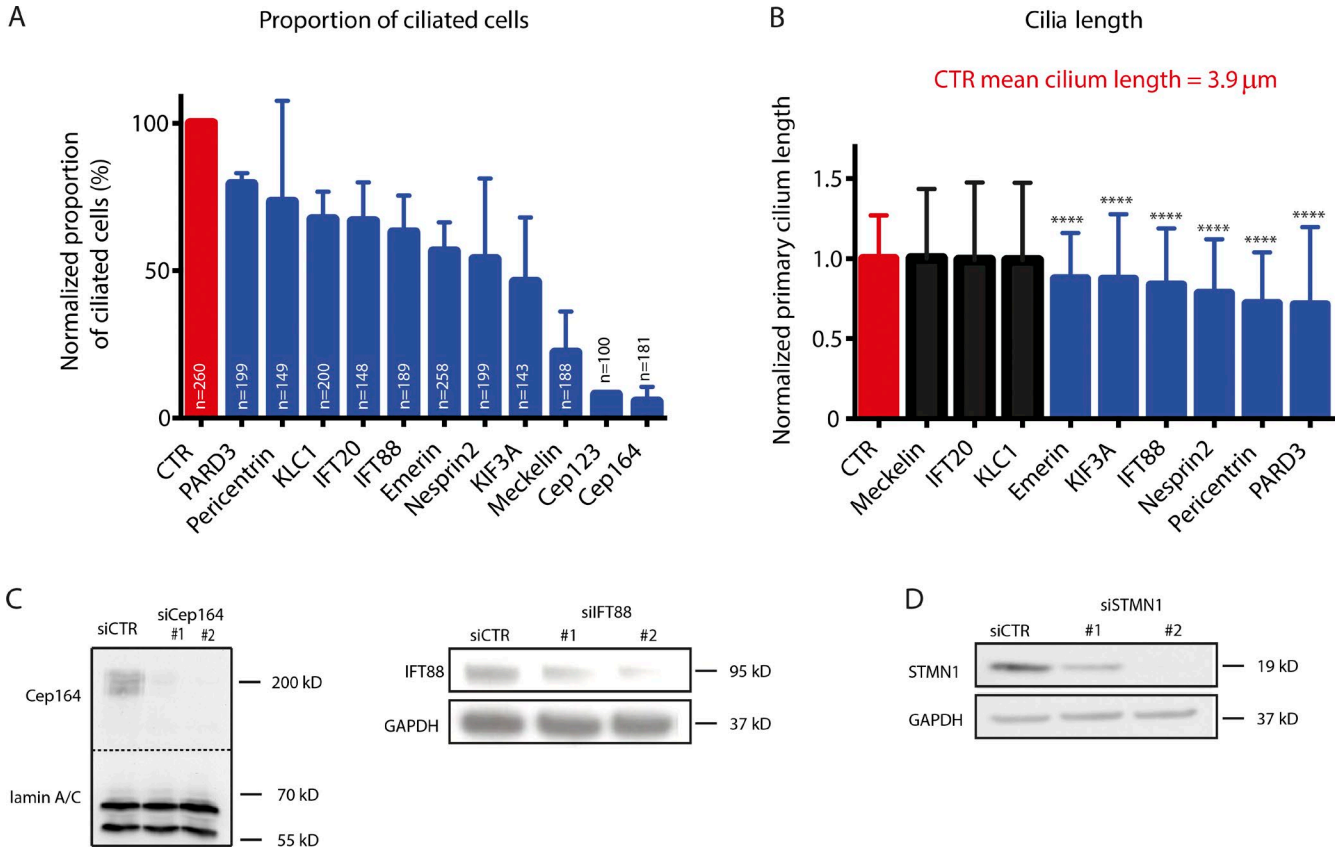


Figure S1. **Validation of siRNAs used in this study.** (A) Control of siRNA efficiency presented in Fig. 2 A by quantification of the number of ciliated cells. Proportions were normalized with respect to nontargeting control siRNA for each condition. Red is the control, and blue means there is a statistically significant difference with respect to control. (B) Control of siRNA efficiency presented in Fig. 2 A by quantification of cilia length. Proportions were normalized with respect to nontargeting control siRNA for each condition. Red is the control, blue means there is a statistically significant difference with respect to control, and black means there is no significant difference with the control. ****, $P < 0.0001$. Error