Supplementary Material
James E. Sillibourne et al. doi: 10.1242/bio.20134457

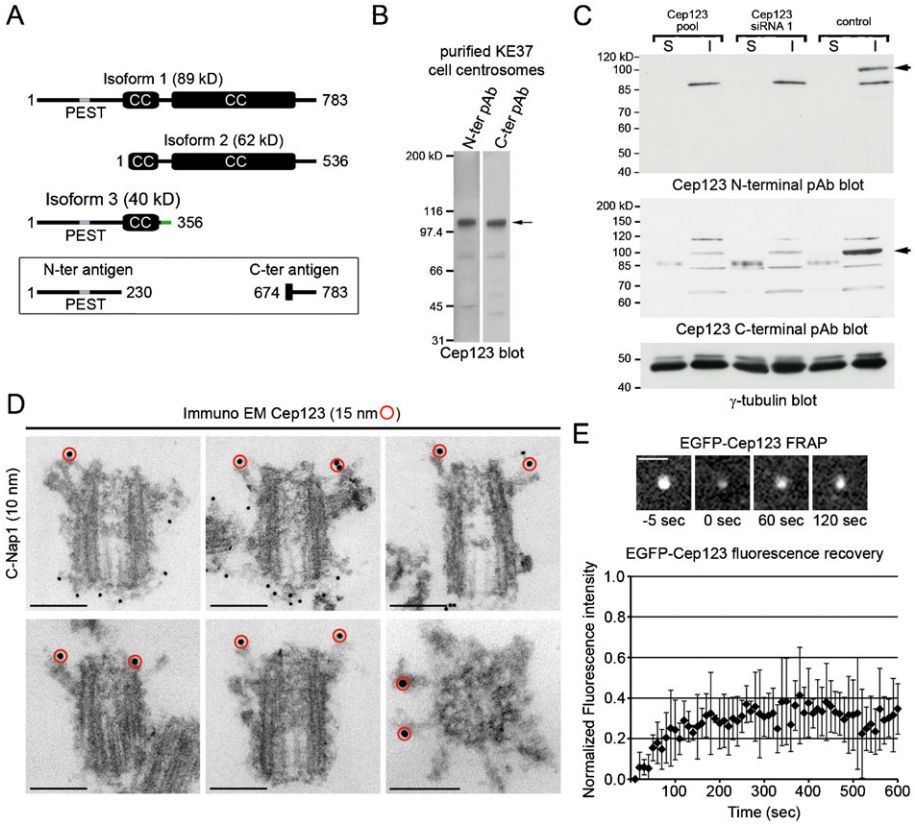


Fig. S1. Characterization of Cep123. (**A**) A diagram showing the location of the coiled-coil domains (CC) and potential PEST sequence (grey line) in the three putative isoforms of Cep123. Isoform 2 is identical to isoform 1 except that lacks residues 1–247, while isoform 3 is identical to the first 356 residues of isoform 1 with the exception of its last 13 amino acids, which are unique (green line). The two different antigens, N-ter and C-ter, used to raise antibodies against Cep123 are also shown. (**B**) Western blotting of fractionated centrosomes purified from KE37 cells with antibodies against the N- and C-terminus of Cep123 indicated that isoform 1 was the predominant centrosomal isoform, migrating with an apparent molecular weight of approximately 100 kD. (**C**) Western blotting of soluble and insoluble fractions from siRNA-treated RPE1 cells with anti-Cep123 N- and C-terminal antibodies demonstrated the specificity of the antibodies and showed that isoform 1 is the predominant cellular isoform of Cep123. (**D**) Electron micrographs of isolated centrosomes stained with the anti-Cep123 N-terminal antibody (15 nm gold particles marked with red circles) and anti-C-Nap1 antibody (10 nm gold particles). (**E**) FRAP analysis of RPE1 cells expressing EGFP-Cep123. Fluorescent images of centrosomal EGFP-Cep123 are shown before and after photobleaching and the results are presented as normalized fluorescent recovery ($n=5$) with the standard deviation shown. The mean $t_{½}$ recovery was calculated as 61.8±40.6 s. Scale bar: 1 µm.

