SUPPLEMENTARY INFORMATION

A Primary Microcephaly Protein Complex forms a ring around parental centrioles

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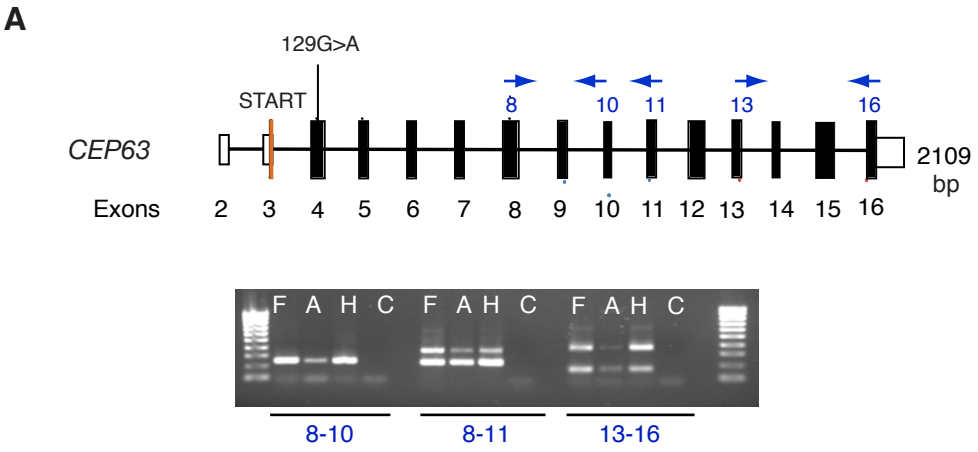
1. Supplementary Note

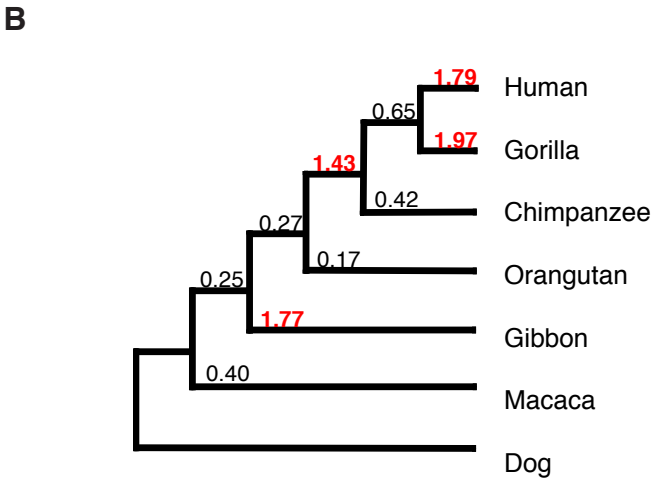
All three cases in the family studied had microcephaly at birth (head circumference -4SD to -6SD; birth weights were not recorded). All had speech delay but did learn to speak by 3y, by 5 years they showed clear evidence of cognitive delay and when seen none could write more than their name; all three were regarded as having mild/moderate mental retardation by their physicians and family; they were not physically retarded. The three affected females were ethnic Pakistani and when seen were able to care for themselves by dressing, washing and simple cooking. When first seen for the study they were: aged 18y with an HC of 43cm (9cm < 3rd centile); aged 16y with a head circumference (HC) of 41.5cm (10cm < 3rd centile); and 7.5y with an HC of 33.5cm (15cm < 3rd centile). The older two females were of reduced height and weight for their ages (-2SD to -3SD), the youngest had always been petite, being born at term at 2kg (for unknown reasons) and at 7.5y having a height and weight -4SD below expected. None had fits or congenital malformations and all were physically healthy. The youngest child had the smallest head size, and we assume that this is the additive result of MCPH and another condition that had lead to her general small size. However, HC is only a crude method of estimating brain volume and there can be considerable variation between the centile HC in affected members of the same family [16]. Chromosome analysis was normal, but brain imaging and a sophisticated metabolic screen could not be performed. The phenotype of primary microcephaly and proportionate short stature lead us to a diagnosis of MCPH or mild Seckel syndrome. Interestingly, bi-allelic mutations in CEP152 were found to cause Seckel syndrome [36].

Informed consent was obtained form the patients and their parents by Pakistani doctors in Urdu. These consents were verbal, not written, as is the request of the family (typical and normal for Pakistani families, whose members do not read or write and are unwilling to sign forms they cannot read).The family agreed to participate in a study of microcephaly and were subsequently visited by a Pakistan-based filed worker who explained the project. The family then gave assent for C.G.W. to visit them at the three locations members lived at. At these visits consent was obtained for pedigree and history details to be recorded, medical examination to be performed, photographs to be taken and blood sample drawn.

