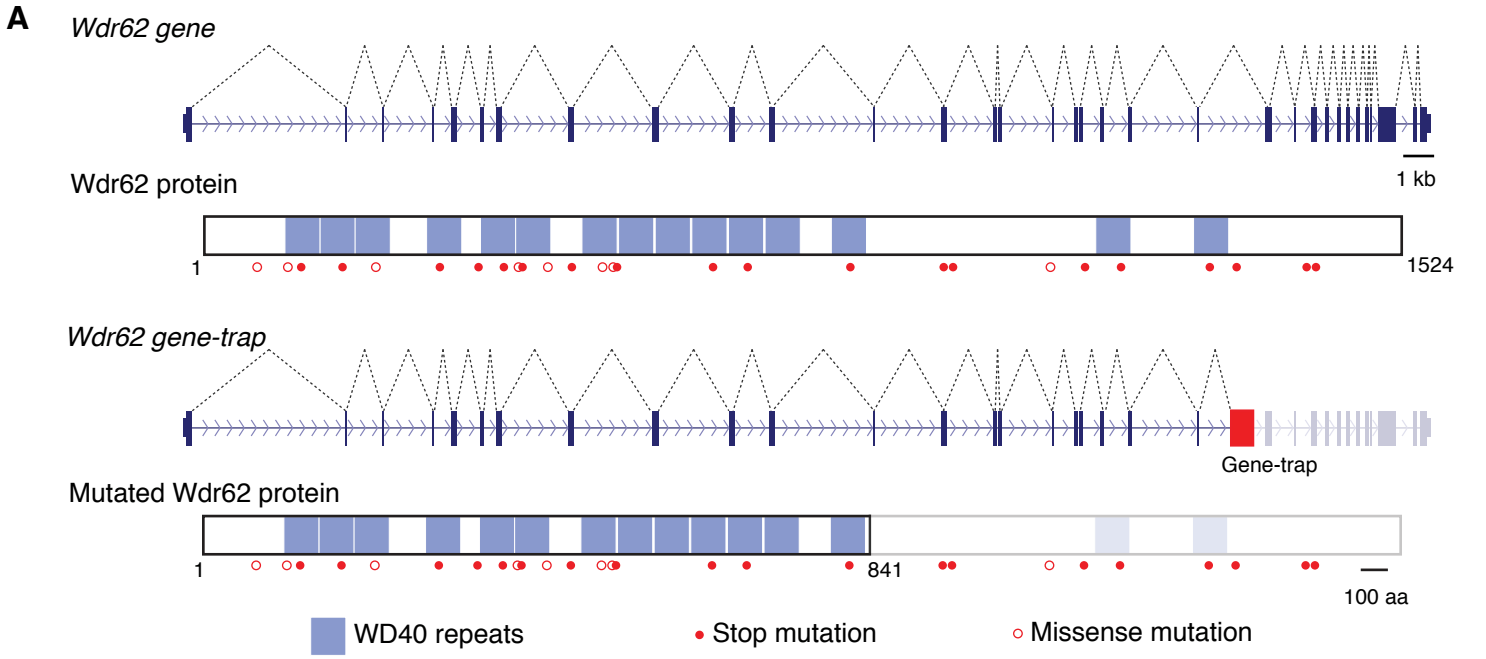
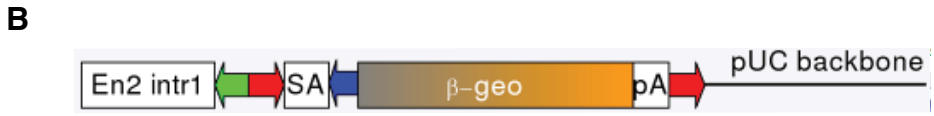
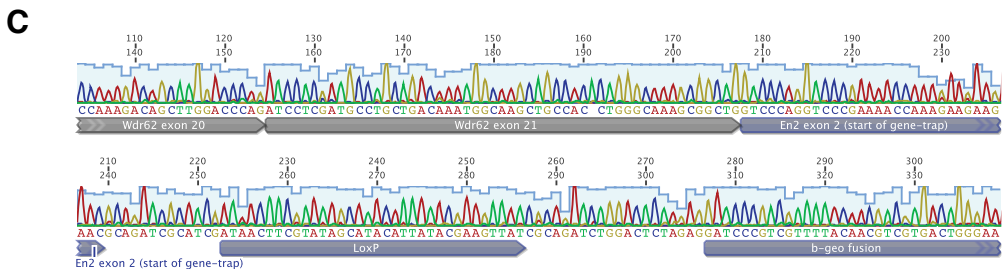
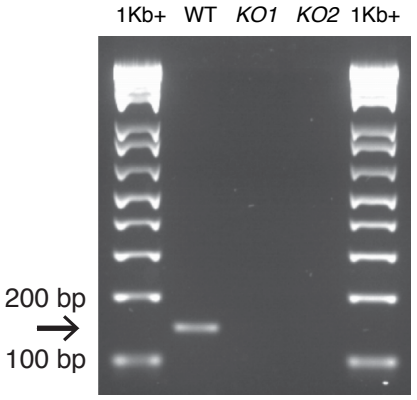
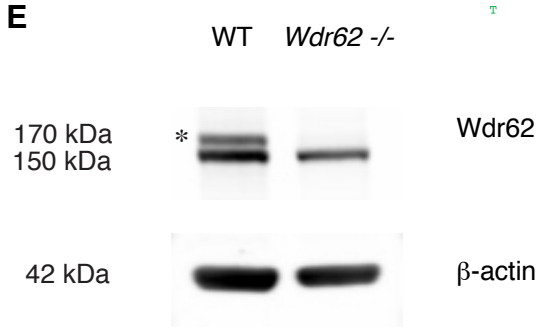
Figure S1, related to Figure 1