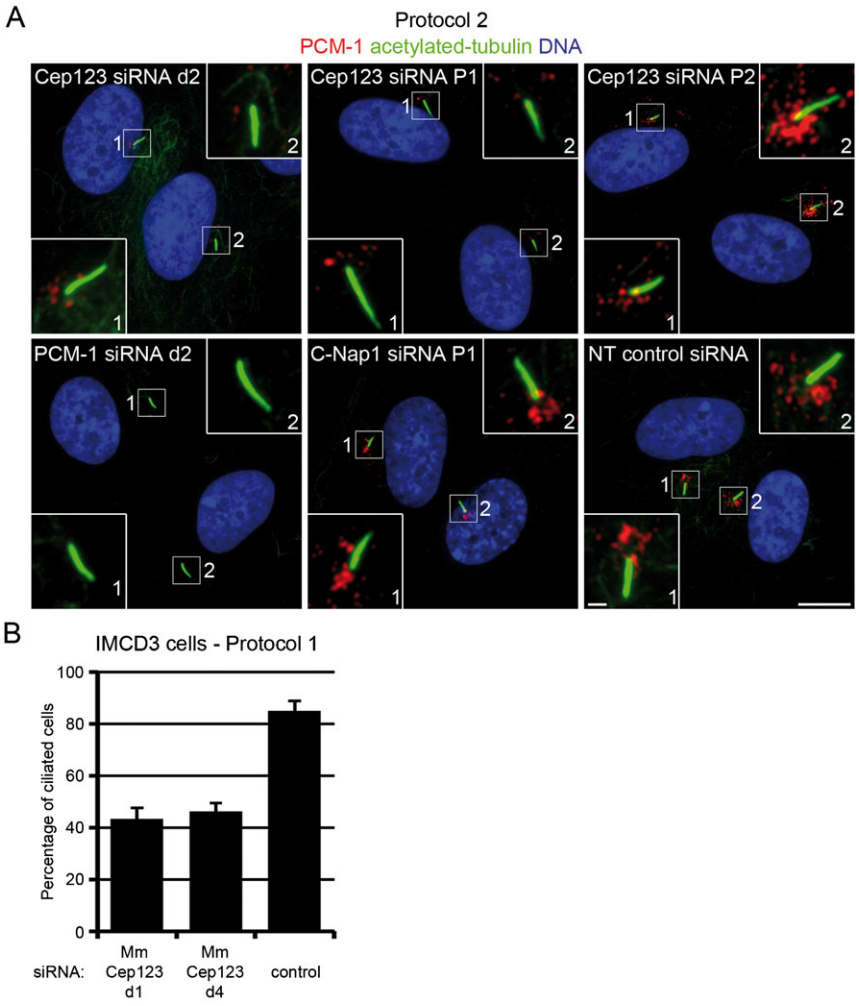


Fig. S2. Cep123 is required for primary ciliogenesis. (**A**) Immunofluorescent images of RPE1 cells treated with siRNA using protocol 2 (Fig. 4A) stained with DAPI to label the DNA (blue) and antibodies against acetylated tubulin (green) and PCM-1 (red). Scale bars: 10 µm and inset 1 µm. (**B**) Quantification of the effect of Cep123 depletion upon primary cilium formation in IMCD3 cells (Cep123 siRNA d1, $P=2.68\times10^{-4}$ and Cep123 siRNA d4, $P=2.24\times10^{-4}$, student's t-test). The results represent the mean of three independent experiments ($n=300$) and the s.d. is shown.