2. **Supplementary Figures and Legends**

SUPPLEMENTARY FIGURE 1

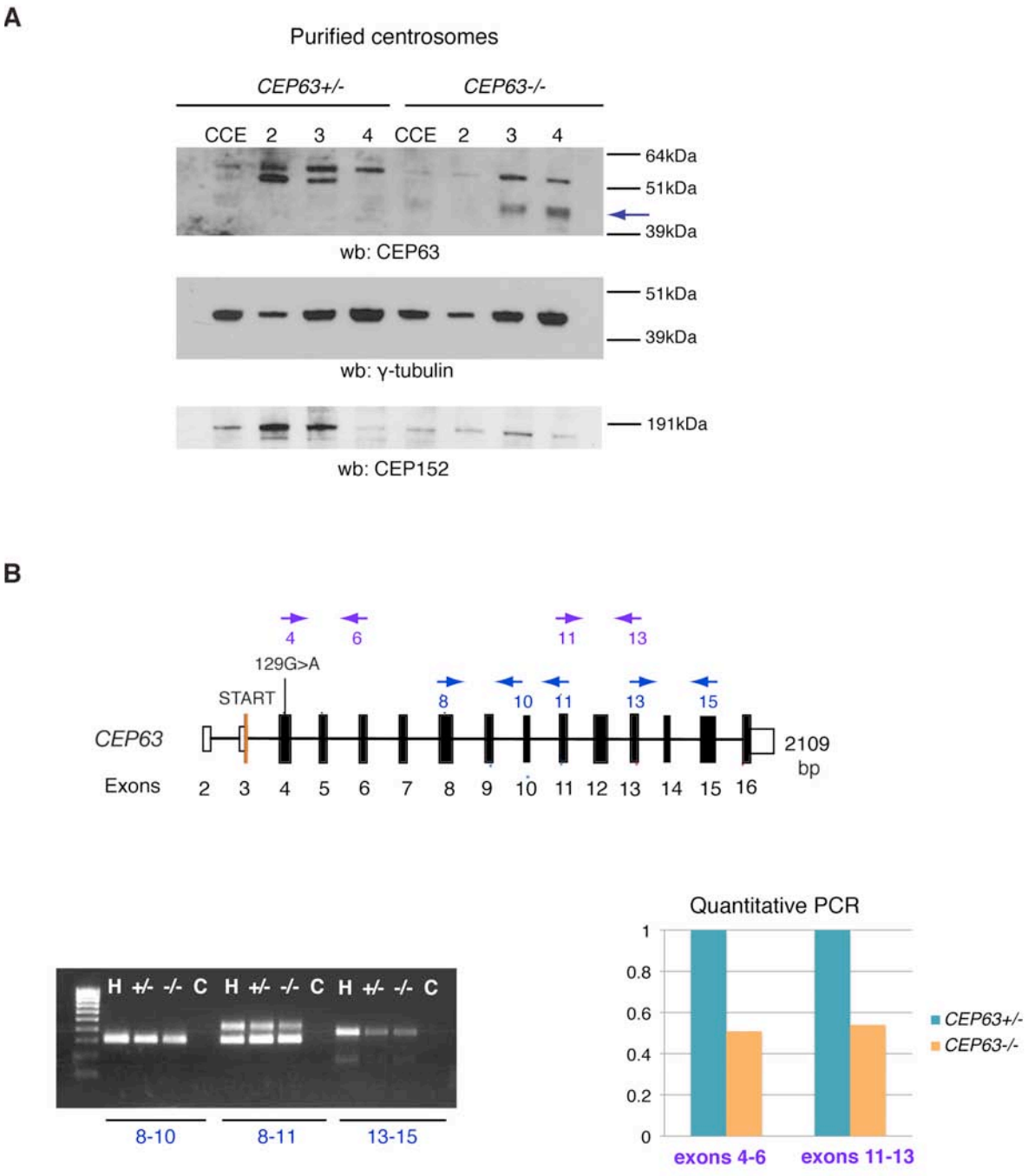




Supplementary Figure 1. *CEP63* gene is expressed in foetal brain and is positively selected for in the higher apes clade. (A) All four spliced forms of *CEP63* are expressed in human foetal brain. PCRs of *CEP63* cDNA from foetal brain (F), adult brain (A) and HeLa cells (H) are shown. A control is included for each set (C). Primer positions in specific exons are indicated in schematics. PCRs with primers 8-10 produce only one specific band. PCRs with primers 8-11 produce two bands indicating that transcripts including (upper, larger band) and excluding (lower, smaller band) exon 10 are present in foetal and adult brain. This was confirmed by direct sequencing. PCRs with primers 13-16 produce two bands indicating that transcripts including exons 14 and 15 and excluding exons 14 and 15 are both present in foetal and adult brain. Note that all splice variants were present in brain as well as in HeLa cells. (B) Genomic DNA of chimpanzee, gorilla, orangutan, and gibbon was sequenced using human *CEP63* primers. The resultant sequences were used to create cDNAs for each species. *CEP63* cDNA for macaque, dog, cow, mouse, rat and chicken were derived from public domain databases. The sequences were then analysed looking at the ratios of synonymous to non-synonymous nucleotide

changes using the “Ka/Ks Calculation tool” at http://services.cbu.uib.no/tools/kaks. The program generates a phylogenetic tree based on similarities and differences between the species. For each “branch” of the tree the Ka/Ks ration is shown. When Ka/Ks >1, there may be a positive selection of the gene in that branch. The four potentially significant results in the higher ape clade are shown in red.

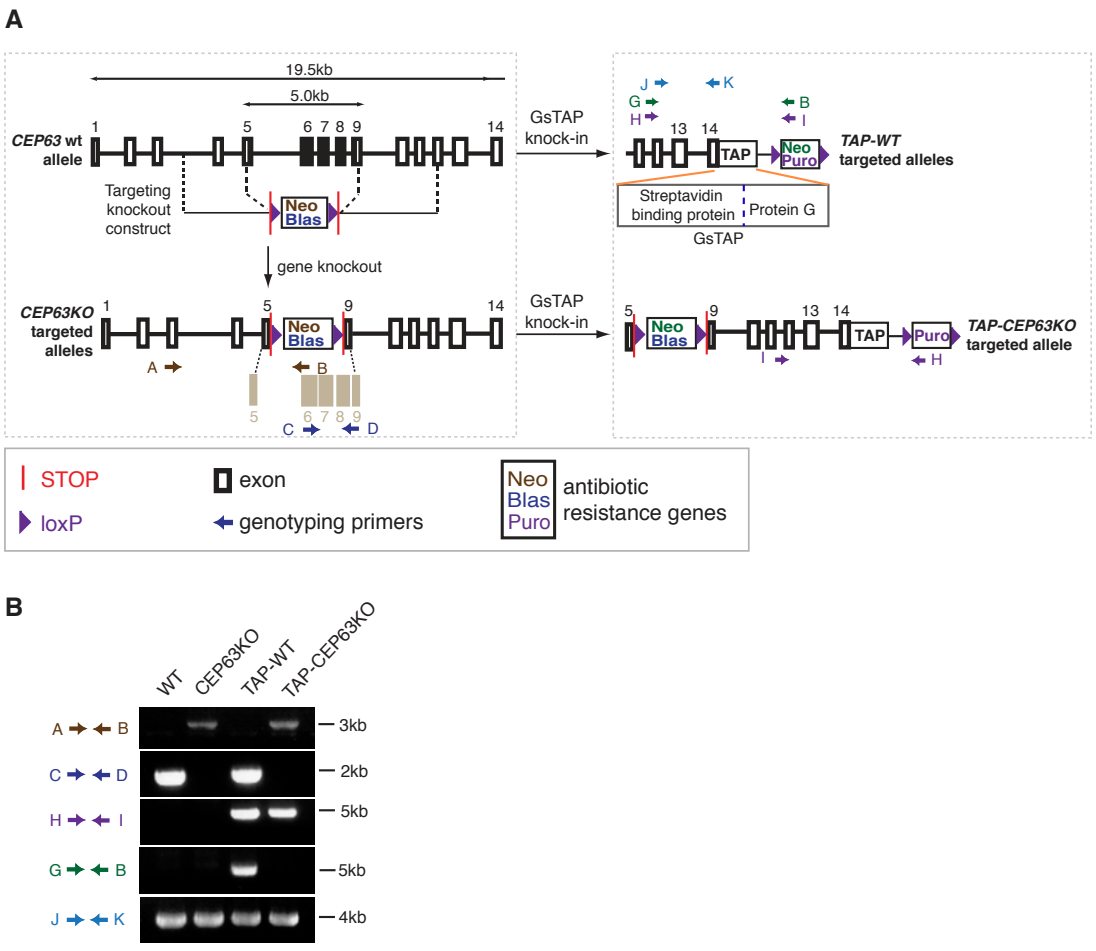
SUPPLEMENTARY FIGURE 2



Supplementary Figure 2. The c.129G>A mutation in *CEP63* is a hypomorph. (A) Cytoplasmic cell extracts (CCE) and centrosome-containing sucrose fractions (2-4) were prepared from parent-of-patient (*CEP63*$^{+/-}$) and patient (*CEP63*$^{-/-}$) cells. Immunoblots were probed with CEP63 antibodies. Note that the two prominent bands are 51kDa and 56kDa in *CEP*63$^{+/-}$ cells, whereas the antibody detects a 53kDa and a 42kDa band (arrow) in *CEP63*$^{-/-}$ cells. Anti-γ-tubulin antibodies indicate centrosome content of fractions. **(B)** *CEP63* transcripts are present in patient cells. On the left: PCRs of *CEP63* cDNA from HEK-293 (H), *CEP63*$^{+/-}$ (+/-) and *CEP63*$^{-/-}$ (-/-) cells. A control is included for each set (C). Primer positions in specific exons are indicated in schematics (in blue). PCRs with

primers 8-11 produce two bands indicating that transcripts including (upper, larger band) and excluding (lower, smaller band) exon 10 are present in all three cell lines. PCRs with primers 13-15 produce two bands indicating that transcripts including or excluding exons 14 are present in the cell lines. On the right: quantitative PCRs of reverse-transcribed cDNA extracted from *CEP63*$^{+/-}$ and *CEP63*$^{-/-}$ cells. Primer positions in specific exons are indicated in schematics (in purple). Values from triplicate reactions were normalised to actin as an invariant control. *CEP63*$^{+/-}$ expression level is set as '1'.