bars represent standard deviation. (C) WBs demonstrating the efficacy of the Cep164 and IFT88 siRNAs. Lamin A/C or GAPDH antibodies were used as loading controls for the blots. (D) WBs demonstrating the efficacy of the stathmin 1 siRNAs. GAPDH antibodies was used as loading controls for the blots.

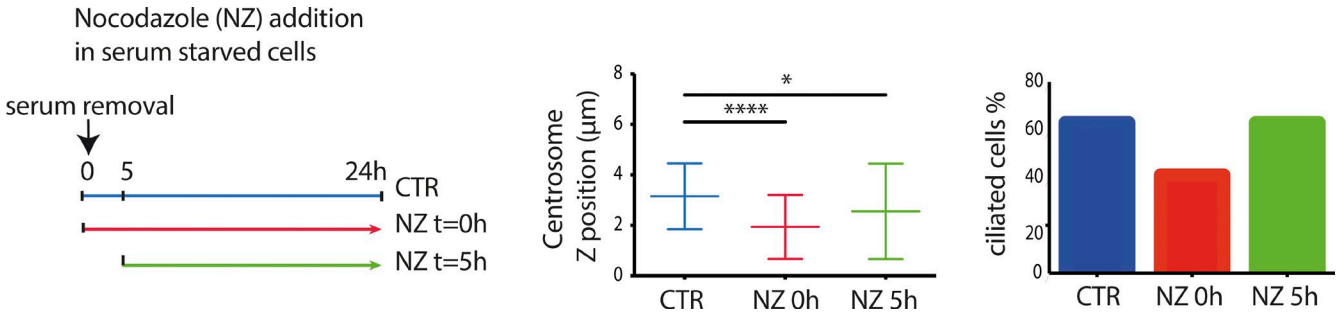


Figure S2. **Nocodazole experiment.** Effect of microtubule depolymerization on centrosome z position and ciliogenesis rate by addition of nocodazole synchronously with (red) or 5 h after (green) serum withdrawal (results of two independent experiments, $n = 75$ cells per condition). Centrosome z position (middle) and ciliated cell percentages (right) are represented for each condition. *, $P < 0.05$; ****, $P < 0.0001$.