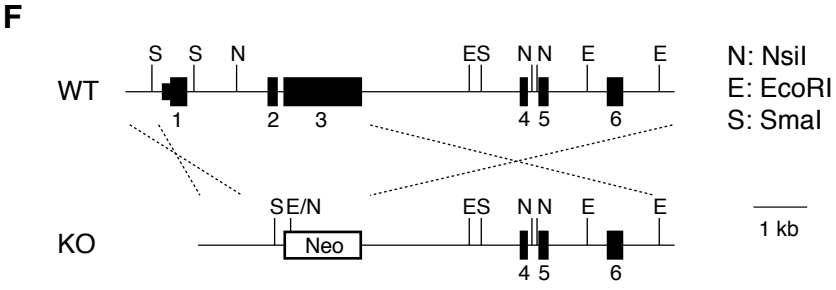


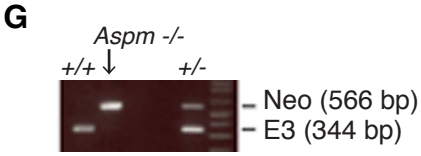


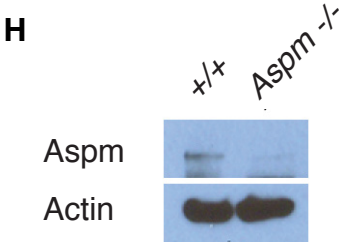


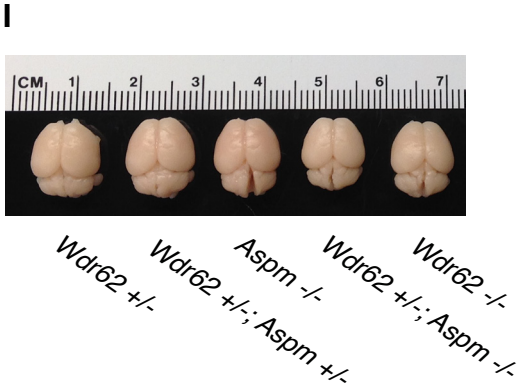












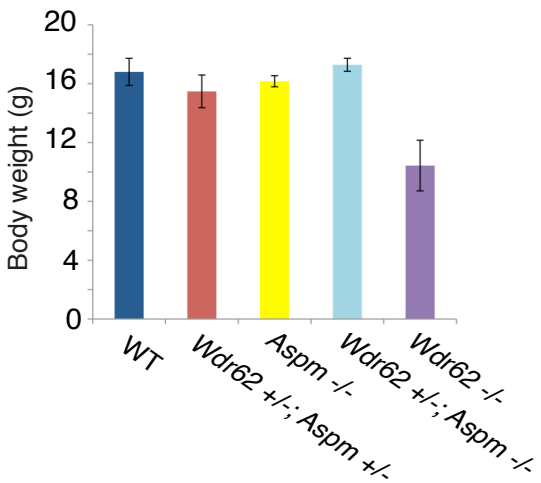


Figure S2, related to Figure 2

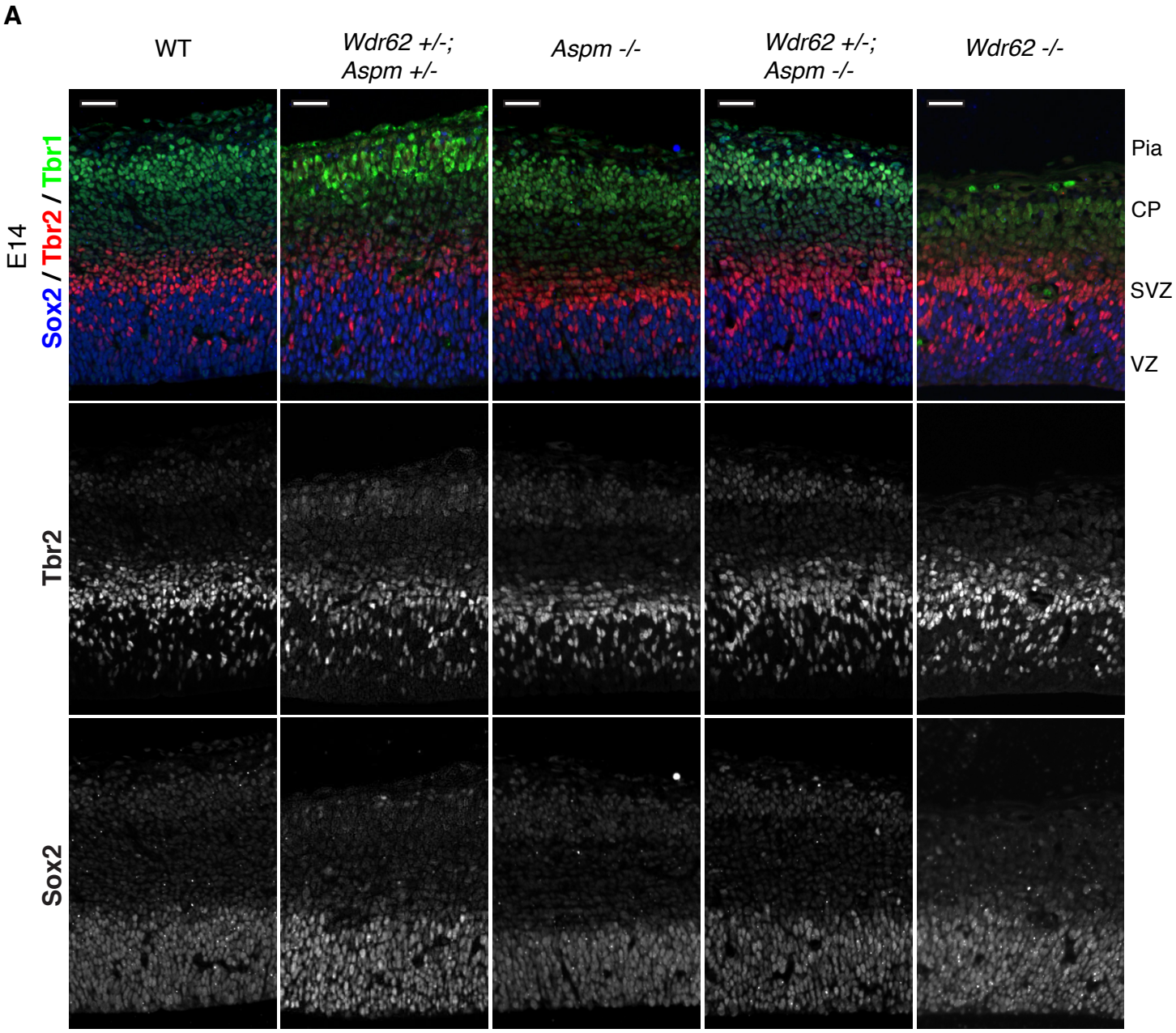
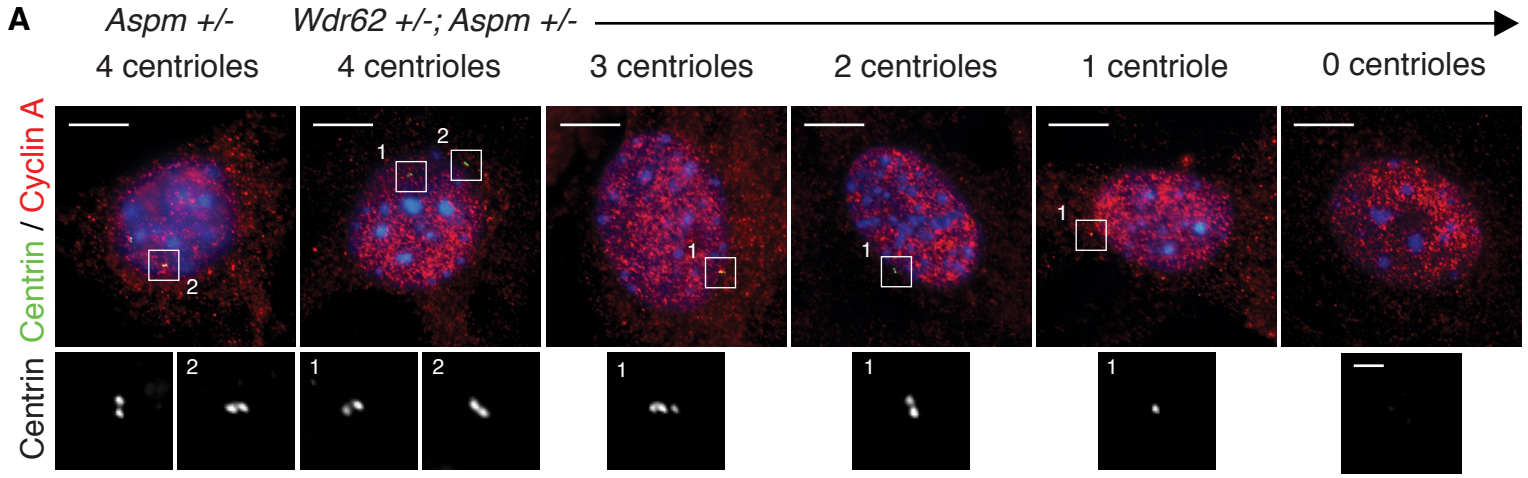
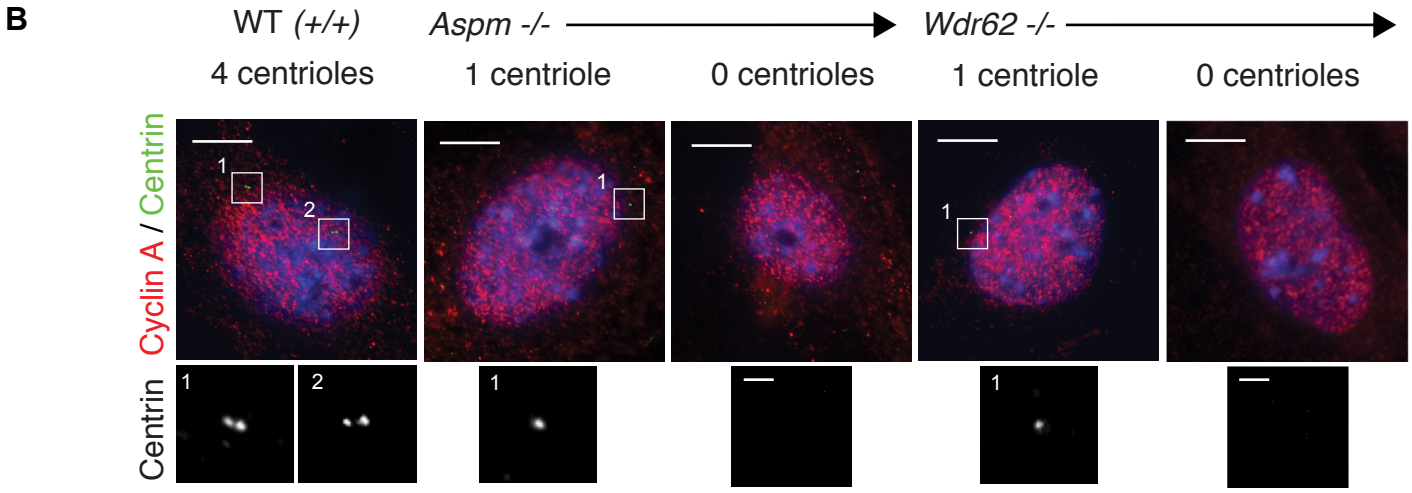


