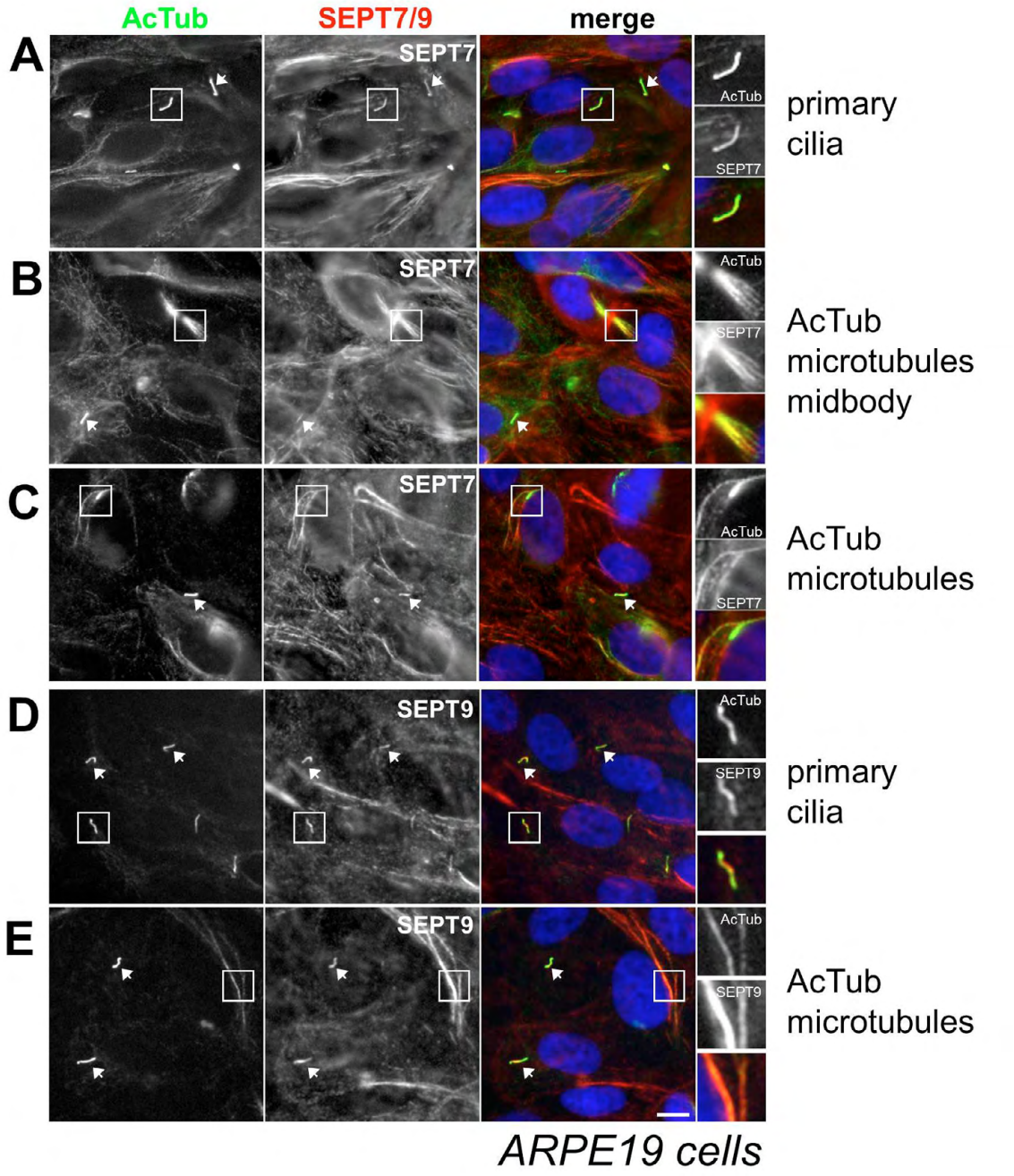


**Fig. S1.** Septins localize to the axoneme of the primary cilium in ARPE19 cells. ARPE19 cells were grown on coverslips to confluence were then transferred in low serum (1%) for 48 hours. After fixation in paraformaldehyde, cells were processed for immunofluorescence using anti-acetylated-tubulin (AcTub, green) and anti-SEPT7 or anti-SEPT9 antibodies (red), and DAPI (blue) to stain nuclei. Panels on the right represent enlarged views (white boxes) of representative cilia (**A,D**) or cytoplasmic AcTub positive microtubules (**B**,**C**,**E**). White arrows pointed to cilia in the same fields. Scale bar: 5 μm.