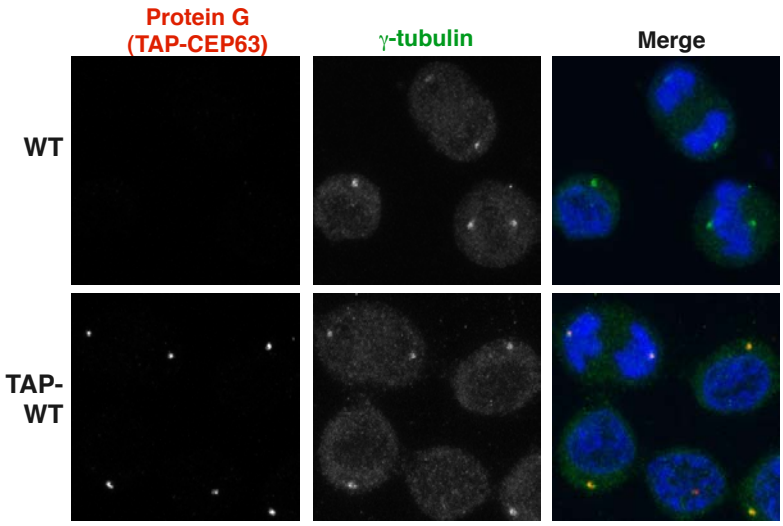
SUPPLEMENTARY FIGURE 3



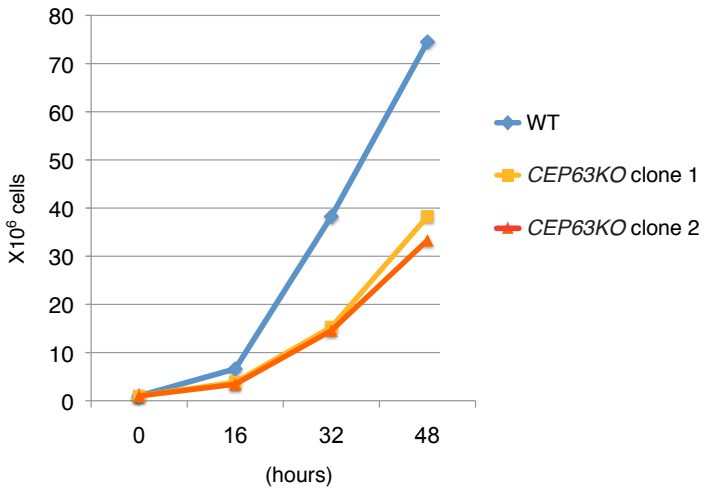
Supplementary Figure 3. Design of *CEP63* knockout strategy in DT40 cells. **(A)** Diagram shows design of homologous gene targeting in DT40 cells. Briefly, parts of exons 5 and 9 and all of exons 6-8 were replaced by neomycin and blasticidin resistance genes flanked by in-frame STOP codons to create the *CEP63KO* alleles. A GsTAP was knocked in in-frame into the last exon of *CEP63* with the concomitant removal of the STOP codon. A GsTAP tag was introduced into both *CEP63* alleles in *WT*, but only into a single allele of *CEP63* in *CEP63KO* cells. **(B)** PCRs of genomic DNA extracted from cells of indicated genotypes. Location of primers is marked in *A*. Briefly, primers C-D map within knockout region and the lack of PCR product confirms the absence of this region in both alleles of *CEP63KO* cells. Primers G-B and H-I confirm biallelic targeting of TAP tag into *TAP-WT* cells, while primer H-I confirm monoallelic targeting of TAP tag in *TAP-CEP63KO* cells. Primers J-K recognise sequences within *CEP63* allele and act as controls.

SUPPLEMENTARY FIGURE 4

A

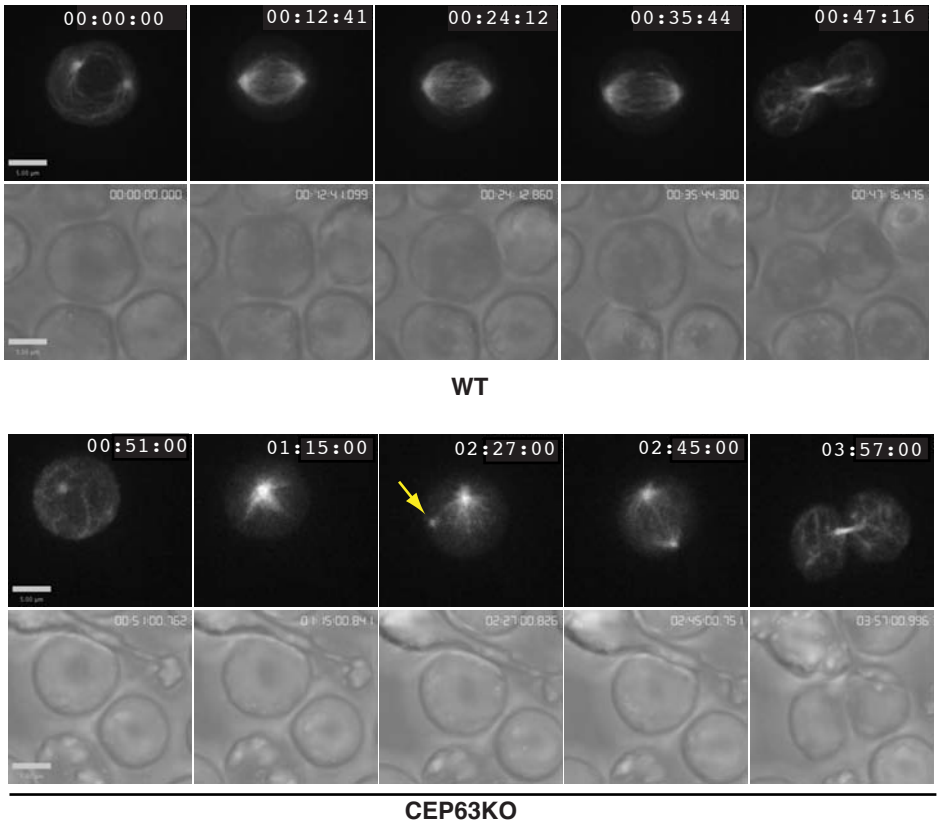


B



Supplementary Figure 4. CEP63 is a centrosomal protein that is required for normal cell growth in DT40 cells. (A) *WT* and TAP cell lines were co-stained with antibodies against protein G (red in merge) and the centrosome marker, γ-tubulin (green in merge). DNA is blue in merged image. Scale bar=5μm. **(B)** Two independent *CEP63KO* clones display growth impairment compared to *WT* cells. Cell numbers were scored in automated fashion with Vi-Cell Cell Viability Analyser.