Figure S3, related to Figure 3





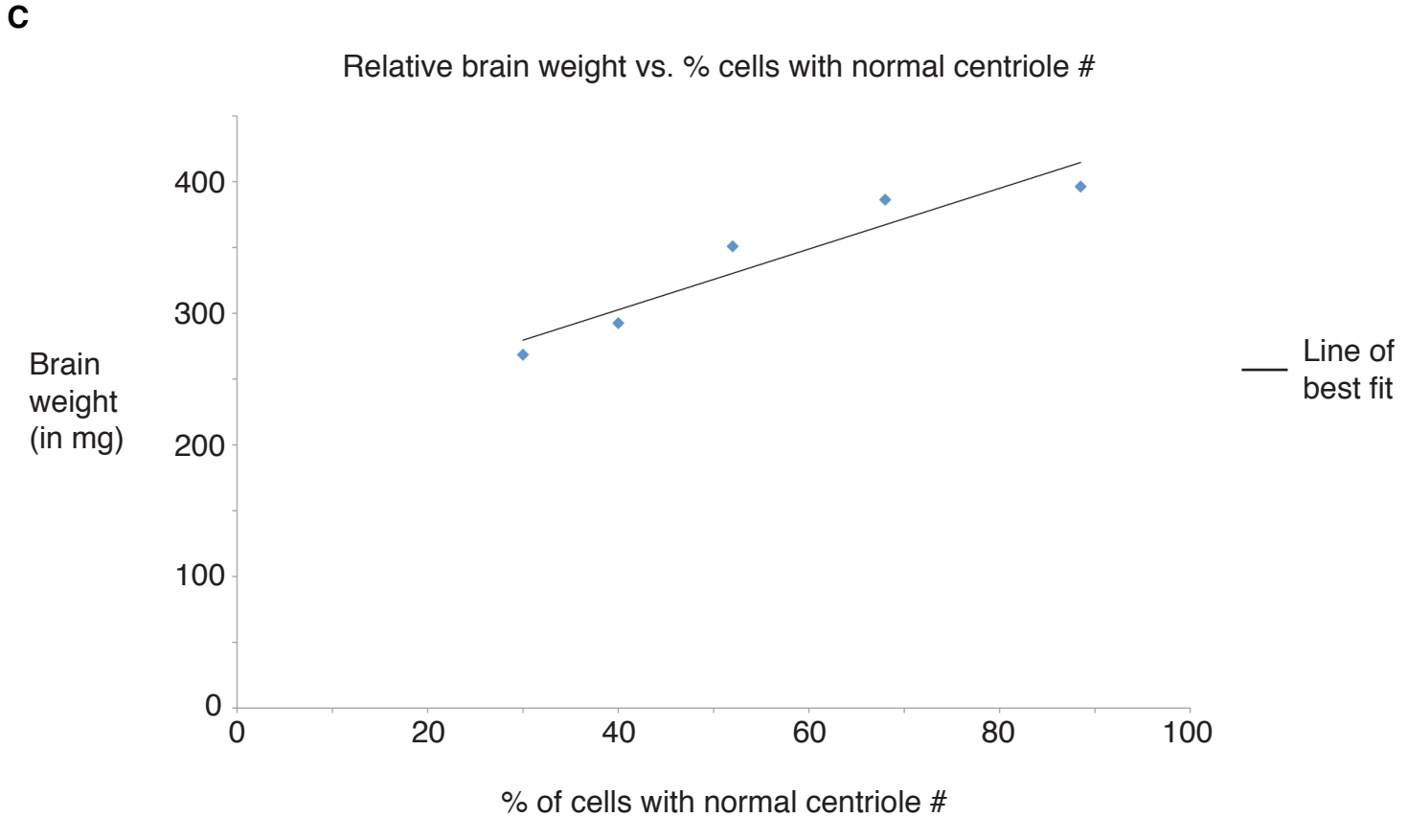
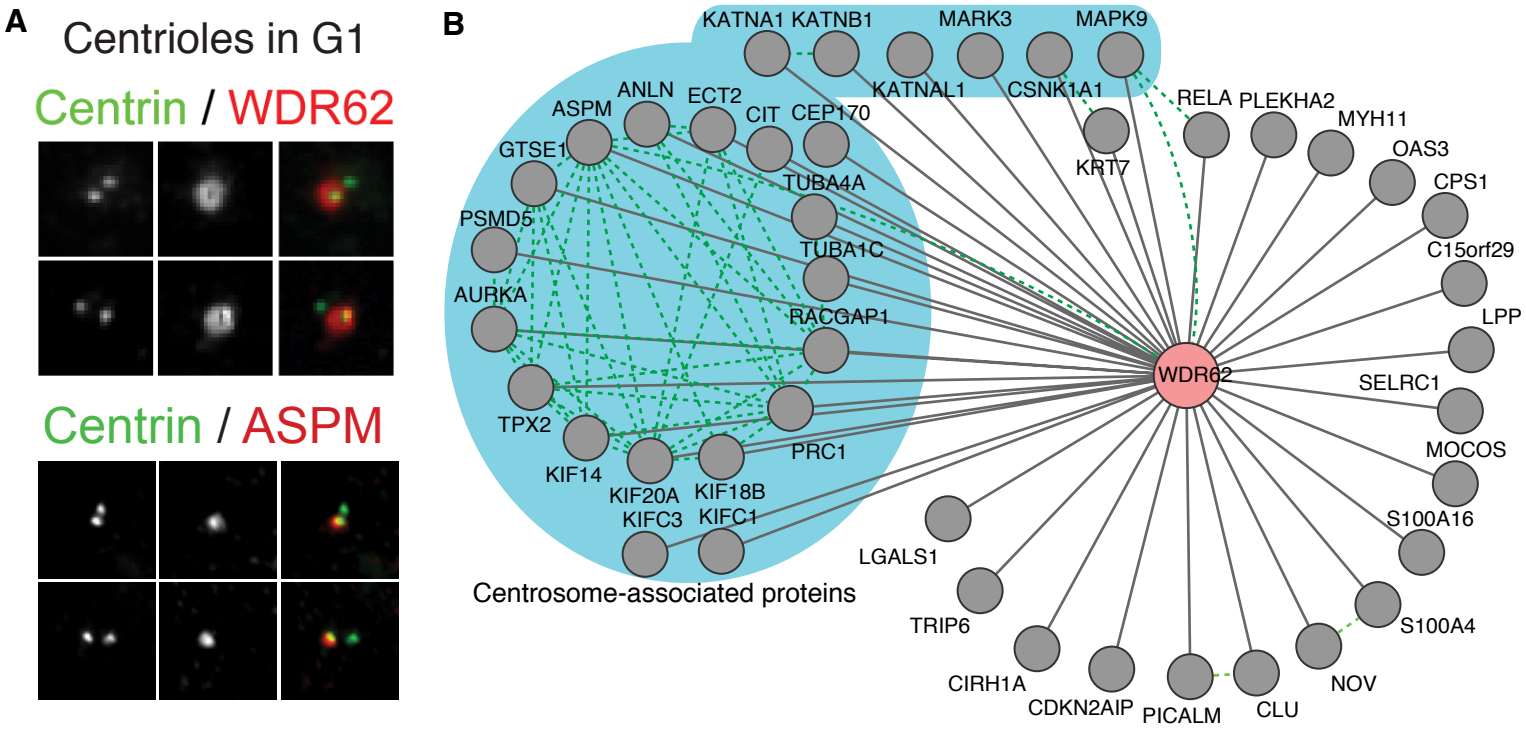
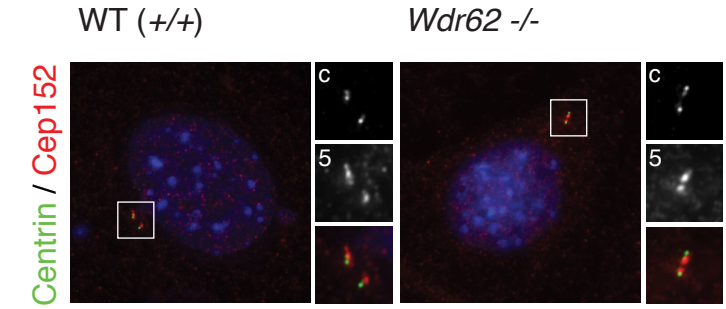