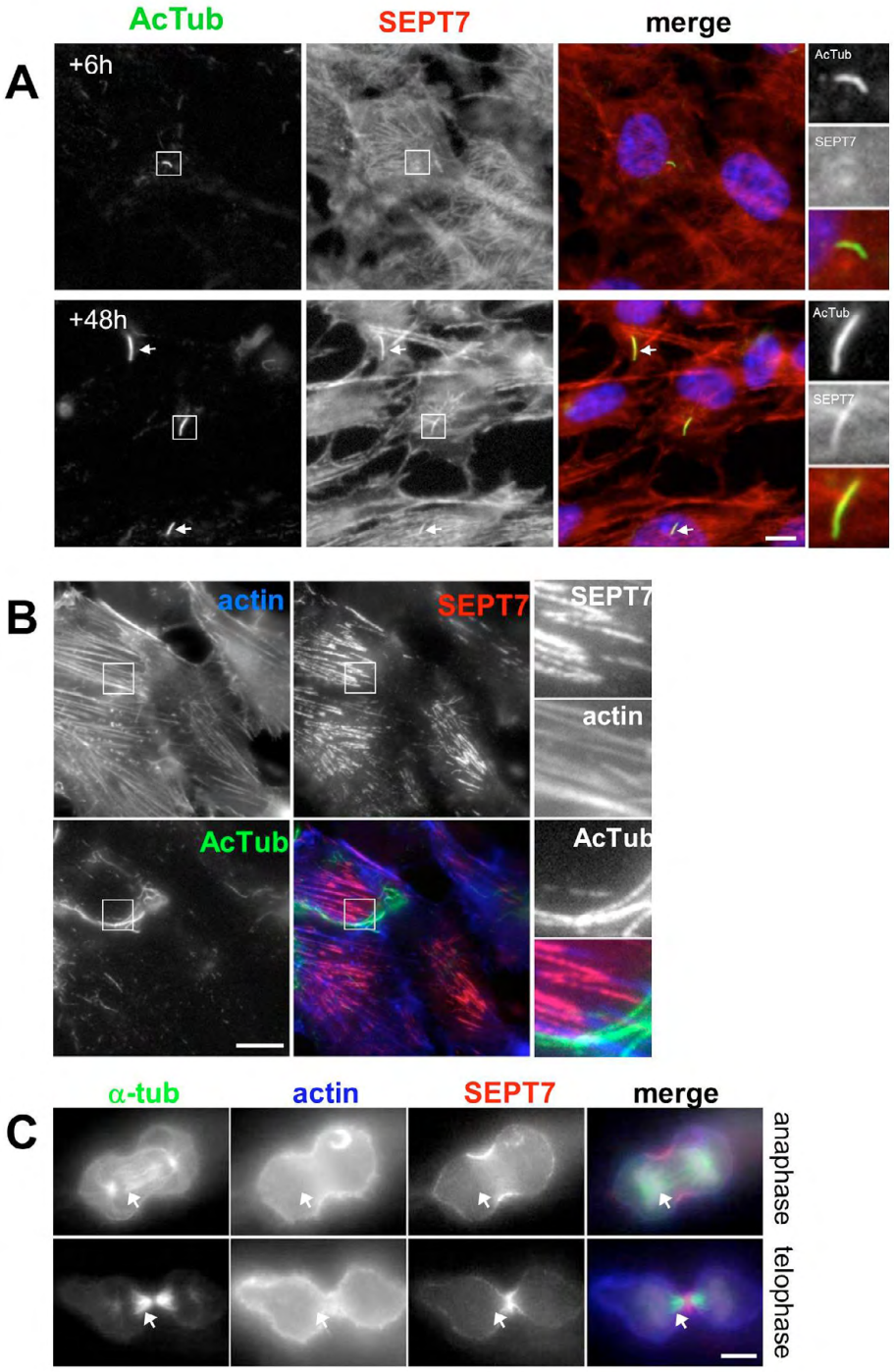


**Fig. S2.** SEPT7 localizes to the axoneme of long mature cilia and actin cytoskeleton in RPE1 cells. (**A**) RPE1 cells, serum-starved for 6 or 48 hours, were processed for immunofluorescence using antibodies against acetylated-tubulin (AcTub, green) and SEPT7 (red). Panels on the right represent enlarged views of representative cilia (white boxes), white arrows stress other cilia in the same field. (**B**) RPE1 cells grown on coverslips in the presence of serum, were fixed and processed for immunofluorescence using antibodies against acetylated-tubulin (AcTub, green; **B**) or α-tubulin to stain microtubules (α-Tub, green; **C**), and SEPT7 (red, **B** and **C**), as well as fluorescent phalloidin to observe F-actin (blue, **B** and **C**) and DAPI to stain the chromosomes (gray, **C**). (**B**) An interphasic cell is shown. (**C**) Different mitotic phases are shown; anaphase (top panel) and telophase (bottom panel). Scale bars: 5 μm.

