Online Methods

Mutation detection

The mapping approach is described in[4]. Briefly, genomic DNA from three affected cousins was analysed using a multiplexed panel of 400 microsatellite markers based on the CHLC/Weber Human Screening Set version 8 (Research Genetics). The region was refined by seeking crossovers in linked families and subsequent minimum region was used in bioinformatics analysis. This yielded three genes which were sequenced; CEP70, ARMC8 and CEP63. For each gene, all exons and splice sites and polyadenylation signal were analysed by bidirectional sequencing of genomic DNA from family members and our Pakistani control panel as in [4]. The *CEP63* c.129G>A mutation was confirmed to segregate correctly within the family and was absent from genome databases and from 360 ethnically matched chromosomes. Primers used to sequence genomic DNA for mutation analysis, screening the control panel and sequencing primates (see Evolutionary analysis) are listed in Supplementary Table 3. All families gave informed consent to enter the study, which had Cambridge Research Ethic Committee approval, Cambridge, UK.

Evolutionary analysis

From genomic DNA of gorilla, chimpanzee, orang-utan and gibbon all exons of *CEP63* were sequenced using the human primers. For assessment of splicing we designed intra-exonic primers and used foetal cDNA as a template. Macaque and other mammals *CEP63* exonic data were obtained from public databases. We compiled each species' *CEP63* cDNA and analysed them using the Ka/Ks calculation tool with the phylogenetic tree (rooted to dog, but same results for other non-ape/monkey mammals) derived from prealigned sequences (http://services.cbu.uib.no/tools/kaks).

Cell culture and drug treatments

DT40 and HeLa cells were cultured as in[26]. Human B lymphocytes were derived from parent of patient (*CEP63*$^{+/-}$) and patient (*CEP63*$^{-/-}$) blood as described in [51] and were cultured in RPMI-1640 containing 10% FBS, 100U/ml penicillin and 100mg/ml streptomycin at 37^{o}C in 5% CO_2. For Fig. 3C cells were treated with 125 μM monastrol (Tocris) for 7 hours. For Fig. 6E, cells were treated with 125 μM monastrol or 300 nM BI-2536 (Axon) for 90 minutes.

Analysing transcripts in human lymphocytes

RNA was extracted from human B-cells using RNeasy kit (Qiagen), according to manufacturer's instructions. cDNA was generated using Superscript III Reverse Transcriptase (Invitrogen). To quantify expression of *CEP63* transcript in human B-cells, the following primers were used in quantitative PCR reactions (Supplementary Fig. 2B): *CEP63* exon 4, *CEP63* exon 6, *CEP63* exon 11 and *CEP63* exon 13 (primer sequences in Supplementary Table 3).

Antibodies and immunofluorescence

Antibodies were raised in rabbits against bacterially expressed and purified hexa-histidine-tagged or maltose binding protein-tagged recombinant proteins that correspond to 200-450aa of *H. Sapiens* CEP63 (ENSP00000426129) [26]. Antibodies were produced by Enzymax (Lexington, USA) and were affinity purified in the lab against the fusion protein. Additional primary antibodies used were CDK5RAP2 and chicken anti-TACC3[26], CEP152 (Bethyl Laboratories), centrin-3 (Abnova), FLAG-M2 (Sigma-Aldrich), Protein G (Abcam), Protein G-HRP (Abcam), ninein (kind gift of R. Rios), α-tubulin (Dm1α; Sigma-Aldrich), γ-tubulin (GTU88; Sigma-Aldrich), Strep-tag II antibody (Abcam) and GT335 (Adipogen).

DNA was stained with Hoescht (Sigma-Aldrich). DT40 and HeLa cells were fixed and stained with antibodies as in[26].

Generating *CEP63KO* DT40 cells

For generating *CEP63KO* targeting construct, homology arms were PCR amplified from DT40 genomic DNA. Primers for left arm (LA) were *CEP63KO* LA F and *CEP63KO* LA R and primers for right arm (RA) were *CEP63KO* RA F and *CEP63KO* RA R (primer sequences in Supplementary Table 3). Two *CEP63* alleles were targeted sequentially with neomycin and blasticidin resistance genes. Gene targeting and expansion of antibiotics resistant clones were performed as described before [26]. Targeted integration of neomycin cassette was screened by PCR using the following primers (letters in parentheses are annotated in Supplementary Fig. 3A): (A) *CEP63KO* check F and (B) Neomycin R. Homozygous clones were identified by screening for absence of exons 6-9: (C) *CEP63KO* exon 6 F and (D) *CEP63KO* exon 9 R.

Generating TAP-*WT* and TAP-*CEP63KO* cells

The following primers were used to amplify the left arm (LA): (J) TAP LA F and (K) TAP LA R (letters indicate corresponding arrows in Supplementary Fig. 3A). Primers to amplify the right homology arm (RA) were TAP RA F and TAP RA R (Supplementary Table 3). Homology arms and selection markers (neomycin or puromycin cassettes) were cloned into a pBluescript II SK^- vector carrying the GsTAP sequence[24] (a plasmid containing the GsTAP sequence was provided by K.J. Patel). Final constructs were transfected into *WT* and *CEP63KO* cells as in [26]. Targeted integration of neomycin or puromycin cassettes was screened by PCR using the following primers: (B) Neomycin R or (I) Puromycin R in combination with (G) TAP check F (Supplementary Table 3).