Figure S4, related to Figure 4





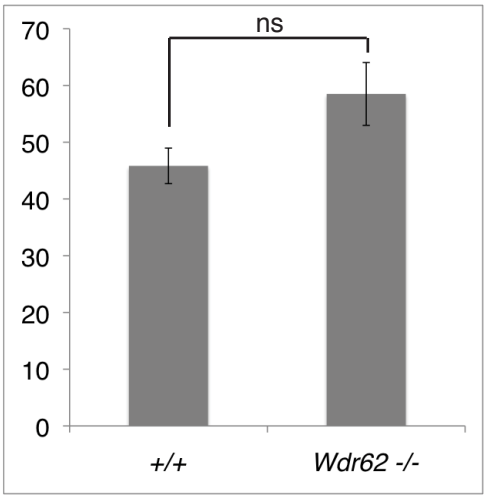


Figure S5, related to Figure 5

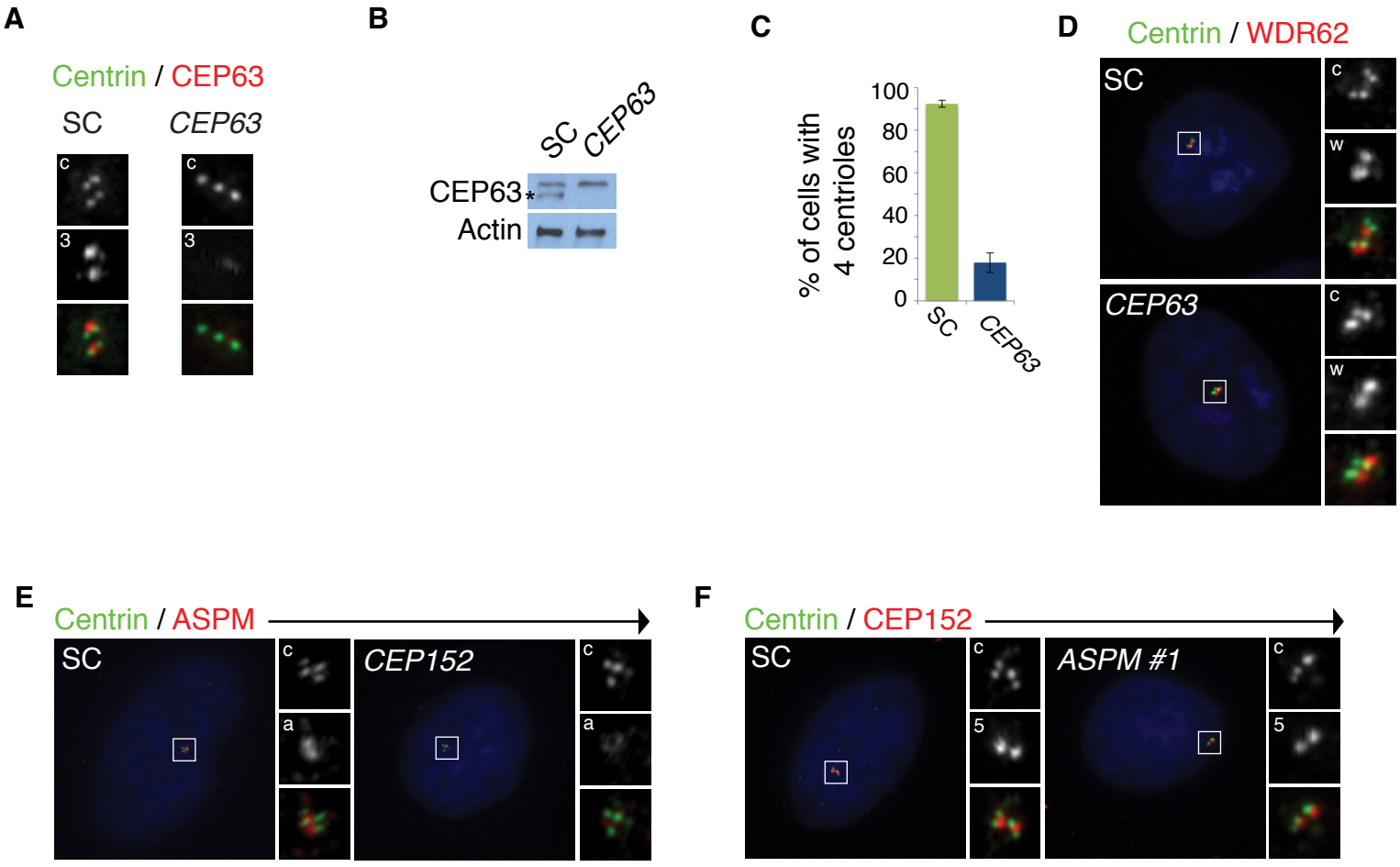
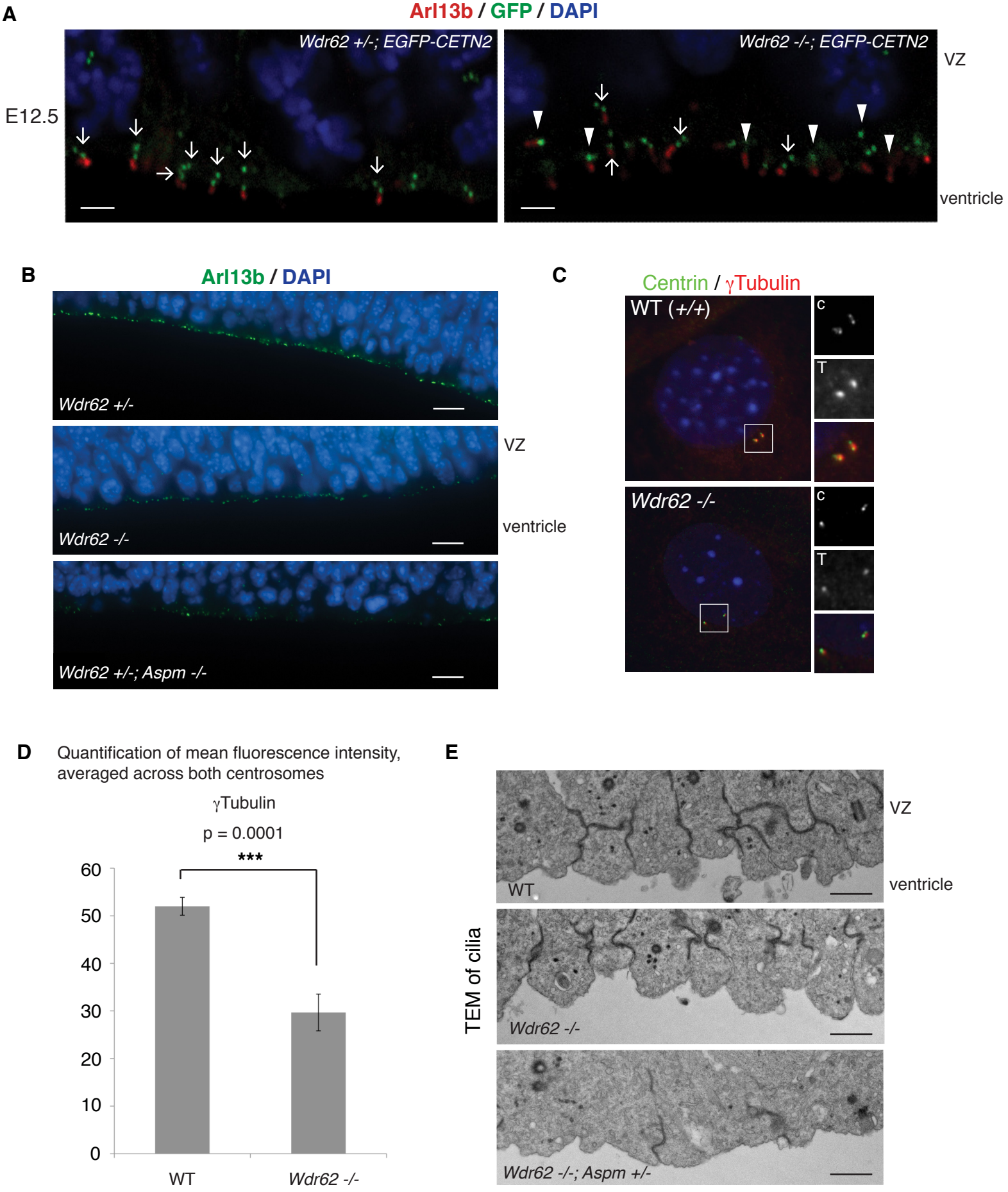