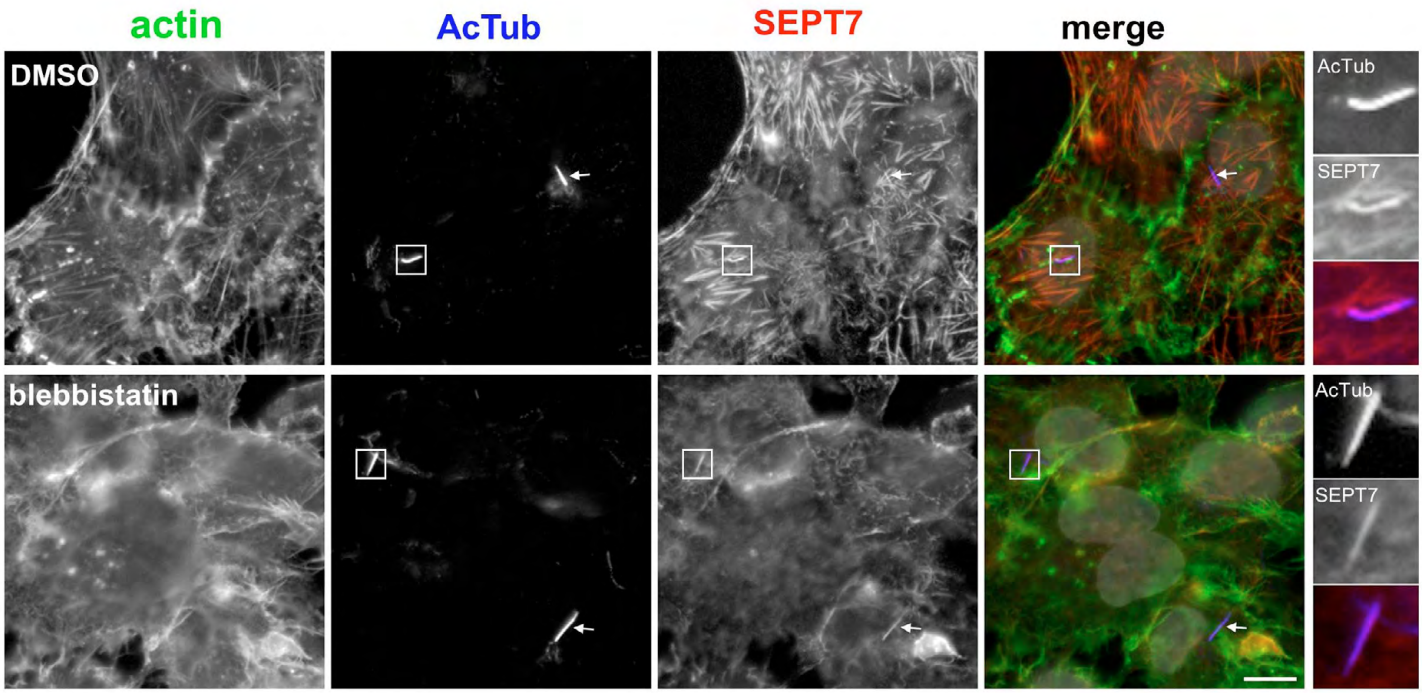


**Fig. S3.** Blebbistatin does not affect localization of SEPT7 to cilia. RPE1 cells were serum starved for 24h and then treated with 1 μM of Blebbistatin or, as a control, with the same final concentration of DMSO for 60 minutes, then fixed and analyzed by immunofluorescence using antibodies against SEPT7 (red) and acetylated-tubulin (AcTub, blue), as well as with fluorescent phalloidin (green) to stain F-actin. Panels on the right represent enlarged views of representative cilia (white boxes). White arrows pointed to cilia in the same fields. Scale bar: 5 μm.