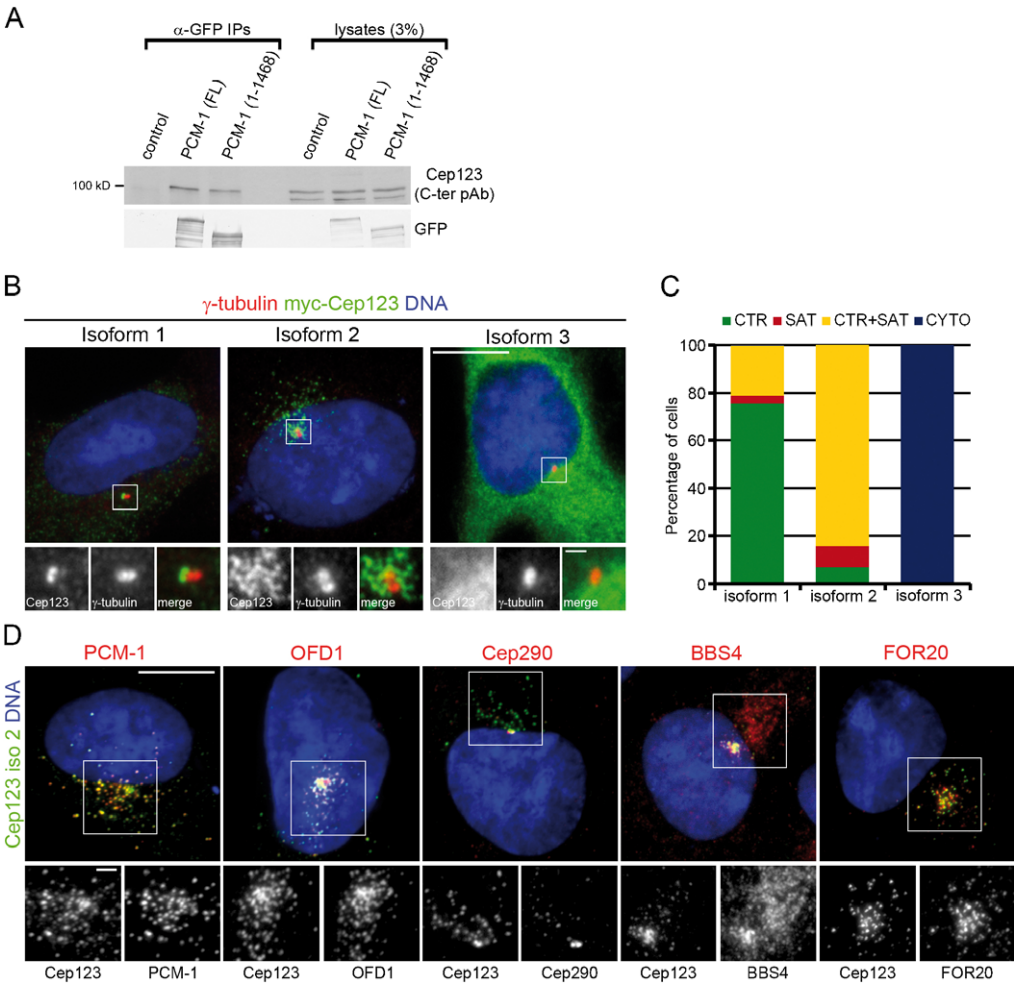


Fig. S3. Centriolar satellite proteins interact with Cep123. (**A**) Western blotting of anti-GFP immunoprecipitates from RPE1 cells transiently transfected with GFP-PCM-1 expression constructs with anti-GFP and anti-Cep123 C-terminal antibodies demonstrated that endogenous Cep123 co-precipitates with over-expressed PCM-1 and it interacts with a domain located between residues 1–1468 of PCM-1. (**B**) Immunofluorescent images of RPE1 cells transiently transfected with myc-tagged Cep123 constructs and stained with DAPI to label the DNA (blue) and antibodies against the myc-tag (green) and γ-tubulin (red). (**C**) Quantification of the number of transfected cells exhibiting Cep123 localized to the distal appendages and/or centriolar satellites. The results represent the mean of two independent experiments ($n=100$). (**D**) Immunofluorescent images of RPE1 cells transiently transfected with myc-tagged isoform 2 of Cep123 constructs and stained with DAPI to label the DNA (blue) and antibodies against the myc-tag (green) and the centriolar satellite proteins PCM-1, OFD1, Cep290, BBS4 and FOR20 (all red). Cytoplasmic particles of Cep123 frequently co-localized with PCM-1 and OFD1, but not so often with BBS4, Cep290 and FOR20. Scale bars: 10 µm and inset 1 µm.