Figure S6, related to Figure 7



SUPPLEMENTAL FIGURE LEGENDS

Figure S1, related to Figure 1. Generation of *Wdr62* intron 21 gene-trap mice and *Aspm* mutant mice
(A) Mouse *Wdr62* encodes a protein of 1,524 amino acids. Purple bars indicate the predicted locations of WD40 repeats. Top: Orthologous locations of human *WDR62* patient mutations aligned with the mouse genomic locus and protein sequence, with missense mutations represented by red outlined circles and "Stop" mutations (including nonsense, frameshift, or splicing alleles that cause frameshifts) represented by red filled circles. Even stop mutations occurring near the C-terminus of the protein result in severe (null) patient phenotypes. Bottom: Genomic structure and predicted protein impact of gene-trapped Wdr62 locus, which leads to early truncation of mouse *Wdr62* mRNA after exon 21, mimicking several null alleles in humans.
(B) *Wdr62* gene-trap vector pGT0lxr contains 1.5 Kb of mouse *En2* intron 1, the splice acceptor (SA) of *En2* exon 2, a fusion of β-galactosidase and neomycin transferase (β-geo cassette), and an SV40 polyadenylation (pA) signal. FRT sites (red arrows) allow post-insertional excision of the SA/β-geo cassette. Lox71/loxP sites (green/blue arrows) allow excision of the SA alone.
(C) cDNA sequence generated by RT-PCR from a *Wdr62* +/- mouse showing the truncation of the *Wdr62* coding sequence after exon 21, followed immediately by En2 exon 2 (the start of the gene-trap), then a loxP site and the start of the β-geo fusion. Image of sequence generated using Geneious version 5.6 software (Biomatters, RRID:SCR_010519), http://www.geneious.com (Kearse et al., 2012).
(D) WT *Wdr62* transcript is absent from homozygous gene-trap brain at E14.5. RT-PCR of RNA from brains of E14.5 male littermates, with the forward primer located in *Wdr62* exon 21 and the reverse primer located in exon 22 (flanking the insertion site of the gene-trap). When PCR products were run out on an agarose gel, a band at the expected size (148 bp) was seen in the WT *(+/+)* but absent from two KO (*Wdr62* -/-) littermates. Lanes (from left to right): Ladder (1 Kb+), *WT, KO # 1, KO # 2* and ladder.
(E) Absence of full-length Wdr62 protein in homozygous gene-trap (*Wdr62* -/-) mice. Top row: Western blot analysis using antibody raised to the C-terminus of mouse Wdr62 yields a band (*) at the expected size of 170 kDa in WT *(+/+)* lysate (left) that is absent from the *Wdr62* -/- lysate (right). Bottom row: β-actin loading control at 42 kDa.
(F) The mouse *Aspm* gene targeting construct, in which a neomycin cassette replaces exons 1-3 of the wild-type *Aspm* gene.
(G) Genotyping of *Aspm* mutant mice. Analysis of PCR by agarose gel electrophoresis confirms the presence of the Neo cassette and absence of wild-type exon 3 (E3) in *Aspm* -/- mice. Wild-type *(+/+)* and heterozygous *(+/-)* controls are also shown.
(H) Loss of full-length Aspm protein in *Aspm* -/- mice. Western blot for Aspm (top row) yields a band at the expected size in WT (+/+) lysate (left). The faint band visible around the same size in *Aspm* -/- lysate (right) is nonspecific, as the antibody used in the blot was raised against amino acids 497-520, i.e., the VTKR epitope (Kouprina et al., 2005), which is part of the deleted region (exons 1-3 replaced by the Neo cassette; see Figure S1G). β-actin is used as a loading control (bottom row).
(I) Image of whole brains (without the olfactory bulbs) shows progressive decrease in brain size with decreasing dosage of *Aspm* and especially *Wdr62*. Arranged in descending order of size, from left to right, are: *Wdr62* +/-, *Wdr62 +/-; Aspm* +/-, *Aspm* -/-, *Wdr62 +/-; Aspm* -/- and *Wdr62* -/-.
(J) Body weight at P30 is not significantly different between WT controls and the various mutant genotypes (*Wdr62 +/-; Aspm* +/-, *Aspm* -/- and *Wdr62 +/-; Aspm* -/-), with the sole exception of *Wdr62* -/-. Error bars indicate mean ± SEM.