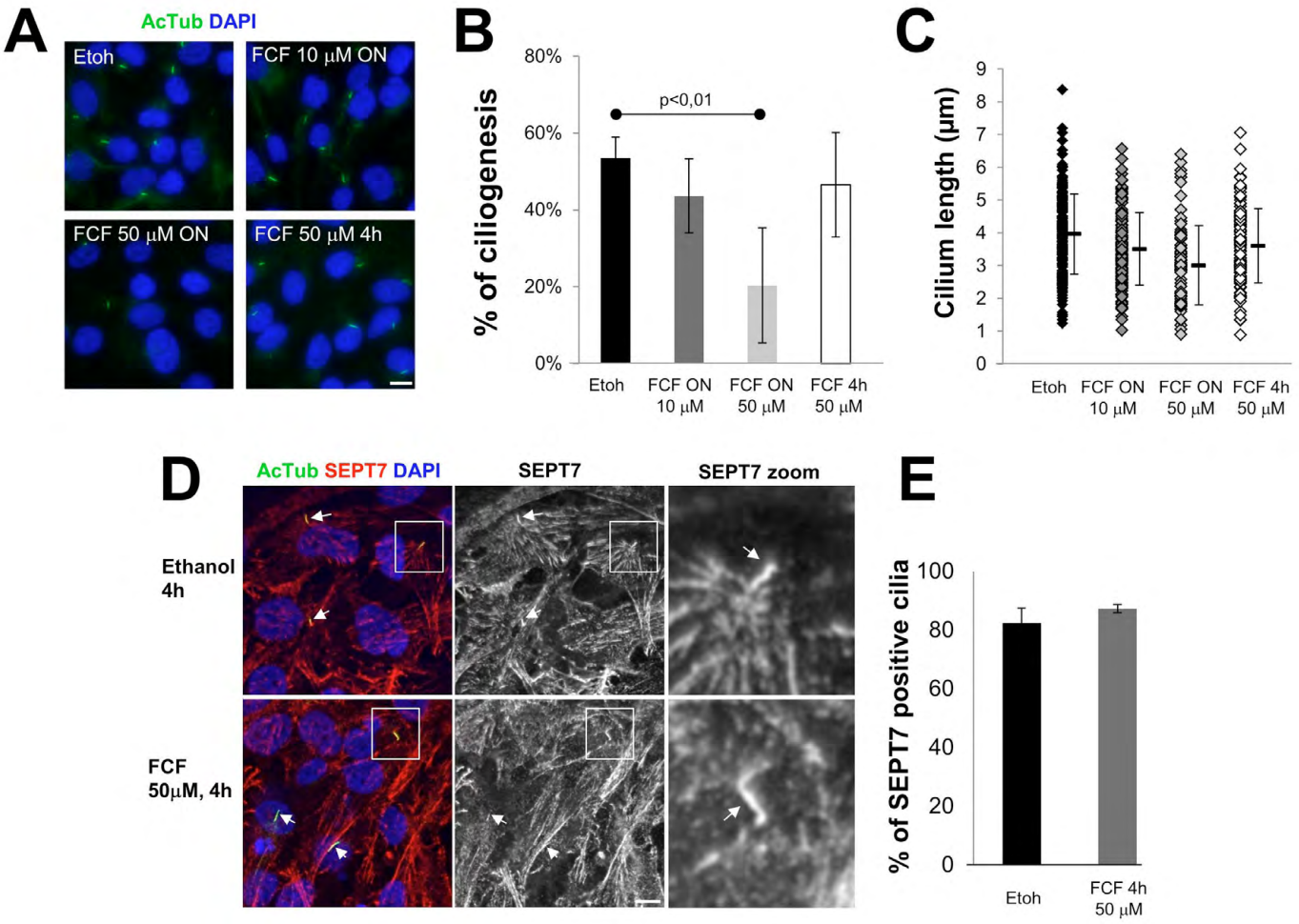


**Fig. S4.** Effect of Forchlorfenuron (FCF) on ciliogenesis in RPE1 cells. (**A-C**) RPE1 cells were treated with or without FCF at different concentrations (10 or 50 µM overnight (ON), or 50 µM for 4 hours) as indicated, then fixed and stained for cilia (AcTub, green) and nuclei (DAPI). The proportion of ciliated cells (B) and the length of cilia (C) were quantified from three independent experiments. (**D,E**) RPE1 cells treated with or without 50 µM FCF for 4 hours were stained for cilia (AcTub, green), SEPT7 (red) and nuclei (DAPI). The proportion of cilia positive for SEPT7 was quantified from 3 independent experiments. Scale bar: 5 µm.