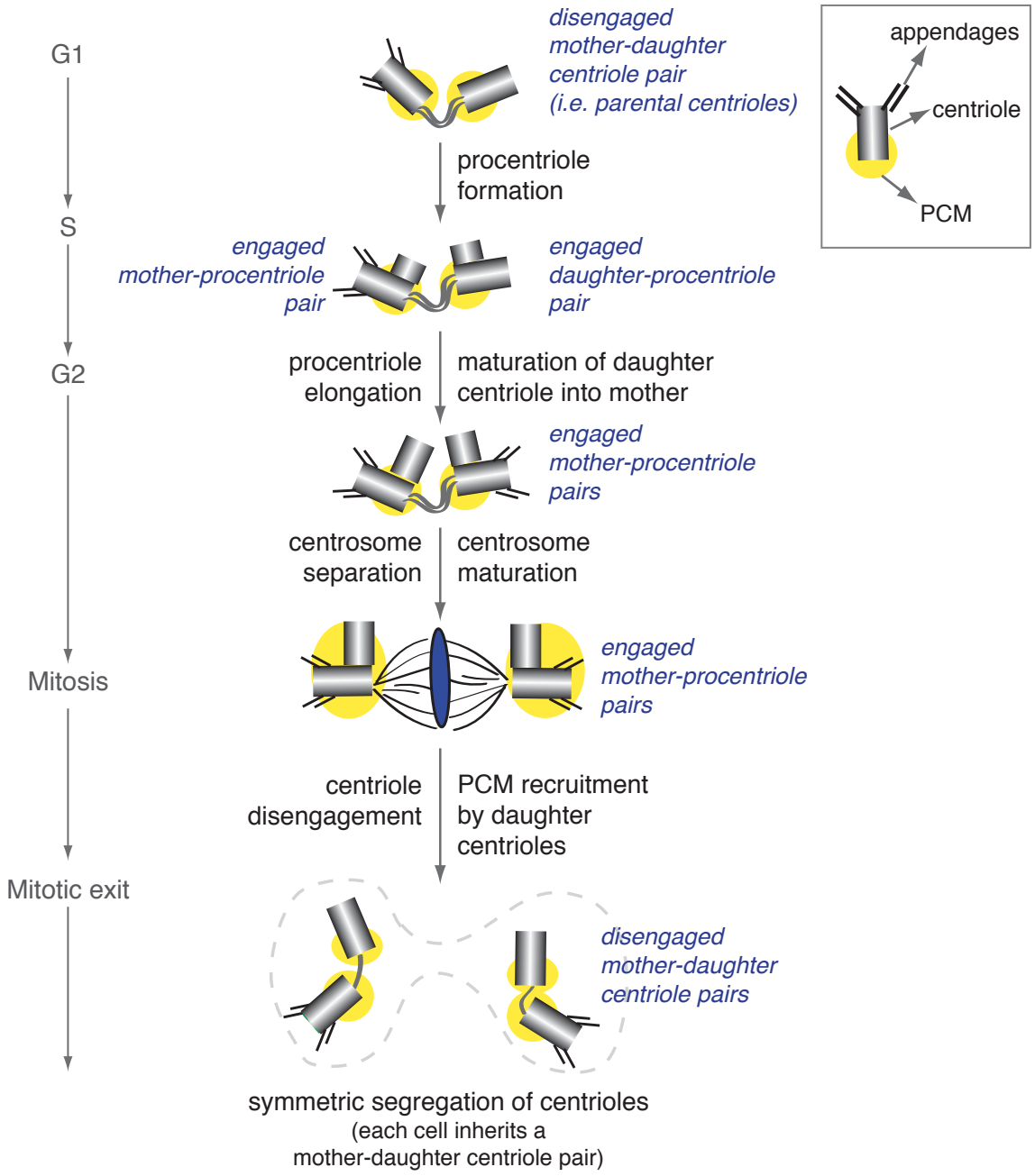
SUPPLEMENTARY FIGURE 5



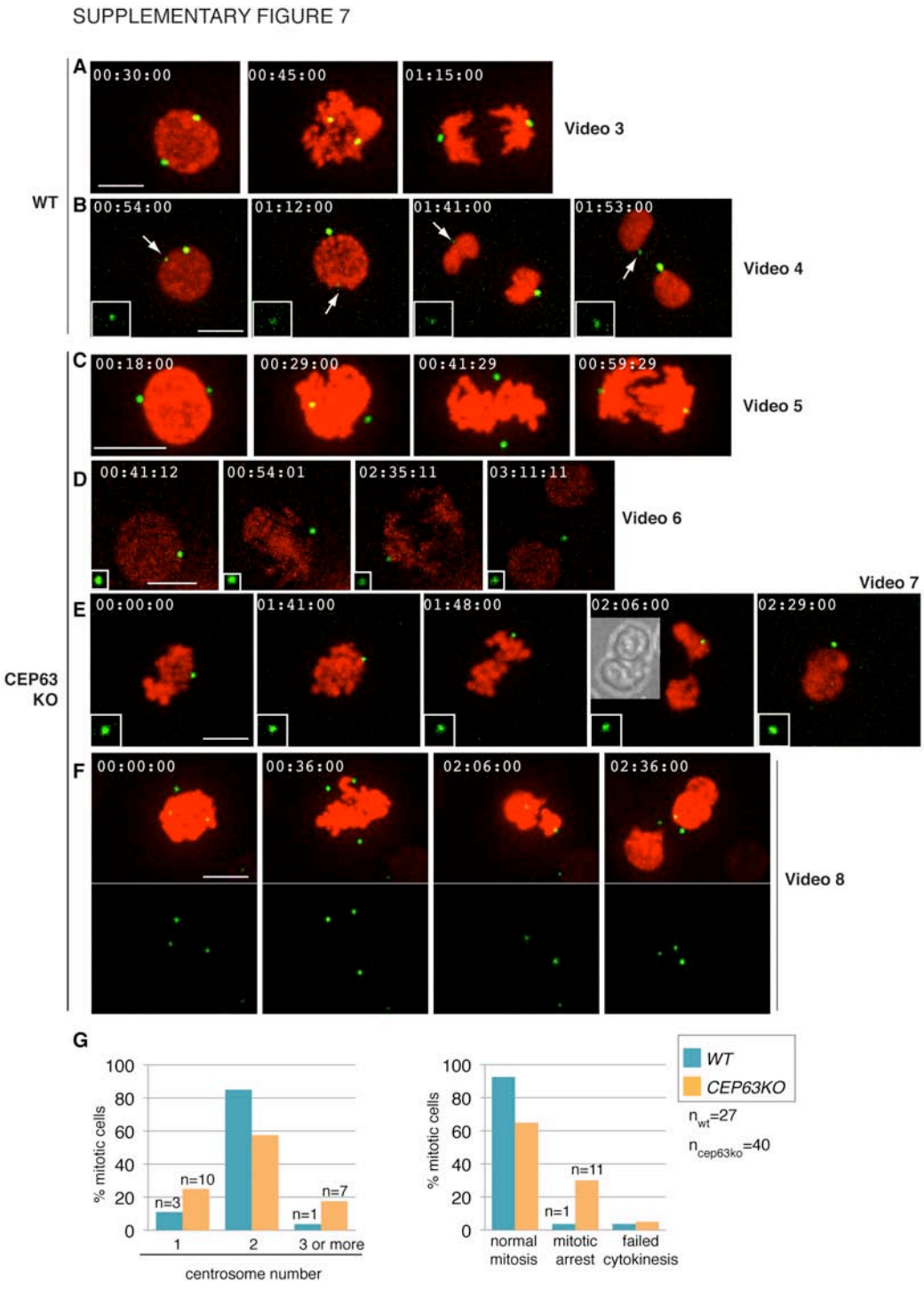
Cell lines	monopolar	monopolar to bipolar	bipolar	multipolar
WT	0	0	100% (11)	0
CEP63KO	7% (3)	15% (6)	66% (27)	12% (5)

Supplementary Figure 5. Time-lapse analysis of mitotic spindle formation in *CEP63KO* cells. Still frames from time-lapse experiments following *WT* and *CEP63KO* cells through mitosis (see Videos 1 and 2). Cells were transiently transfected with GFP-tubulin and imaged at frame rate of 3 minutes. *CEP63KO* cell assembles a monopolar spindle, but 1.5 hours after entry into mitosis the spindle becomes bipolar (arrow marks appearance of second pole). Table shows summary of spindle phenotypes observed during filming. Number of cells with particular phenotype is in parantheses. 'Monopolar to bipolar' depicts phenotype transition**.** Cells that formed multipolar spindles during this analysis entered mitosis already containing multiple microtubule asters.