Figure S2, related to Figure 2. *Aspm* -/-, *Wdr62* -/- and *Wdr62 +/-; Aspm* -/- neocortex show an increase in basal progenitors relative to apical progenitors.
(A) Tbr2+ basal progenitors are increased in the *Wdr62* -/- and *Wdr62 +/-; Aspm* -/- cortex at E14.5. Left to right: WT, *Wdr62 +/-; Aspm* +/-, *Aspm* -/-, *Wdr62 +/-; Aspm* -/- and *Wdr62* -/- cortex stained for Sox2 (blue), Tbr2 (red) and Tbr1 (green) to label apical progenitors (APs), basal progenitors (BPs) and neurons in layer VI of the cortical plate, respectively. Top panel: multi-channel image. Middle panel: single-channel image showing Tbr2 immunostaining. Bottom panel: single-channel image showing Sox2 immunostaining. Scale bar = 25 μm.

Figure S3, related to Figure 3. Depletion of Wdr62 and Aspm impairs centriole duplication, with the severity of the cellular defect proportional to the severity of the microcephaly

(A) *Aspm* +/- and *Wdr62 +/-; Aspm* +/- mouse embryonic fibroblasts (MEFs) stained with Centrin (green) and Cyclin A (red) to mark centrioles and S-phase/G2 cells, respectively. Insets (in grayscale) show Centrin staining at higher magnification except for the last panel on the right showing zero centrioles. Scale bar = 10 μm.
(B) Mouse embryonic fibroblasts (MEFs) stained with Centrin (green) and Cyclin A (red) to mark centrioles and S-phase/G2 cells, respectively, reveals that some *Aspm -/-* and *Wdr62 -/-* cells are acentriolar. Left to right: Wild-type (WT), *Aspm -/-* and *Wdr62 -/-*. Insets (in grayscale) show Centrin staining at higher magnification, except for panels showing zero centrioles. Scale bar = 10 μm.
(C) The severity of the centriole duplication defect correlates well with the severity of the microcephaly phenotype. The mean brain weight (in mg) of a given genotype at P30, plotted against the percentage of S-phase/G2 cells of that genotype with 4 centrioles, is shown as blue dots. Genotypes corresponding to each dot, from left to right: *Wdr62 -/-*, *Wdr62 +/-; Aspm -/-*, *Aspm -/-*, *Wdr62 +/-; Aspm* +/-, and WT. The line of best fit (linear regression) is shown in black: $y = 2.3x + 210$, $R^2 = 0.9$.

Figure S4, related to Figure 4. WDR62 forms a physical complex with ASPM.
(A) Top: Two different HeLa cells in late G1 stained for endogenous WDR62 and Centrin (to label centrioles) show a ring-like staining pattern around the mother centriole, which is more prominent in appearance than the daughter centriole. Bottom: Two different U2OS cells in G1 stained for ASPM and Centrin. ASPM localizes asymmetrically to the larger and brighter (maternal) centriole.
(B) Schematic of the WDR62 'interactome' determined via IP-mass spectrometry, using WDR62-HA as bait. Gray lines connect to WDR62 interactors that meet the threshold for significance (NWD-score > 1), including ASPM. Predicted or known interactions between various interacting partners of WDR62, based on the STRING protein-protein interaction database (von Mering et al., 2005), are indicated as green dotted lines. Centrosomal-associated proteins are indicated in the blue shaded area.
(C) Wdr62 is not required for centrosomal localization of Cep152. Wild-type (WT) and *Wdr62 -/-* MEFs stained with Centrin (green), to mark centrioles, and Cep152 (red). Insets: c = Centrin and 5 = CEP152.
(D) Quantification of mean Cep152 fluorescence intensity across a fixed area, averaged across both centrosomes, with background fluorescence subtracted in WT and *Wdr62 -/-* MEFs. Mean ± SEM, ns = not significant, p = 0.07.

Figure S5, related to Figure 5. Microcephaly-associated proteins WDR62, CEP63 and ASPM assemble at the centrosome in a sequential manner
(A) CEP63 is a proximal centriole protein that is required for centriole duplication in human cells. During S-phase, CEP63 can be detected between each centriole pair in scrambled control (SC) transfected cells. The centrosomal signal is abolished using siRNA against human *CEP63*, and the majority of CEP63-depleted cells had two or three centrioles. c = Centrin and 3 = CEP63.
(B) CEP63 antibody specifically detects endogenous CEP63 (band marked with asterisk *) by Western in scrambled control (SC) transfected cells; signal decreases upon knockdown with *CEP63* siRNA.
(C) Quantification of percentage of scrambled control (SC) and *CEP63* knockdown cells with 4 centrioles. Mean ± SEM.
(D) WDR62 localization is not dependent on CEP63. Top to bottom: WDR62 localizes to centrosomes in scrambled control (SC) transfected cells, and is unperturbed in cells treated with *CEP63* siRNA. Insets: c = Centrin and w = WDR62.
(E) Centrosomal localization of ASPM is dependent on CEP152. Top to bottom: ASPM localizes to centrosomes in scrambled control (SC) transfected cells. ASPM is absent from centrosomes in cells treated with *CEP152* siRNA. Insets: c = Centrin and a = ASPM.
(F) ASPM is not required for the centrosomal localization of CEP152. Left to right: Cells were treated with scrambled control (SC) or *ASPM* siRNA (*ASPM #1*), and then stained for Centrin and CEP152. CEP152 localization at the centrosome was unchanged in ASPM-depleted cells. Insets: c = Centrin and 5 = CEP152.

Figure S6, related to Figure 7. Reduction in centrosomes and cilia in mice lacking *Wdr62* and *Aspm*.
(A) Immunohistochemistry in *Wdr62 -/-; EGFP-Centrin* transgenic mice shows reduction in centrioles *in vivo*. Immunostaining for Arl13b (to label cilia) in *Wdr62 +/-; EGFP-Centrin* mouse brain (top) reveals cilia associated with pairs of centrioles (marked with arrows) in an orderly manner along the ventricular surface. Arl13b staining in *Wdr62 -/-; EGFP-Centrin* mouse brain (bottom) shows cilia associated with odd numbers of centrioles or no centrioles (marked with arrowheads), as well as pairs of centrioles (marked with arrows). Scale bar = 2.5 μm.

(B) Arl13b+ cilia are dramatically reduced in single and double mutant cortex at E14. Top to bottom: *Wdr62 +/-* (negative control), *Wdr62 -/-* and *Wdr62 +/-; Aspm -/-* cortex stained for Arl13b (green) and counterstained with DAPI. Scale bar = 10 μm.
(C) Wdr62 is required for adequate centrosomal accumulation of γ-tubulin. Wild-type (WT) and *Wdr62 -/-* mouse embryonic fibroblasts (MEFs) stained with Centrin (green), to mark centrioles, and γ-tubulin (red). Insets: c = Centrin and T = γ-tubulin.
(D) Quantification of mean γ-tubulin immunofluorescence intensity across a fixed area surrounding the centrosome, averaged across both centrosomes, with background fluorescence subtracted. Shown here is a comparison of WT and *Wdr62 -/-* MEFs. Mean ± SEM, *** p = 0.0001.
(E) Representative electron micrographs of WT (top), *Wdr62 -/-* (middle) and *Wdr62 -/-; Aspm +/-* (bottom) brain sections at E12.5 show a gene-dose dependent paucity of centrioles and cilia in the ventricular zone of each mutant. Scale bar = 1 μm.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animals

All animal experimentation was carried out under protocols approved by the IACUC of Boston Children's Hospital. Targeted *Wdr62* gene-trap ES cells (MMRRC Cat# 021135-UCD, RRID: MMRRC_021135-UCD) from the Sanger Institute Gene Trap Resource (SIGTR) were injected into 129Sv blastocysts to generate chimeric mice. Male chimeras were bred to WT 129Sv background females (Charles River) to transmit the gene-trap allele. The mice were later outcrossed to C57Bl/6 background females (Charles River) and are maintained separately on 129Sv and C57Bl/6 backgrounds, having been back-crossed for at least 5 generations.