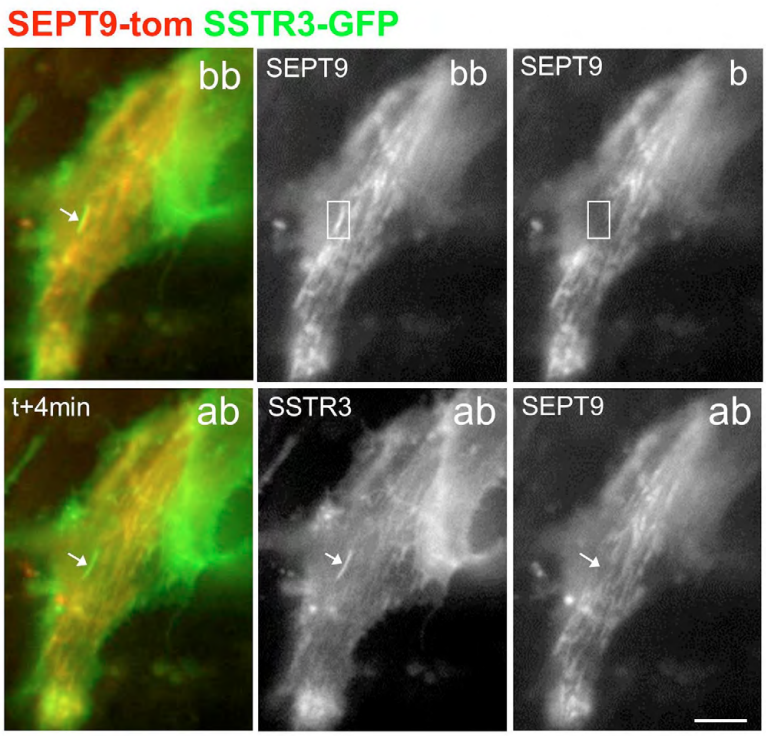


**Fig. S5.** SEPT9 forms a stable pool at the cilium of RPE1 cell. RPE1 cells were transiently co-transfected with plasmids coding for SSTR3-GFP (green) and SEPT9-tomato (red) fusions then serum-starved for 48 hours and the dynamics of SEPT9 at the cilium was analyzed by FRAP. SEPT9 fluorescence in the cilium and surrounding cytoplasmic area was bleached (white box) and recovery of the fluorescence was followed for 4 min. Fluorescence of the indicated markers were followed before bleach (bb) just after the bleach (b) and 4 minutes after bleach (ab). Scale bar: 5 µm.

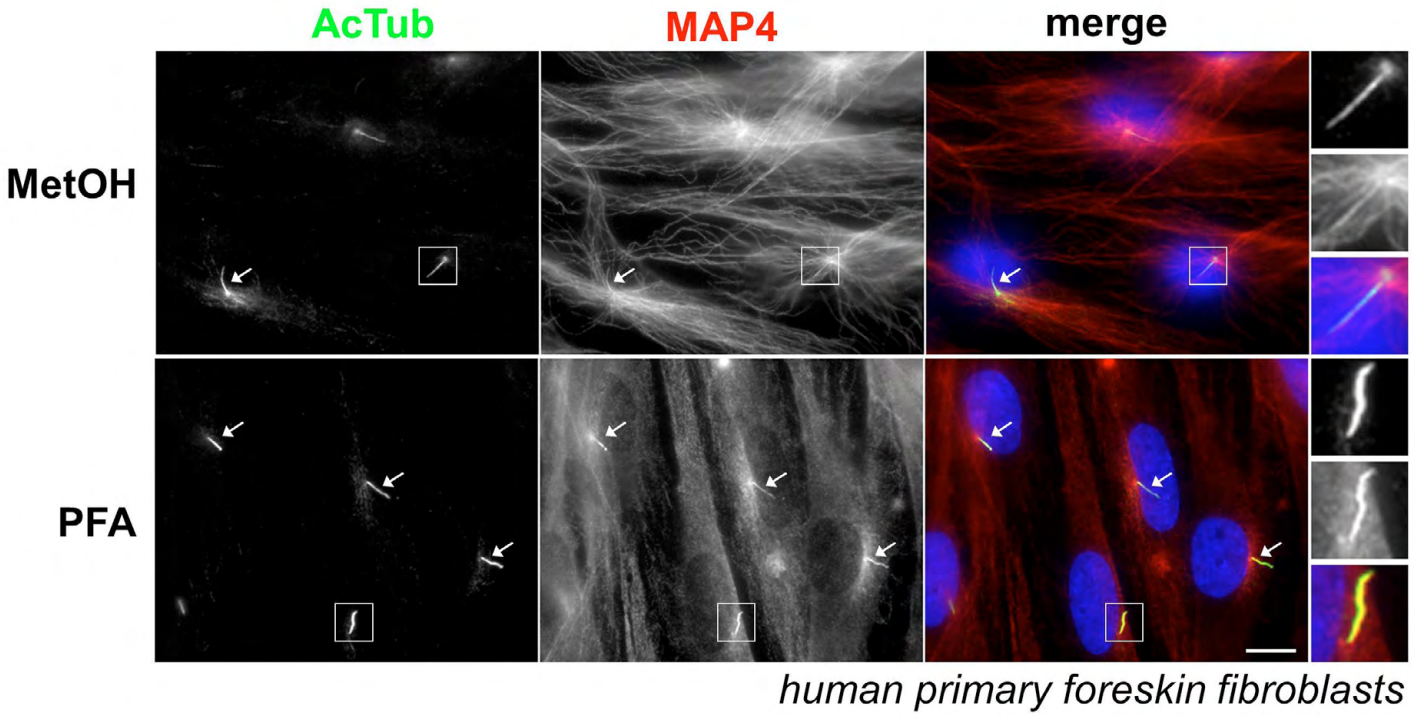


**Fig. S6.** MAP4 localizes to the primary cilium in human primary fibroblasts. Human primary foreskin fibroblasts were grown on coverslips, serum starved for 48 hours and then fixed with methanol (MetOH) or paraformaldehyde (PFA) and processed for immunofluorescence using antibodies against acetylated-tubulin (AcTub, green) and MAP4 (red), and DAPI to stain nuclei (blue). Panels on the right represent enlarged views of representative cilia (white boxes), white arrows pointed to cilia in the same fields. Scale bar: 5 µm.