SUPPLEMENTARY FIGURE 6



Supplementary Figure 6. Centrosome duplication cycle.



Supplementary Figure 7. Time-lapse analysis of centrosome behaviour during mitosis in *CEP63KO* cells. (A-F) Still frames from time-lapse experiments following *WT* and *CEP63KO* cells through mitosis. Cells were transiently transfected with Ruby-histone H2B and GFP-PACT (a centrosomal targeting signal derived from the centrosomal scaffolding protein, AKAP450 [37]) and imaged at frame rate of 6 minutes. Numbering of corresponding video files is shown on right. Note that videos are maximal projections of z-sections and include the whole cell. **(A)** A *WT* cell undergoes normal bipolar cell division with two centrosomes segregating into daughter cells. **(B)** In this *WT* cell the two centrosomes incorporate differential amount of GFP-PACT. This asymmetry was observed in around 20% of cells and in half of these cells only one centrosome was visible (3 out of 27 *WT* cells).

All these cells completed mitosis with normal timing, so we suspect that they contained two centrosomes. Instead, GFP-PACT signal intensity may correlate with when exactly GFP-PACT expression starts in the centrosome cycle and/or the age of centrioles present in centrosomes. **(C)** *CEP63KO* cell displays normal bipolar cell division with two centrosomes segregating into daughter cells. **(D)** *CEP63KO* cells displays bipolar cell division in the presence of 1 centrosome, thus asymmetrically segregating the centrosome. Note that mitosis takes longer in this cell than in *WT*. **(E)** *CEP63KO* cell undergoes bipolar cell division in the presence of 1 centrosome, but fails cytokinesis. Note that this cell was already in mitosis at the start of filming, yet it initiates anaphase only after 1 hour 48 minutes. **(F)** *CEP63KO* cell undergoes multipolar cell division in the presence of 3 centrosomes leading to centrosome missegregation. Note that anaphase transition is initiated after 2 hours in this cell. **(G)** Graphs show summary of centrosome numbers per mitotic cell (left) and outcomes of mitosis (right) from experiments in *A*-*F*. 'Normal mitosis' means bipolar cell division and includes cells with a single centrosome. 'Mitotic arrest' means that cell did not initiate anaphase until end of filming (minimum 1 hour after nuclear envelope breakdown). 'Failed cytokinesis' means that cells initiated cytokinesis but then the furrow regressed. 12 *CEP63KO* cells arrested in mitosis: 4 contained 1, 2 contained 2 and 6 contained supernumerary centrosomes. There were 3 *WT* cells displaying only 1 centrosome and all completed mitosis normally. The only wt cell arrested in mitosis contained 2 centrosomes and may have suffered laser damage.

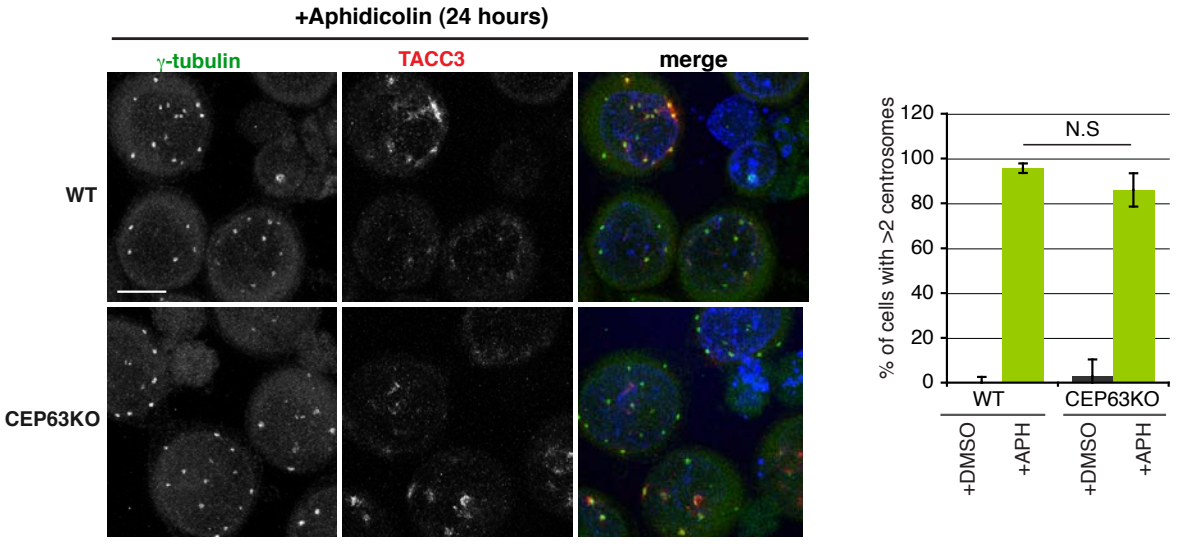
SUPPLEMENTARY FIGURE 8

A

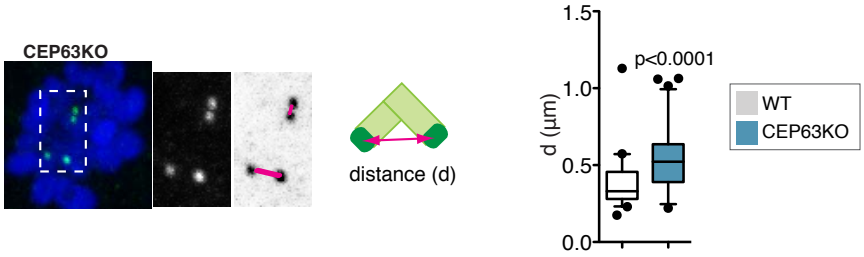
		% monopolar spindles
CEP63KO1	Day 0	34
	Day 3	40
	Day 10	40