Aspm gene targeting was carried out commercially by InGenious Targeting Laboratory, Inc using a targeting vector, in which a Neo cassette with 1.5-kb and 8.0-kb homology arms replaced exons 1-3 of *Aspm*. This targeting vector, which was generated by the authors, was first electroporated into 129/Sv embryonic stem (ES) cells, followed by selection in G418. Surviving clones were expanded for PCR analysis to identify recombinant ES clones, which were then injected into C57Bl/6 blastocysts. Male chimeras were then bred with wild-type C57Bl/6 females to ensure germline transmission.

Wdr62 het mice and *Aspm* het mice were bred to generate *Wdr62* +/-; *Aspm* +/- (trans het) mice; these trans het animals were subsequently cross-bred to each other to generate double mutant (*Wdr62 +/-; Aspm -/-*) mice along with control, *Wdr62 -/-* and *Aspm -/-* littermates for comparison.

Transgenic *EGFP-Centrin2* mice, henceforth referred to as *EGFP-Centrin*, were obtained from Jackson Laboratory (JAX strain name CB6-Tg(CAG-EGFP/CETN2)3-4Jgg/J; IMSR Cat# JAX:008234, RRID:IMSR_JAX:008234) by rederivation from cryopreserved sperm. The *EGFP-Centrin* transgene was designed with cytomegalovirus immediate early enhancer/ chicken β-actin promoter and intron (CAG promoter) upstream of an EGFP-Centrin 2 fusion protein (containing the full length human *Centrin*-2 cDNA cloned in-frame into the C-terminus of Enhanced Green Fluorescent Protein), and followed by a β-globin polyA sequence. Therefore, the transgenic mice express Enhanced Green Fluorescent Protein-labeled human *Centrin-2* driven by the CAG promoter (Higginbotham et al., 2004). After rederivation, transgenic mice were crossed to *Wdr62* het mice to generate *EGFP-Centrin; Wdr62 +/-* mice, which were then cross-bred to each other to generate *EGFP-Centrin; Wdr62 -/-* mice and transgenic control (*EGFP-Centrin; Wdr62 +/-* and *EGFP-Centrin; +/+*) littermates for comparison.

For histological analysis, brains were perfused with PBS followed by 4% paraformaldehyde (PFA) in PBS. Fixed brains underwent either paraffin embedding and sectioning at 5 μm, or embedding in 4% agarose in PBS and vibratome sectioning at 50-100 μm. Genotyping primers are available upon request.

Cell Culture

Primary mouse embryonic fibroblasts (MEFs) were isolated from E14.5 embryos and dissociated by trypsinization. To establish immortalized cell lines, MEFs were derived from *Wdr62* and *Aspm* single- and double-mutant embryos at E9.5 or E14.5 and transformed with SV-40 retrovirus using the Phoenix packaging system (RRID:SCR_003163). Both primary and immortalized MEFs were maintained in Dulbecco's Modified Eagle Medium, High Glucose (HyClone) with 15% fetal bovine serum (FBS, Gibco) and 1mM penicillin, streptomycin and L-glutamine.

HeLa (UCSF Cell Culture Facility, RRID:CVCL_0030) and U2OS cells (ATCC Cat# HTB-96, RRID:CVCL_0042) were grown in Dulbecco's Modified Eagle Medium, High Glucose (HyClone) with 10% fetal bovine serum (FBS, Gibco), 1mM penicillin, streptomycin and L-glutamine. For siRNA transfections, HeLa and U2OS cells (UCSF tissue culture facility) were cultured in Advanced DMEM (Invitrogen) supplemented with 2% FBS (Invitrogen) and Glutamax-I (Invitrogen). Cells were transfected with siRNA using Oligofectamine (Invitrogen) according to the manufacturer's instructions and analyzed 48 hrs later. Human Stealth siRNAs to *WDR62* (Cat# HSS138565), *CEP63* (Cat# HSS188410), *ASPM* (Cat# HSS138111) and *CPAP* were obtained from Life Technologies. *CEP152* siRNAs were synthesized by Life Technologies. Sequences for the siRNA oligonucleotides (5' to 3') used in this study are described below.

CEP152 #1: CAGCUCUUUGAGGCUUAUGAG (Cizmecioglu et al., 2010)
CEP152 #2: GCGGAUCCAACUGGAAAUCUA (Cizmecioglu et al., 2010)
WDR62 #1: CCAACUGCAUGAAGCAGCACUUGCU
WDR62 #2: AGCAAGUGCUGCUUCAUGCAGUUGG
CEP63 #1: CAGGGAAGAUCGGUCUGAAAUUGAG
CEP63 #2: CUCAAUUUCAGACCGAUCUUCCCUG
ASPM #1: GGAGAGAGAGAAAGCUGCAAGAAUU

ASPM #2: AAUUCUUGCAGCUUUCUCUCUCUCC
Scrambled control: AAACTAAACTGAGGCAATGCC

Quantitative RT-PCR

For RT-PCR, RNA was harvested using RNeasy Mini Kit (Qiagen). DNA was removed using TURBO DNA-free DNase kit (Ambion) and then 500 ng of RNA was used to synthesize cDNA using Superscript III First Strand Synthesis System for RT-PCR (Invitrogen). qPCR was carried out using KAPA SYBR Green (2X) master mix (Kapa Biosystems) and the following primers:

Forward (*Wdr62* exon 21): 5' – GCTGACAAATGGCAAGCTG – 3'
Reverse (*Wdr62* exon 22): 5' – GATGGTCTTGAGGGGTTCCT – 3'

Western blotting

To detect endogenous WDR62 or WDR62-HA, lysate was prepared using either lysis buffer containing 0.5% NP-40, 150 mM NaCl, 200 mM Tris pH 8 and 1X Dulbecco's Phosphate Buffered Saline (DBPS), or IP buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Triton X-100; see (Caspi et al., 2000), to which Halt protease/ phosphatase inhibitor (Thermo Scientific) and cOmplete mini EDTA-free PI tablets (Roche) were added. Protein concentrations were normalized using the bicinchoninic acid (BCA) assay (Thermo Scientific). Samples were diluted in 4X NuPAGE LDS sample buffer (Life Technologies) and were not boiled but heated to 50°C for 5 min. Extracted proteins were separated by electrophoresis on NuPAGE Novex Bis-Tris (4-12%) polyacrylamide gels (Life Technologies), followed by overnight transfer at 4°C onto polyvinylidene difluoride (PVDF) membrane (Millipore).

For quantitative immunoblotting, PVDF membranes were blocked for 1 hr at room temperature in Odyssey Blocking Buffer (LI-COR Biosciences), incubated with primary antibodies for ~1.5 hrs at room temperature or O/N at 4°C, and then incubated with fluorescent-dye-conjugated secondary antibodies (LI-COR Biosciences). Bands were detected, normalized and quantified with the Odyssey Infrared Imaging System (LI-COR Biosciences).

To detect endogenous WDR62, CEP63 and ASPM after immunoprecipitation, samples were incubated in 2x Laemmli reducing buffer but were not boiled. Samples were separated on 4-15% gradient TGX precast gels (Bio-Rad), transferred onto Protran nitrocellulose membrane (Whatman) and then subjected to immunoblot analysis using ECL Lightening Plus (Perkin-Elmer).