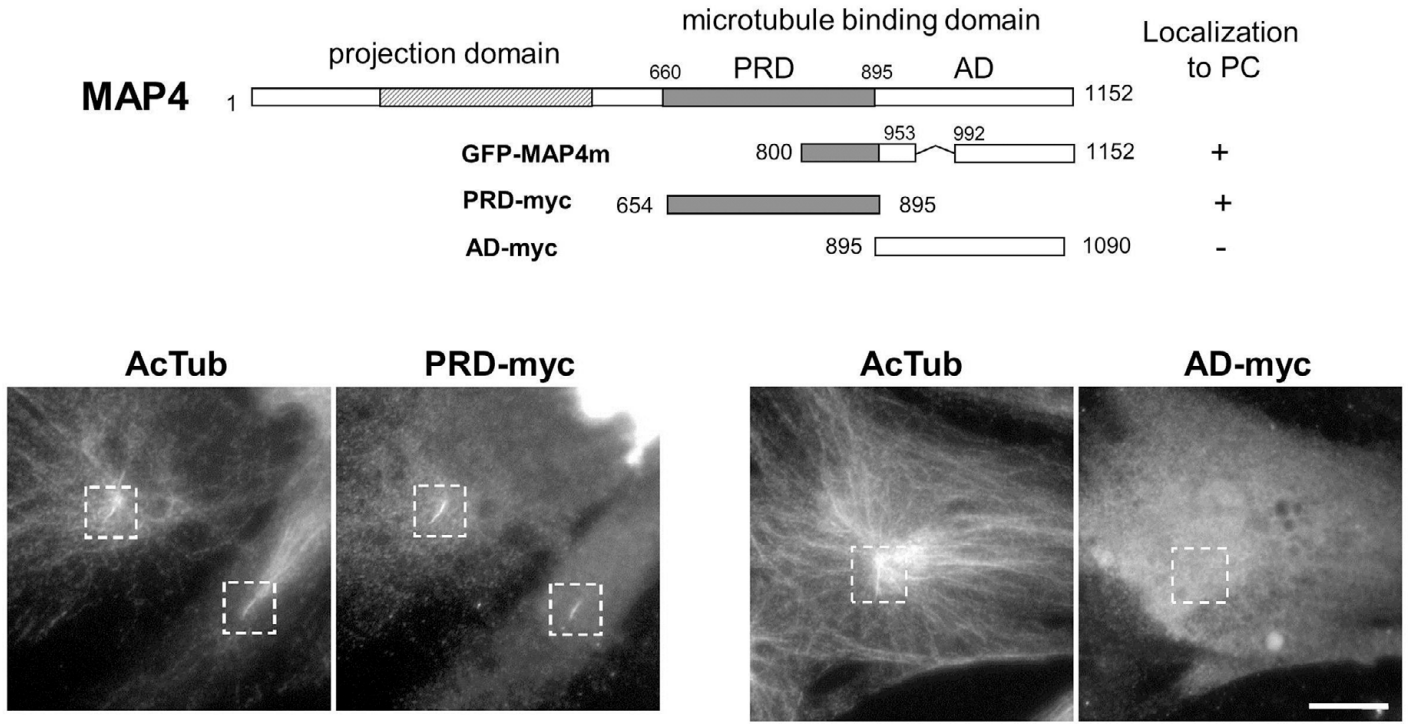


**Fig. S7.** The proline-rich domain of MAP4 contains a cilium targeting domain. Schematic representation of MAP4 showing its functional domains and the region encoded by the GFP-MAP4m fusion and the myc-tagged PRD and AD constructs. PRD: proline-rich domain; AD: affinity domain. RPE1 cells were transiently transfected with either PRD or AD myc-tagged-MAP4 constructs as indicated, fixed and processed for immunofluorescence using anti-acetylated-tubulin (AcTub, left panels) and anti-Myc (right panels). Cilia are boxed in white. Scale bar: 5 μm.