		% monopolar spindles
CEP63KO2	Day 0	43
	Day 17	31
	Day 20	35

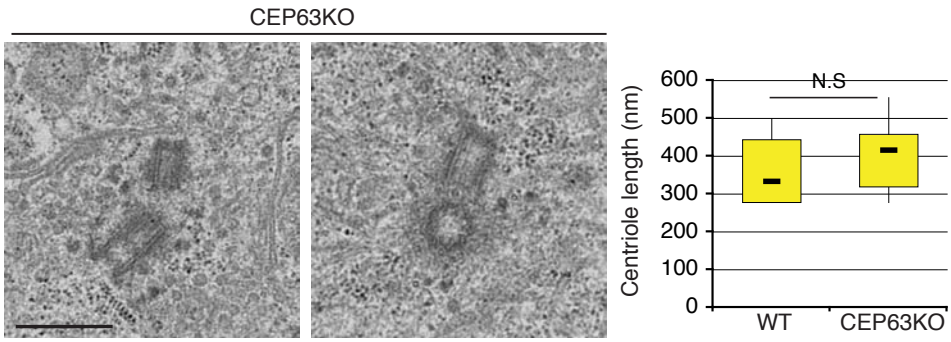
B



C



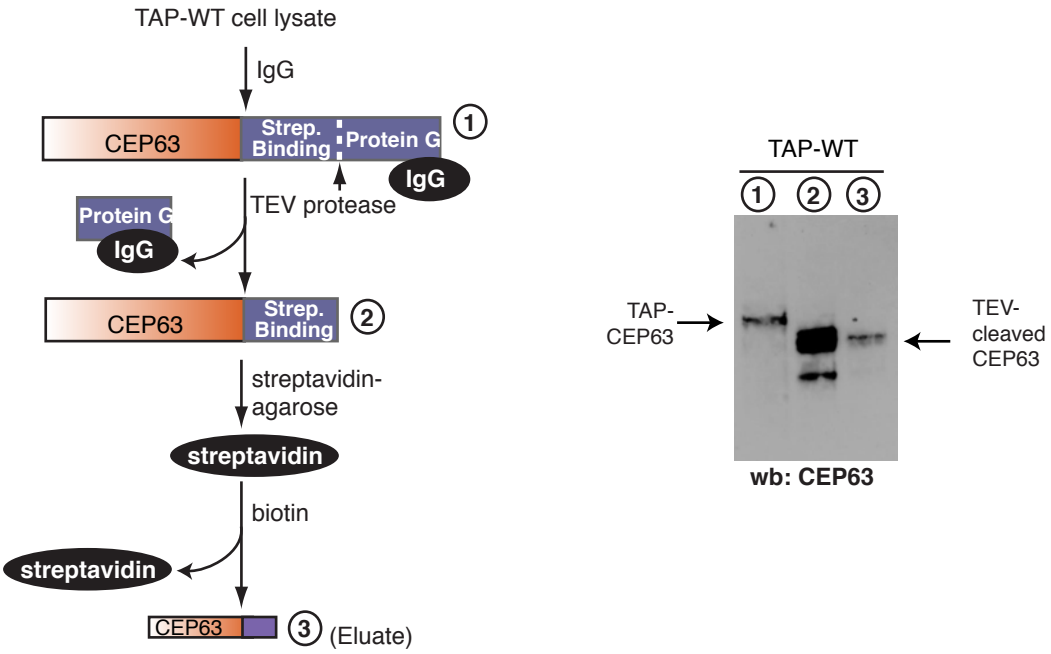
D



Supplementary Figure 8. CEP63 is not a core centriole duplication factor. **(A)** Percentages of monopolar spindles were scored on indicated days for two independent *CEP63KO* cell populations continuously cultured over time periods stated (at least 200 cells per timepoint). **(B)** Cells were treated with DMSO only (black bars in graph) or aphidicolin (green bars in graph) for 24 hours. Images show representative fields of cells stained with γ-tubulin (green in merge) and TACC3 antibodies (red in

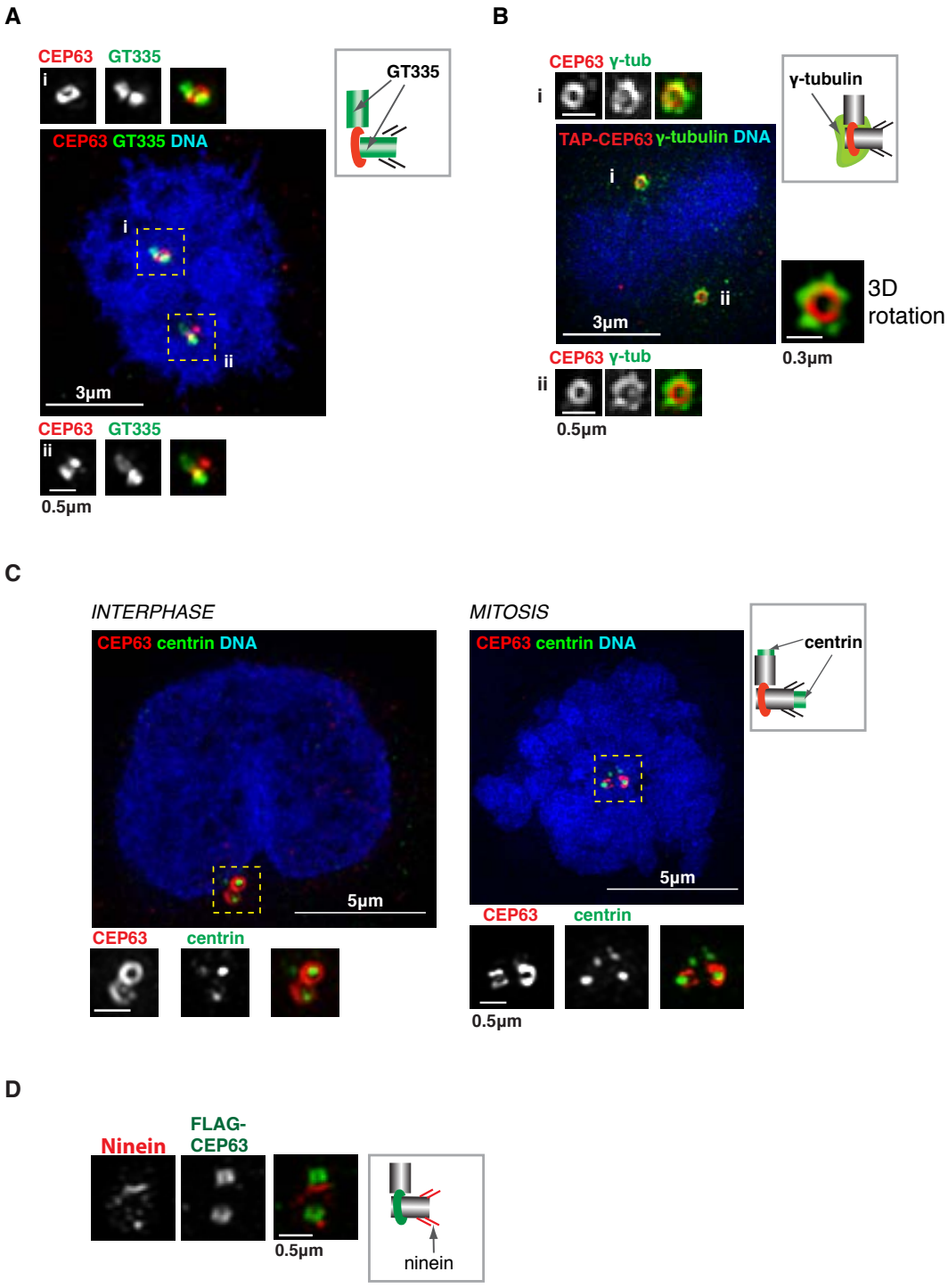
merge). DNA is blue in merge. Scale bars=5μm. Graph shows percentage of cells with more than 2 centrosomes (3 experiments, at least 200 cells per experiment, bars show mean±s.d.). N.S. not significant: p=0.1 (Student's T test, type 2, unpaired). **(C)** Distances (d) between centrioles within individual centriole pairs were measured in mitotic cells using the distal centriole marker, centrin (dark green in schematics). Only cells with bipolar spindles containing 2 centrioles per spindle pole were included. Examples of a *CEP63KO* cell stained with anti-centrin-3 antibody (green in merge). DNA is in blue. Framed area is shown in higher magnification with corresponding centrin signal. Inverted image is included to aid visualisation. Box plot represents distribution of distances (n=47 wt and n=62 *CEP63KO* centriole pairs). Distances were measured in 3D using Volocity. Box plots: length of whiskers is at 5th and 95th percentiles, the box shows interquartile (25-75) range and horizontal line represents the median. p value was obtained by two-tailed, unpaired Student t test with Welch correction. Scale bar=0.5μm **(D)** Transmission electron micrographs of *CEP63KO* centrosomes; on the left a disengaged centriole pair are visible whereas on the right a pair of engaged centrioles are shown. Note that structure of centrioles appears normal. Box plot shows distribution of centriole lengths in centriole-containing but otherwise random sections of *WT* and *CEP63KO* cells (wt: n=14, *CEP63KO*: n=20 centrioles). N.S. not significant: p=0.4 (Student's T test, type 2, unpaired). Box plots: length of whiskers is at minimum and maximum of data and the box shows the interquartile (25-75) range, while horizontal line represents the median. Scale bar=500nm.