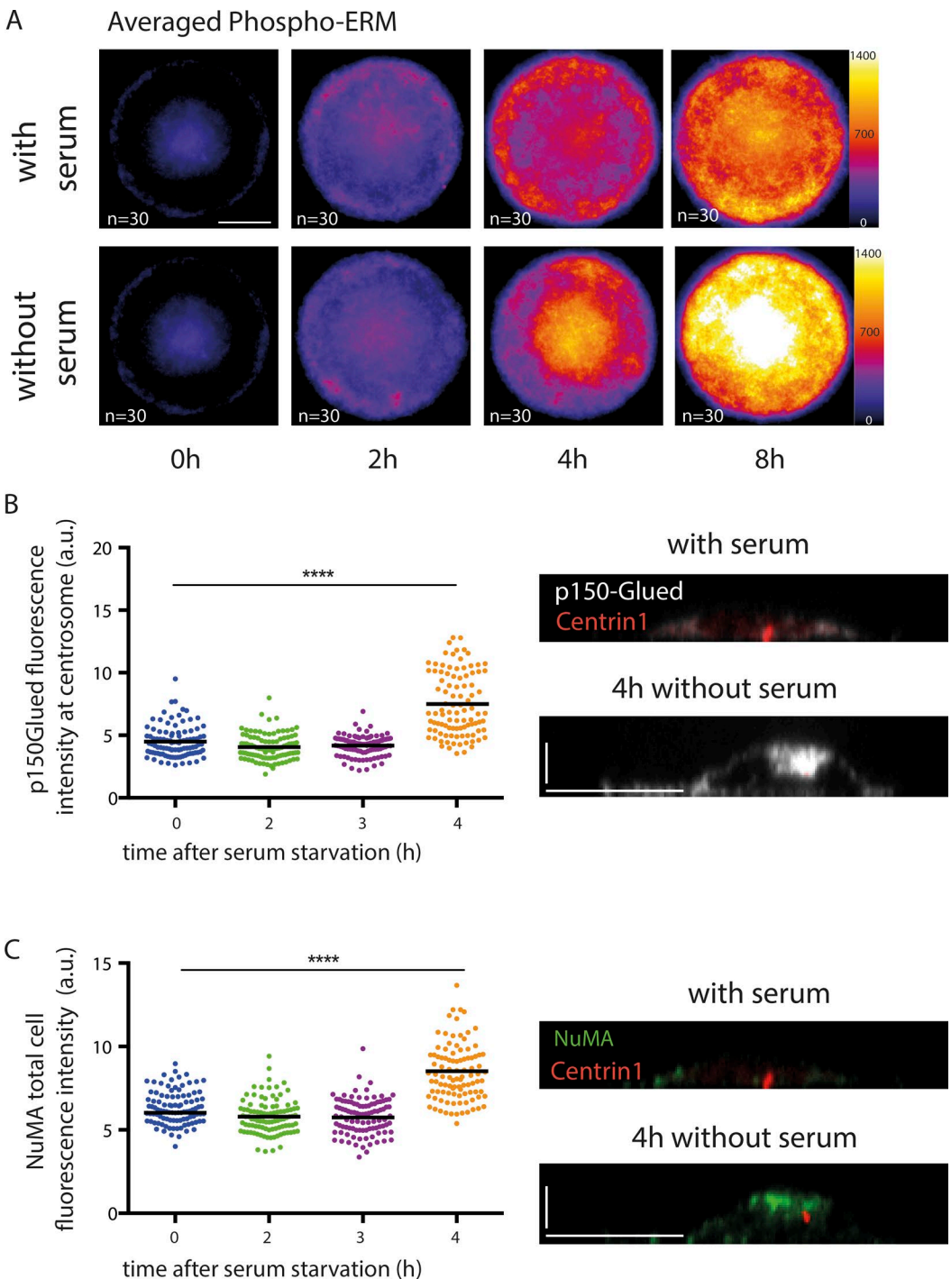


Figure S3. **Recruitment of apical markers during centrosome migration.** (A) Averaging of phospho-ERM fluorescence intensity levels (LUT fire) in serum-fed and serum-starved cells obtained by stacking and averaging 30 images per condition. (B) The graph shows the measurements of p150Glued fluorescence intensity at the centrosome in RPE1 EGFP-centrin1 cells after serum starvation (results of two independent experiments, *n* = 100 cells per condition). Side views of RPE1 cells expressing EGFP-centrin1, cultured in the presence or absence of serum, stained with antibody to p150Glued. (C) The graph shows the measurements of NuMA fluorescence intensity at the centrosome in RPE1 EGFP-centrin1 cells after serum starvation (results of two independent experiments, *n* = 100 cells per condition). Side views of RPE1 cells expressing EGFP-centrin1, cultured in the presence or absence of serum and stained with antibody to NuMA (green; same cells as B). Total NuMA fluorescence intensity was measured on maximal projection of z stacks. Bars: (x and y) 10 µm; (z) 3 µm. ****, $P < 0.0001$. a.u, arbitrary units.