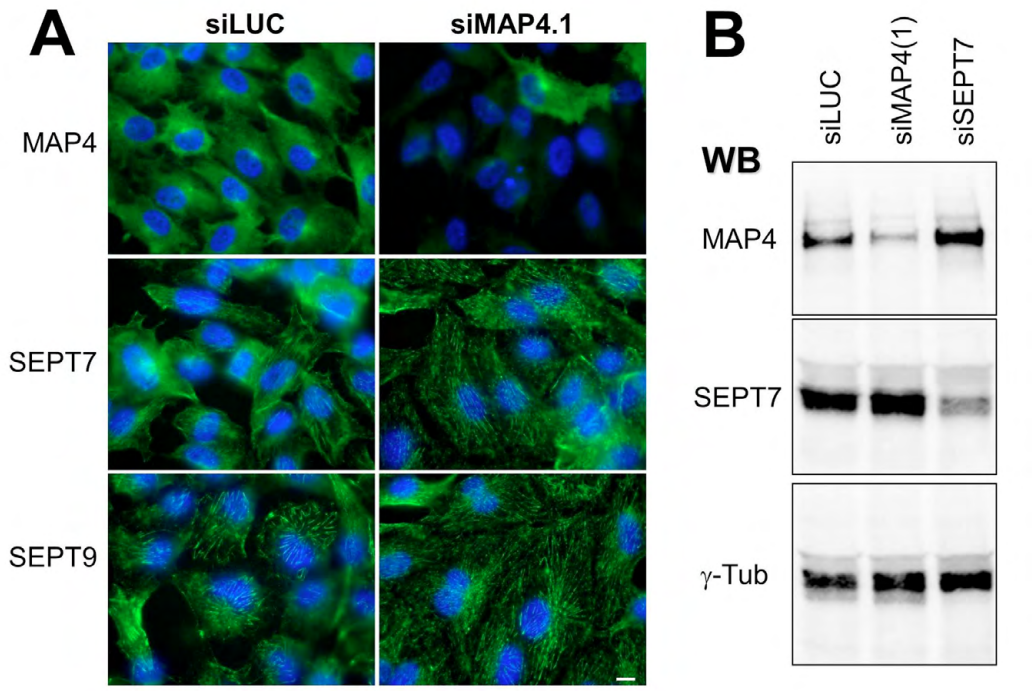


**Fig. S8.** Depletion of MAP4 does not affect expression of septins and vice versa. (**A**) RPE1 cells were treated with MAP4-specific siRNA (siMAP4.1) or luciferase targeting siRNA (siLUC) as in Figure 8. Expression of MAP4, SEPT7 and SEPT9 was analyzed by immunofluorescence. Scale bar: 5μm. (**B**) RPE1 cells were treated with siLUC, siMAP4(1) or siSEPT7(1). Expression of MAP4 and SEPT7, and α-tubulin (α-tub) as a control, was analyzed by western blotting.