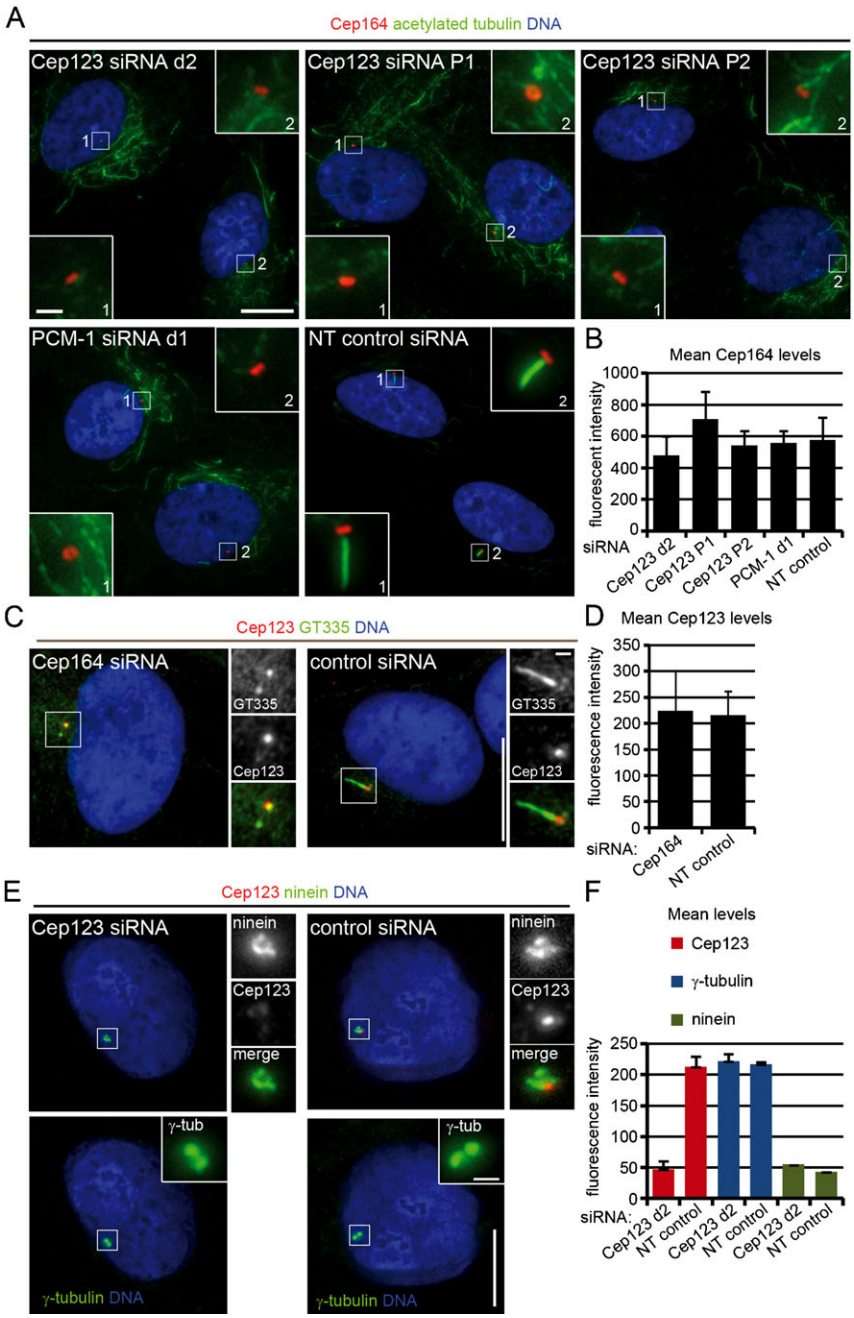
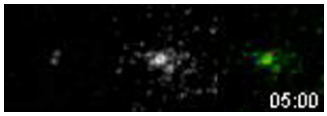