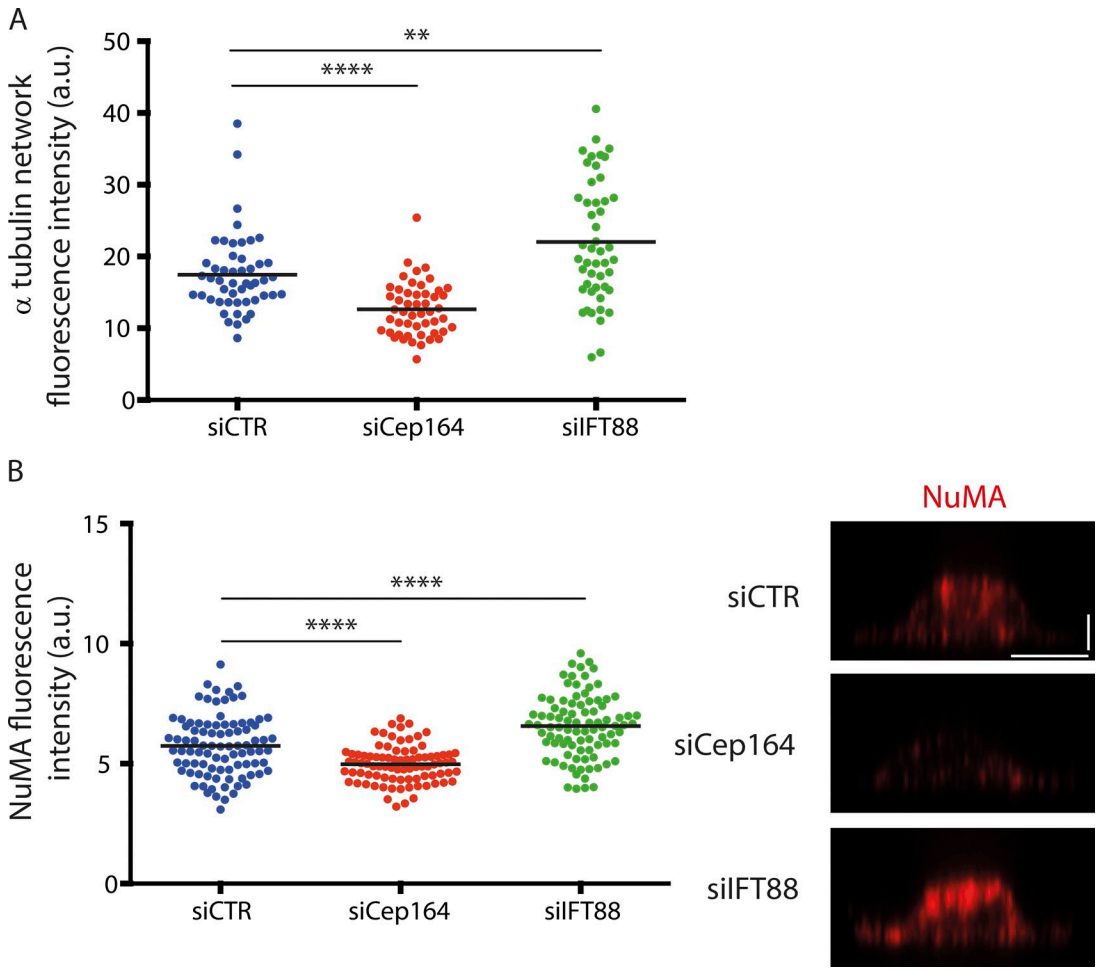
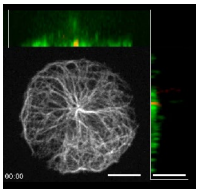
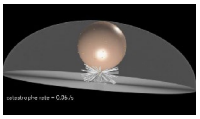