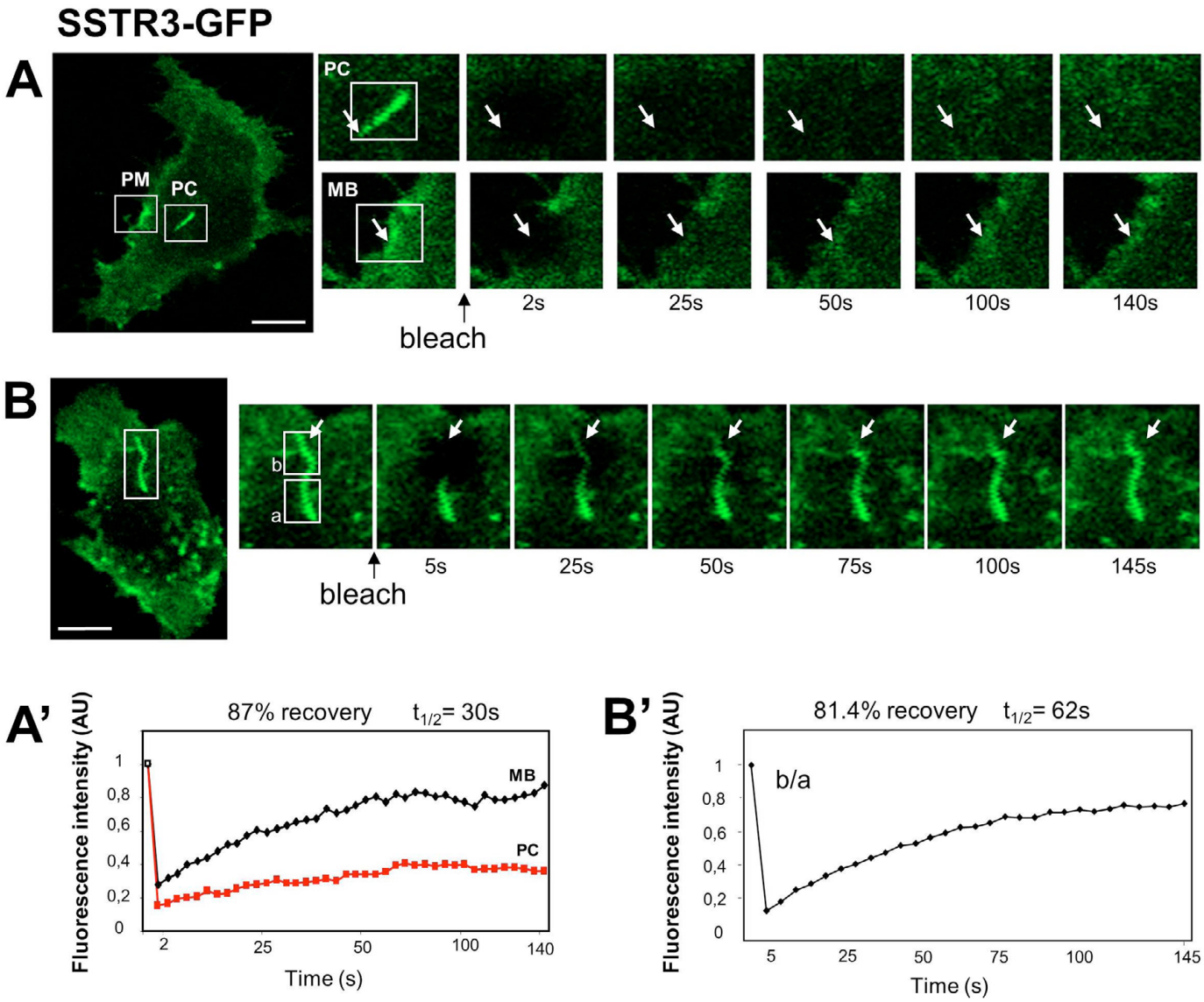


**Fig. S9.** Dynamics of the ciliary membrane marker SSTR3 in RPE1 cilia. (**A**,**B**) RPE1 cells transiently expressing SSTR3-GFP were used for FRAP studies. (**A**) The whole cilium (PC) and a representative region of the plasma membrane (MB) of the same cell were photobleached and fluorescence recovery was followed for 140 sec. (**B**) A region corresponding to half of the cilium was photobleached and fluorescence recovery was followed for 145 sec. (**A′**) Quantification of fluorescence recovery of SST3R-GFP when the whole cilium was photobleached (n=20). (**B′**) Quantification of fluorescence recovery of SST3R-GFP within the photobleached region (b), normalized to the signal present in the region of the cilium which was not photobleached (a) (n=20). Scale bars: 5 μm.

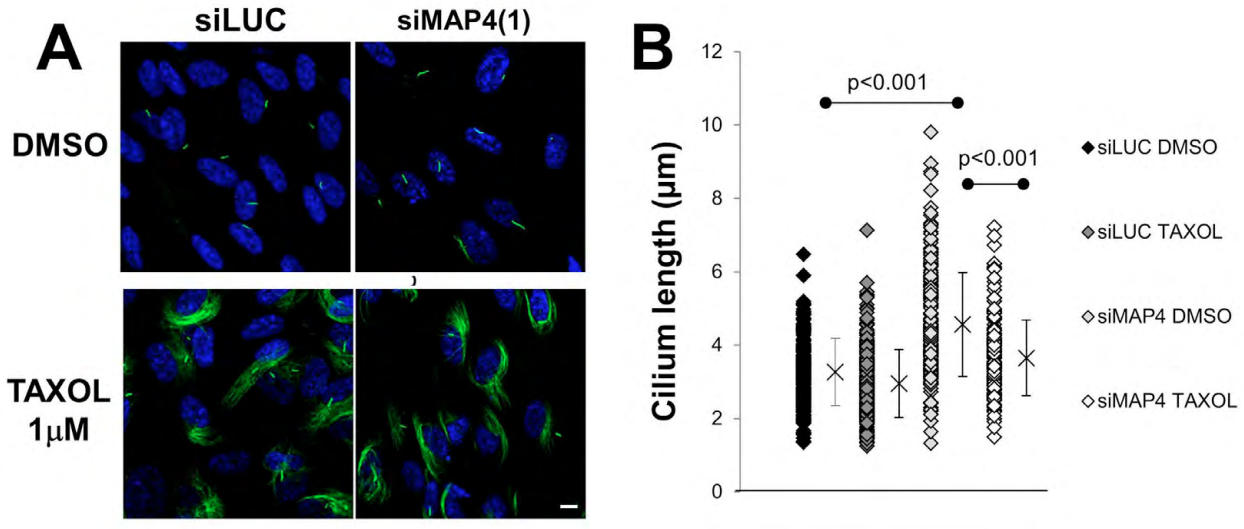


**Fig. S10.** Effects of MAP4 on the length of cilia is inhibited by taxol. RPE1 cells treated with MAP4-specific siRNA (siMAP4(1)) or luciferase targeting siRNA (siLUC) as in Fig. 8, were incubated with taxol (1 μM, 2 hours) or with DMSO as a control. Cells were fixed and analyzed by immunofluorescence (**A**), using an antibody against acetylated-tubulin (AcTub, green). The length of cilia was measured as in Fig. 8 (**B**). Statistical analysis was performed with the Fischer's test. Scale bar: 5 μm.