SUPPLEMENTARY FIGURE 9



Supplementary Figure 9. Affinity-purification of GsTAP-tagged CEP63 from *TAP-WT* cells.Tandem affinity purification of GsTAP-tagged CEP63 protein is shown. IgG beads were used to immunoprecipitate GsTAP-tagged CEP63 from cell lysate. IgG beads coupled with CEP63 were washed and then incubated with TEV protease to remove the protein G moiety of the GsTAP tag and thus release CEP63 from IgG beads. Supernatant was then incubated with streptavidin-agarose resin and biotin was used to elute tagged CEP63 as before (Eluate=E). Western blots were probed with anti-CEP63 antibodies. Encircled numbers on immunoblot correspond to particular steps in schematics when samples were collected. Note that a degradation product is visible after the TEV protease treatment, but this band is no longer present after elution from streptavidin-agarose resin.

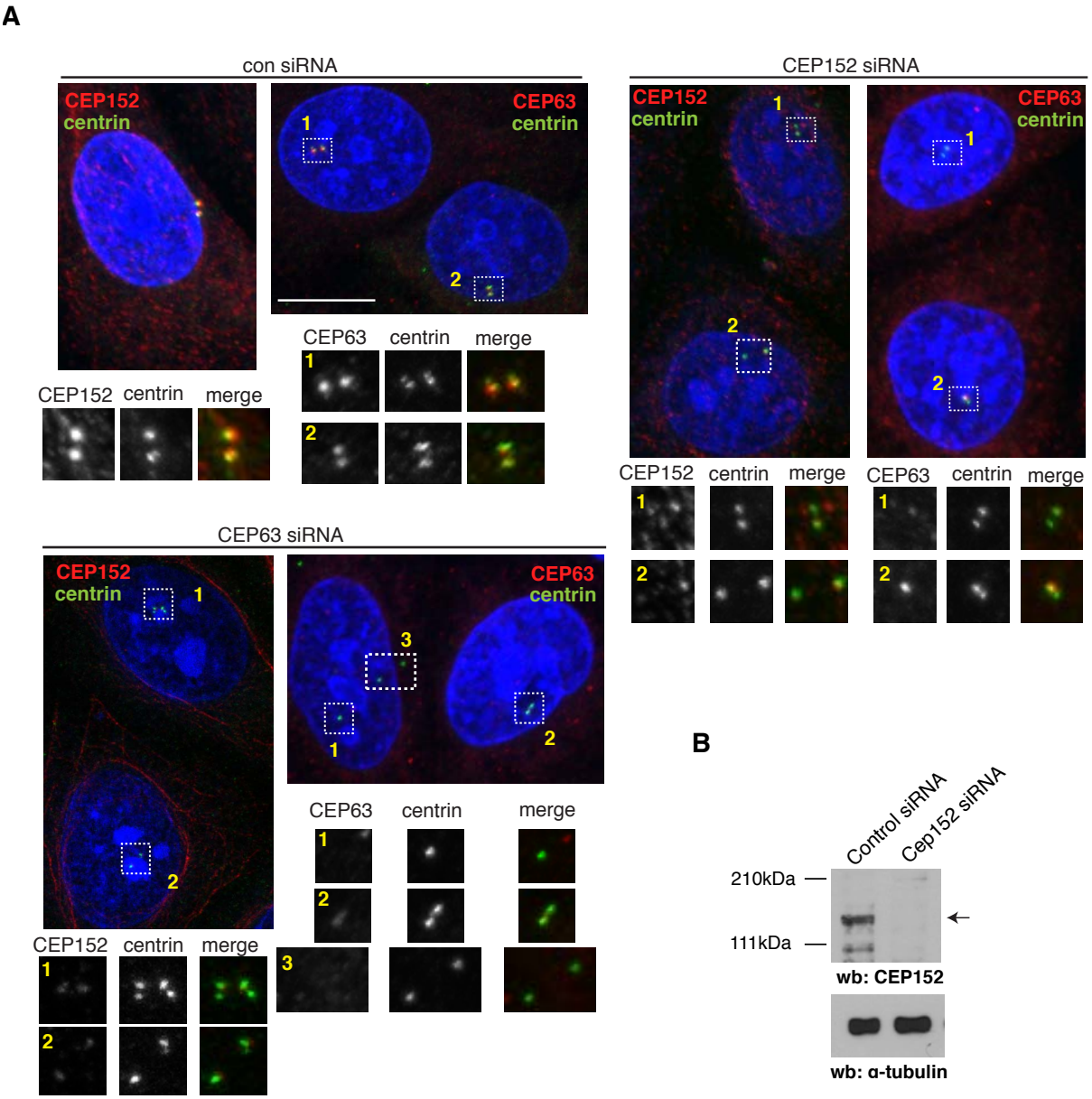
SUPPLEMENTARY FIGURE 10



Supplementary Figure 10. CEP63 forms a ring around parental centrioles. (A) 3D-SIM image of *TAP-WT* DT40 cell co-stained with antibodies against protein-G (i.e. CEP63; red in merge) and polyglutamylated tubulin, a centriole marker (green in merge). Schematic shows relative positions of polyglutamylated tubulin and CEP63. **(B)** 3D-SIM image of *TAP-WT* DT40 cell co-stained with antibodies against protein-G (i.e. CEP63; red in merge) and γ-tubulin, a PCM marker (green in merge). Schematic shows relative positions of γ-tubulin and CEP63. **(C)** 3D-SIM image of *CEP63*$^{+/-}$ human lymphocyte co-stained with antibodies against CEP63 (red in merge) and centrin-3 (green in