Fig. S4. Cep123 and Cep164 localize to the distal appendages independently of each other and Cep123 depletion does not affect ninein localization. (**A**) Immunofluorescent images of siRNA-treated RPE1 cells stained with DAPI to label the DNA (blue) and antibodies against acetylated tubulin (green) and Cep164 (red). (**B**) Quantification of centrosomal Cep164 fluorescent intensity levels showed that Cep123 depletion had no impact upon the localization of Cep164 to the centrosome with its centrosomal levels remaining unchanged (n=20–32). (**C**) Immunofluorescent images of Cep164 or NT control siRNA treated RPE1 cells stained with DAPI (blue) and antibodies against polyglutamylated tubulin (GT335, green) and Cep123 (red). (**D**) Quantification of centrosomal Cep123 fluorescent intensity showed that depletion of Cep164 had no impact upon the centrosomal localization of Cep123 (n=15). (**E**) Cep123 or NT control siRNA-treated RPE1 cells stained with DAPI (blue) and antibodies against γ-tubulin (green), ninein (green) and Cep123 (red). (**F**) Quantification of Cep123, γ-tubulin and ninein centrosomal fluorescent intensities demonstrated an efficacious depletion of Cep123 and showed that γ-tubulin levels and ninein levels were largely unaffected by the ablation of Cep123. Scale bars: 10 µm and inset 1 µm.



Movie 1. Live imaging of RPE1 cells transfected with mCherry-centrin-1 (left panel and red in merged images) and EGFP-Cep123 (center panel and green in merged images). Time is in minutes and seconds. Scale bar: 1 µm.

Table S1. Cep123 cloning and GeneSOEing primers.

Cep123 cloning	GeneSOEing primers
Cep123-S01	5′-GTCGACATGCTCCTGGGATTTCGGAGAG-3′
Cep123-AS01	5′-GGATCCCTAGCAGGTGGGGGCATGAGAC-3′
Cep123 GS-Fw	5′-GCCAAATGCCAAGAACTCGCTCATCAGTACCTGGCAAACC-3′
Cep123 GS-Rv	5′-TGCCAGGTACTGATGAGCGAGTTCTTGGCATTTGGCACG-3′

Table S2. SiRNA sequences.

C-Nap1 siRNA P1 (Human Cep250 Dharmacon ON-TARGET plus SMART pool siRNA)	
J-012364-05	5′-GAGCAGAGCUACAGCGAAU-3′
J-012364-06	5′-GGACCUCGCUGAACAACUA-3′
J-012364-07	5′-AAGCUGACGUGGUGAAUAA-3′
J-012364-08	5′-GAGAAUAUGAUCCAAGAGA-3′
PCM-1 siRNA d1 and d2 (Qiagen)	
Hs PCM-1_1	5′-CAGUAUCACAUCUGAACUAAA-3′
Hs PCM-1_2	5′-CAGGCUUUAACUAAUUAUGGA-3′
Cep123 siRNA d2 (Qiagen)	
Hs Cep123_2	5′-CACCCUGGUUGUUGGAUAUAA-3′
Cep123 P1 (Human CCDC123 Dharmacon siGENOME pool siRNA)	
D-021334-01	5′-CCGCAGGAGUCAUUUCAAA-3′
D-021334-02	5′-CUCAAGAACUGGUGGAUGA-3′
D-021334-03	5′-UAGAGCACGUCAAAGAUAU-3′
D-021334-17	5′-GGUGAACAGUGAAGACGAU-3′
Cep123 P2 (Human CCDC123 Dharmacon ON-TARGET plus SMART pool siRNA)	
J-021334-18	5′-CCGCAGGAGUCAUUUCAAA-3′
J-021334-19	5′-CACCAUUGUUGCUGGCUUA-3′
J-021334-20	5′-AAAAGGAGCUGGCGGAGAA-3′
J-021334-21	5′-GGUGAACAGUGAAGACGAU-3′
Mouse Cep123 siRNA d1 and d4 (Qiagen)	
Mm_2610507l03Rik_1	5′-CCAGAUGUAGCUGGUAGAAUA-3′
Mm_2610507l03Rik_4	5′-UACAUCAAAGAUAUACAGAAA-3′
Cep164 [(Graser et al., 2007); Qiagen]	
Hs Cep164_278	5′-CAGGUGACAUUUACUAUUUCA-3′
Hs Cep164_279	5′-ACCACUGGGAAUAGAAGACAA-3′
Control [(Azimzadeh et al., 2009); Qiagen]	
GL2 control	5′-CGUACGCGGAAUACUUCGA-3′