Figure S4. **Implication of ciliogenesis effectors in microtubule network remodeling and apical NuMA recruitment.** (A) The graph shows the measurements of tubulin fluorescence intensity at the centrosome in RPE1 EGFP-centrin1 cells 4 h after serum starvation (results of two independent experiments, *n* = 100 cells per condition). (B) The graph shows the measurements of NuMA fluorescence intensity at the centrosome in RPE1 EGFP-centrin1 cells 4 h after serum starvation (results of two independent experiments, *n* = 100 cells per condition). Images show representative side views of NuMA staining in response to siRNA treatments. Total NuMA fluorescence intensity was measured on maximal projection of z stacks. Bars: (x and y) 10 µm; (z) 2.5 µm. **, P < 0.01; ****, P < 0.0001. a.u., arbitrary units.



Video 1. **Microtubule reorganization and centrosome motion upon serum starvation.** This video shows x, y, and z projections of z stacks obtained during live imaging of micropatterned RPE1 stably expressing the centrosomal marker EGFP-centrin1 cells and transduced with MAP4-RFP to label the microtubules. In the left cell, microtubule bundling led to centrosome apical motion. In the right cell, microtubules did not form any bundles, and the centrosome remained close to the basal pole. Magenta arrows point at the bundle of microtubules, and the red arrows point at the centrosomes. Bars: (xy) 10 µm; (xz and yz) 5 µm.



Video 2. **Numerical simulation of microtubule network reconfiguration and centrosome motion in 3D.** Simulations were performed with Cytosim. Little dots represent dyneins. They were present in equal concentrations in both conditions. Microtubule minus ends were bound to the centrosome. Only the microtubule length was varied by modulating the microtubule catastrophe rate (0.06 s^{-1} on the left and 0.007 s^{-1} on the right).

Table S1. **Sequences used in this study**

Gene name	Target sequence (5′ to 3′)
siCTR	AATTCTCCGAACGTGTCACGT
siCep164 sequence 1	CAGGTGACATTTACTATTTCA
siCep164 sequence 2	ACCACUGGGAAUAGAAGACAA
siIFT88 sequence 1	TTCCGGCTATAATGACTACAA
siIFT88 sequence 2	ATGCAGGTGGTCAGTGTTATT
siIF20 sequence 1	AAGGTATCGGGTTGAATATGA
siIFT20 sequence 2	TCGGAACTTGCTCAAATCTAT
siPARD3 sequence 1	ATCGACAAATCTTATGATAAA
siPARD3 sequence 2	TGGAGTAGATTTAGTGGGCAA
siKIF3A sequence 1	AAGACCTGATGTGGGAGTTTA
siKIF3A sequence 2	CTGGTTCAGAAAGACAGGCAA
siPericentrin (PCNT) sequence 1	TTGGACGTCATCCAATGAGAA
siPericentrin (PCNT) sequence 2	AGCGACGATTGCCGAGAGAAA
siMeckelin (TMEM-67) sequence 1	TGGCTAGCCATTGGAATTATA
siMeckelin (TMEM-67) sequence 2	CAGTTAGGATTAGCACCTCAA
siKLC1 sequence 1	CACGTTGTGTGCGATAACGTA
siKLC1 sequence 2	CCCGATGTTGCCAAGCAGTTA
siEmerin (EMD) sequence 1	CAGGTGCATGATGACGATCTT
siEmerin (EMD) sequence 2	CCCAAGAAAGAGGACGCTTTA
siCep123 pooled sequences	CCGCAGGAGTCATTTCAAA
	CACCATTGTTGCTGGCTTA
	AAAAGGAGCTGGCGGAGAA
	GGTGAACAGTGAAGACGAT
siNesprin2 sequence 1	AACGAGAGAACCGCACCGACA
siNesprin2 sequence 2	AAGAGAATTCGTAGACGGACA
siStathmin1 sequence 1	AAGCTGAGGTCTTGAAGCAGC
siStathmin1 sequence 2	AAGAAATTAGAAGCTGCAGAA

The sequences used in the candidate-based siRNA screen.