Plasmids and transfection

To generate FLAG-CEP63 and FLAG-CEP63-CT, 1-703aa and 382-703aa of human CEP63 cDNA were cloned respectively into pCMV-Tag2 (Stratagene). To generate STREP-CEP152, 1-1654aa of human CEP152 cDNA was cloned into pEXPR-IBA105 (IBA). To generate STREP-CEP152-PACT, PACT was cloned downstream of CEP152 cDNA in pEXPR-IBA105. For transient transfection of GFP-tubulin, GFP-PACT (kind gift of S. Munro) and Ruby-H2B (kind gift of J. Pines), electroporation was performed as in[26]. For generating *CEP63KO* DT40 cells stably expressing FLAG-CEP63, STREP-CEP152 or STREP-CEP152-PACT, *CEP63KO* cells were electroporated with puromycin resistance gene and respective constructs (as in Fig. 5B). Transient transfection of HeLa cells was carried out with Lipofectamine (Invitrogen) following manufacturer's instructions.

RNA interference and transfection in mammalian cells

CEP63 and CEP152 siRNAs (on-Target SMART pools, Dharmacon) were transfected into HeLa cells using Oligofectamine (Invitrogen) following manufacturer's instructions.

Immunofluorescence in mouse brain

Mouse brain sections were stained as described before [6].

Image acquisition and analysis

Imaging of fixed cells was performed on a Nikon Eclipse 90i scanning confocal microscope. Images presented are 3D projections of z-sections taken every 0.5 μm across the cell. Images of any individual Figures were acquired using the same settings and were imported into Volocity and Adobe Photoshop

before being processed in the same way. Super-resolution microscopy was carried out with a Structured Illumination Microscope (SIM) by API OMX Deltavision. Cells were imaged with 100X 1.4NA Olympus objective. Data was reconstructed using API SoftWorx software. Time-lapse imaging of DT40 cells was performed using a spinning-disc confocal system (PerkinElmer) as in [26]. 2D volume-rendered image sequences were exported as QuickTime files. For still images of videos, snapshots were taken in Volocity and processed in Photoshop. Quantifications were performed in Volocity 5. Distances between centrioles within each pair were determined using the 'line' tool in 3D volumes. CEP152 signal in Fig. 6E was quantified as follows. Based on centrin staining, a circle of 1.4μm diameter was placed over the centrosome to define a 3D volume across which mean intensities of CEP152 signal were measured automatically. Images for this quantification all contained the same number of Z sections. Electron micrographs of DT40 cells were obtained as described before [26].

Cell cycle analysis

DT40 cells for cell cycle analysis were prepared as described previously [26].

Centrosome purification

Centrosome purification from human B-cells was performed as in[26].

Immunoprecipitation

Cell lysates of HeLa cells expressing FLAG-CEP63 or FLAG-CEP63-CT were prepared as in [26]. For immunoprecipitation, cell lysates were incubated with Anti-FLAG M2 Affinity Gel (Sigma) according to manufacturer's instructions.

Statistical analysis

Statistical analysis and graphs were carried out using Microscoft Excel or Prism (Graphpad Software, Inc.). Equality of variance among datasets was tested with F test. Data sets with equal variance were compared by two-tailed unpaired Student's *t*-test or non-parametric Mann-Whitney U tests, whereas datasets with unequal variance were compared with unpaired *t*-test with Welch's correction.

Identifying CEP63-interactors with Stable Isotope Labelling by Amino Acids in Cell Culture (SILAC)

WT and *TAP-WT* cells were cultured in lysine and arginine free RPMI-1640 medium containing 10% dialysed FBS (Invitrogen), 100U/ml penicillin, 100mg/ml streptomycin and 1mM sodium pyruvate (Invitrogen) and supplemented with either a) 800μM light C^{12} lysine and 482μM C^{12} arginine for *WT* cells or b) 800μM C^{13} lysine and 482μM C^{13} arginine for *TAP-WT* cells. Equal numbers of labelled *WT* and *TAP-WT* cells were pooled and lysed in buffer containing 10mM Tris-HCl (pH7.4), 150mM NaCl, 0.25% NP-40, 1mM DTT, 100μM PMSF, protease and phosphatase inhibitors (Roche). The lysate was clarified by ultracentrifugation (50000xg) and incubated with Streptavidin Plus UltraLink beads (Pierce). Bound protein complexes were eluted with 2mM D-biotin (Invitrogen). Proteins were separated by SDS-PAGE and prior to analysis by mass spectrometry were in-gel digested as described [52]. Peptides were analysed by nanoLC-MSMS on a LTQ Velos-Orbitrap mass spectrometer (Thermo) coupled to a RSLC nanoLC (Dionex) fitted with a 0.1 x 20mm C18 guard column and a 0.075 x 150mm PepMap C18 column (Dionex) and separated using a discontinuous gradient of acetonitrile over 60 min [52]. Mass spectra were acquired using Xcalibur 2.1.0 software. Raw files were processed and C^{12}:C^{13} ratios calculated using Mascot distiller and searched using an in house mascot server running Mascot 2.3

(MatrixScience) against IPI chicken database. LTQVelos Orbitrap settings and Mascot search parameters were as previously described [53].