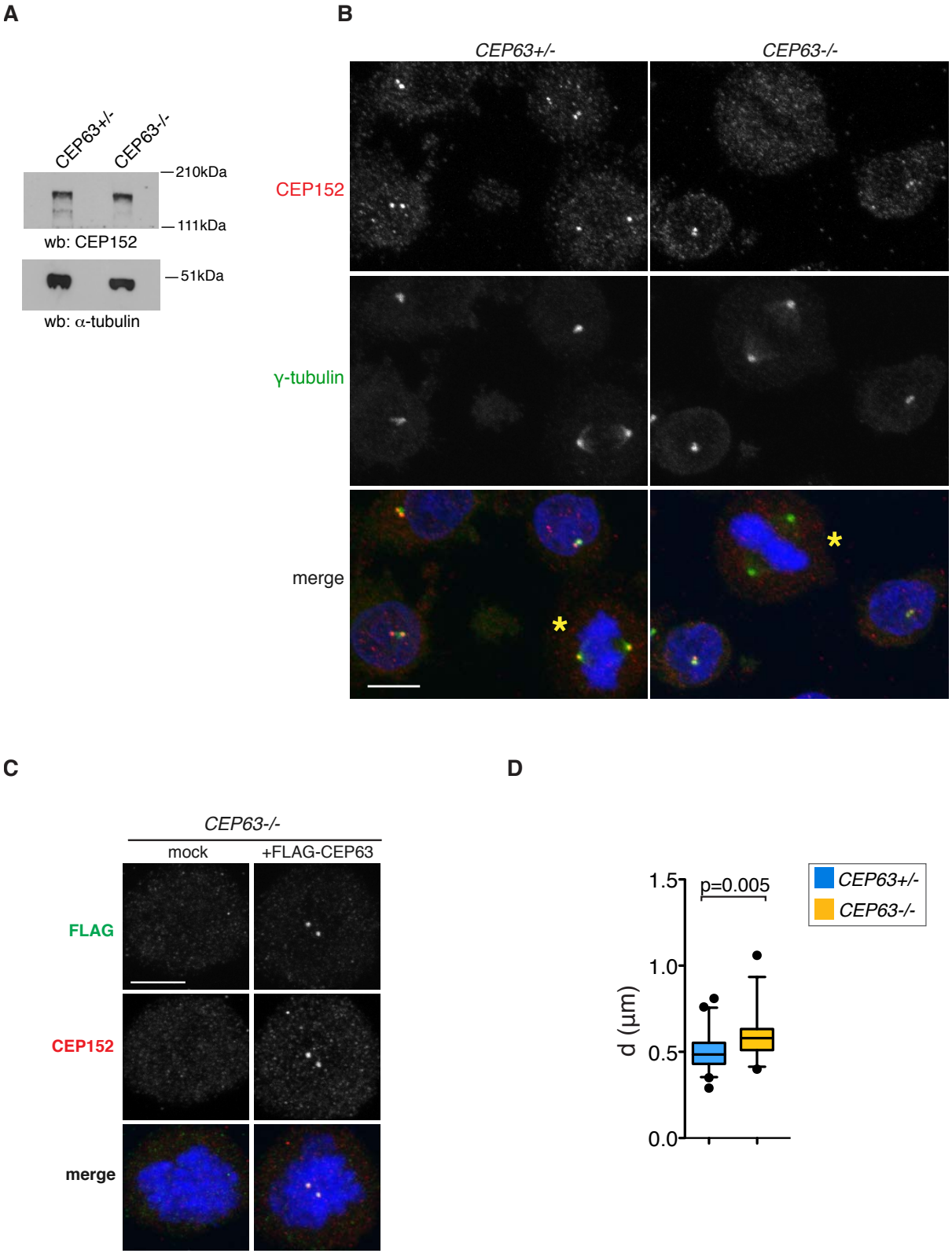
merge). Schematic shows relative positions of centrin-3 and CEP63. **(D)** 3D-SIM image of centrosomes of FLAG-CEP63-expressing HeLa cell co-stained with antibodies against FLAG (green in merge) and ninein (red in merge) antibodies. Ninein recognises subdistal appendages of mother centrioles as shown by schematic.

SUPPLEMENTARY FIGURE 11



Supplementary Figure 11. CEP63 and CEP152 are interdependent for their centrosomal localisation. (A) RNA interference-mediated depletion of CEP63 and CEP152 is shown. Cells depleted of negative control siRNA (con), CEP63-depleted or CEP152-depleted cells were co-stained with antibodies against centrin-3 (green in merge) and CEP152 or CEP63 (red in merge). Higher magnifications of framed regions are shown. Note that in CEP152-depleted cells, CEP63 levels are different on two centrioles (arrows). **(B)** Cytoplasmic cell extracts were prepared from control- or CEP152-depleted HeLa cells. Immunoblots were probed with antibodies against CEP152 and α-tubulin.

SUPPLEMENTARY FIGURE 12



Supplementary Fig. 12. CEP63 is required for centrosomal accumulation of CEP152. (A) Cytoplasmic cell extracts (CCE) were prepared from parent-of-patient (*CEP63*$^{+/-}$) and patient (*CEP63*$^{-/-}$) cells. Immunoblots were probed with CEP152 antibodies. α–tubulin serves as loading control. **(B)** Fields of cells of indicated genotypes were co-stained with anti-γ-tubulin (green in merge) and anti-CEP152 (red in merge) antibodies. DNA is in blue. Mitotic cells are marked by asterisks.

Note that CEP152 signal is absent in the mitotic *CEP63*$^{-/-}$ cell. **(C)** FLAG-CEP63-transfected *CEP*63$^{-/-}$ cells were co-stained with FLAG (green in merge) and CEP152 (red in merge) antibodies. DNA is blue. Centrosomal CEP152 was detected in 0/23 FLAG-negative and 11/11 FLAG-positive mitotic *CEP63*-/- cells. **(D)** Distances (d) between centrioles within individual centriole pairs were measured in parent-of-patient (*CEP63*$^{+/-}$) and patient (*CEP63*$^{-/-}$) mitotic cells as in Supplementary Fig. 10C (*WT*: n=46, *CEP63KO*: n=30 centrosomes). Box plots: length of whiskers is at 5th and 95th percentiles, the box shows interquartile (25-75) range and horizontal line represents the median. P value was obtained by two-tailed, unpaired Student t test.

3. Supplementary Table Legends and Table 2

Supplementary Table 1. is provided as a separate Excel file.

Supplementary Table 1. Spreadsheet of SILAC results. Proteins with L/H ratio 0.5 or less are shown. Worksheets each correspond to a gel slice in descending order (#HS1 was the top of the gel; CEP152 is in #HS2). Note that CEP63 and CEP152 are 10-20-fold enriched in heavy fraction, whereas the next best candidates are only 2-3 fold enriched.

SUPPLEMENTARY TABLE 2

CEP152 peptides	Mascot Score (expect score)	L/H
VSDASTEELAR	79 (4.1e-007)	0.04802
TLIELECLLR	64 (1.2e-005)	0.04509
SPLYNTGQQAETQR	95 (1.2e-008)	0.09504
SALLLNSDLPTGVEYAK	78 (7.2e-007)	0.0728
ALETQIQTLTTNEEQILK	117 (9.5e-011)	0.03962

Supplementary Table 2. CEP152 peptides identified by mass spectrometry. Table shows amino acid sequences of 5 doubly charged peptide ions that identified CEP152 as an interacting partner of GsTAP-CEP63 using the experimental scheme from Figure 4A. Individual Mascot scores greater than 24 indicate extensive homology ($p<0.1$). The SILAC ratios (light to heavy; L/H) for individual peptides are shown.

Supplementary Table 3. is provided as a separate Excel file.

Supplementary Table 3. Primer sequences.

4. Supplementary Video Legends

Video 1. Mitosis in *WT* DT40 cells expressing GFP-α-tubulin. Images were acquired at a rate of 3 minutes/frame. Note that software failed to assign certain frames with correct timestamps.

Video 2. Mitosis in *CEP63KO* DT40 cells expressing GFP-α-tubulin. Images were acquired at a rate of 3 minutes/frame.

Video 3. Mitosis in *WT* DT40 cells expressing GFP-PACT and Ruby-Histone H2B. Images were acquired at a rate of 6 minutes/frame. GFP is green, ruby is red.

Video 4. Mitosis in *WT* DT40 cells expressing GFP-PACT and Ruby-Histone H2B. Images were acquired at a rate of 6 minutes/frame. Note that one centrosome is weaker. GFP is green, ruby is red.

Video 5. Mitosis in *CEP63KO* DT40 cells expressing GFP-PACT and Ruby-Histone H2B. Images were acquired at a rate of 6 minutes/frame. GFP is green, ruby is red.

Video 6. Mitosis in *CEP63KO* DT40 cells expressing GFP-PACT and Ruby-Histone H2B. Images were acquired at a rate of 6 minutes/frame. GFP is green, ruby is red.

Video 7. Mitosis in *CEP63KO* DT40 cells expressing GFP-PACT and Ruby-Histone H2B. Images were acquired at a rate of 6 minutes/frame. GFP is green, ruby is red.

Video 8. Mitosis in *CEP63KO* DT40 cells expressing GFP-PACT and Ruby-Histone H2B. Images were acquired at a rate of 6 minutes/frame. GFP is green, ruby is red.