Antibodies: ASPM, human-specific, VTKR epitope (kind gift of Victor Larionov); Aspm, mouse-specific, 1:1,000 (Bethyl Laboratories Cat# IHC-00058, RRID:AB_2060292); β-actin, clone AC-15, 1:10,000 (Abcam Cat# ab6276, RRID:AB_2223210); CEP63, 1:1000 (Millipore Cat# 06-1292, RRID:AB_10918481); HA, clone 3F10 1:3000 (Roche Cat# 11867431001, RRID:AB_390919); WDR62, human-specific, 1:3000 (Bethyl Laboratories Cat# A301-560A, RRID:AB_1040044); Wdr62 C-term, mouse-specific (Bethyl Laboratories, generated by the authors), and c-Myc (Santa Cruz Biotechnology Cat# sc-764, RRID:AB_631276).

To generate more ASPM antibody for IP and WB, a synthetic peptide consisting of amino acids 497–520 (VTKRKATCTRENQTEINKPKAKR) from the ASPM sequence was conjugated to Keyhole Limpet Hemocyanin (KLH) and used to immunogenize rabbits (Cocalico Biologicals) as described previously (Kouprina et al., 2005).

To generate antibody against the C-terminus of mouse Wdr62 protein for WB, a synthetic peptide comprising amino acids 1513-1524 (LVQAVRRKARGD) from the RefSeq protein sequence (NP_666298) was conjugated to KLH and injected into rabbits. The resulting antisera then underwent affinity purification.

Mass spectrometry (WDR62-HA overexpression)

Lentivirus carrying a C-terminally tagged WDR62-HA construct (Yu et al., 2010) was generated and used to infect HeLa S3 cells, which subsequently underwent puromycin selection to generate a cell line stably expressing WDR62-HA (confirmed by Western blot). Cell pellets were lysed in MCLB lysis buffer (50 mM Tris pH 7.8, 150 mM NaCl, 0.5% NP40) containing cOmplete mini EDTA-free protease inhibitor tablets (Roche). Lysates were incubated with HA-agarose beads (Sigma Cat# A2095, RRID:AB_257974) at 4°C overnight. The beads (and bound immunocomplexes) were washed several times in lysis buffer and PBS, and then eluted with HA peptide. Following TCA precipitation and trypsin digest, the peptides were purified using StageTips (Rappsilber et al., 2007) and then underwent LC-MS/MS, using an LTQ linear ion trap mass spectrometer (ThermoFinnigan). Methods, including data analysis, were carried out as previously described (Sowa et al., 2009). Data were deposited at http://www.peptideatlas.org/PASS/PASS00927 (PeptideAtlas: PASS00927).

Immunoprecipitation (endogenous)

Asynchronous HeLa cells (UCSF Cell Culture Facility, RRID:CVCL_0030) were incubated on ice for 5 min in chilled Dulbecco's PBS (DPBS, Invitrogen) lacking Ca^{2+} and Mg^{2+}, harvested using a cell scraper and lysed on ice for 10 min in lysis buffer (50mM Tris-HCl pH7.4, 266mM NaCl, 2.27mM KCl, 1.25mM KH_2PO_4, 6.8mM $Na_2HPO_4{}^-7H_2O$ and 1% NP-40) supplemented with protease and phosphatase inhibitors (Calbiochem and Thermo Fisher Scientific Inc.). Lysates were clarified at 13,000 rpm for 10 min at 4°C and the Bradford assay (Bio-Rad) was used to quantify protein concentrations. 500 µg of total lysate was incubated with 2 µg of antibody for 2 hrs and then incubated with protein G-Sepharose for an additional 1 hr at 4°C. Immunocomplexes were washed 3x in lysis buffer and subsequently boiled in 2x Laemmli reducing buffer.

Immunofluorescence

To visualize centrosomal proteins, cells were fixed in 100% chilled methanol at -20°C for 3 min. Following fixation, cells were incubated in blocking buffer consisting of 2.5% BSA, 0.1% Triton-X-100, 0.03% NaN_3 in Dulbecco's Phosphate Buffered Saline (DPBS) overnight at 4°C. Primary and secondary antibodies were diluted in blocking buffer and incubated with cells at room temperature for 1 h. Coverslips were mounted using Gelvatol or Prolong Gold (Life Technologies) mounting media. Fluorescence measurements were made with Image J software, by recording the mean fluorescence intensity in a circle centered on the centrosome, with the area kept constant.

For immunohistochemistry at postnatal ages (P10-P30), pups were perfused with PBS followed by 4% PFA in PBS. Brains were then dissected out, drop-fixed in 4% PFA for 16hrs at 4°C, washed in PBS and embedded in 4% agarose in PBS for vibratome sectioning at 50 µm. Matched sections were blocked overnight at 4°C in 0.03% BSA, 0.3% Triton-X-100, 0.1% sodium azide in 1X PBS and then incubated in primary antibody in blocking buffer at 4°C for 48-72 hrs. Sections were washed with PBS (3 x 10 min) and then incubated in secondary antibody in blocking buffer at 4°C overnight. Sections were washed again with PBS (3 x 10 min) before mounting with Fluoromount-G (Southern Biotech) mounting media.

For immunohistochemistry at E14.5, embryos were either drop fixed in 4% PFA overnight at 4°C or perfused with PBS followed by 4% PFA in PBS. Paraffin sections (5 µm) of brains were rehydrated and subjected to antigen retrieval with Retrievagen A (BD Pharmingen), followed by blocking (5% normal donkey serum in PBS), permeabilization (0.1% Triton X-100), and antibody incubation overnight at 4°C.

For staining Arl13b and γ-tubulin in E12.5 brains, embryos were drop-fixed in 4% PFA overnight at 4°C. The brains were dissected out, washed in PBS and embedded in 3% agarose in PBS for vibratome sectioning at 50 µm. Matched sections were then co-stained for Arl13b and γ-tubulin as previously described (Paridaen et al., 2013). Sections were washed with 0.3% Triton-X-100 in PBS (2 x 5 min); permeabilized with 0.3% Triton-X-100 in PBS (30 min); quenched in 0.1M Glycine, pH 7.4 (30 min) and then washed with TxBuffer (3 times for a total of 10 minutes). Sections were incubated in primary antibody in TxBuffer (PBS, 0.2% gelatin, 300mM NaCl, 0.3% Triton-X-100) overnight at 4°C and then washed with TxBuffer (5 x 5 min). Sections were incubated in secondary antibody in TxBuffer (1 hour at RT); then washed with TxBuffer (5 x 5 min) and washed in PBS (3 x 1 min), before mounting with Fluoromount-G (Southern Biotech) mounting media. A similar protocol was used for staining Arl13b in PFA-fixed *EGFP-Centrin* transgenic brains.

For 3D mitotic spindle orientation (*en face*) analysis, the brains of E13.5 embryos were dissected out, washed in PBS and drop-fixed in 100% methanol at -20°C. The hemispheres were separated; the cerebellum, midbrain and hindbrain were removed, and a cut was made separating the medial-most part of the cortex from the rest of the cortex. The samples were then stained for γ-tubulin and α-tubulin. Samples were permeabilized for 15 minutes in PBS + 1%Triton X, and then blocked for 1 hour in PBS + 0.3% Triton X + 3% bovine serum albumin (BSA) + 1% donkey serum (DS). Samples were incubated in primary antibody in PBS + 0.3% Triton X + 3% BSA + 1% DS at room temperature overnight and then washed in PBS + 0.3% Triton X + 3% BSA + 1% DS (2 x 15 minutes, then 2 x 1 hour). Samples were incubated in secondary antibody and counterstained in Hoescht (1:1000) in PBS + 0.3% Triton X + 3% BSA + 1% DS at room temperature overnight and washed in PBS + 0.3% Triton X + 3% BSA +1% DS (2 x 15 minutes, then 2 x 1 hour). The ventral forebrain structures (including the ganglionic eminences) were dissected away, keeping only the lateral part of the cortex with the olfactory bulb for mounting with Prolong R Gold anti-fade reagent (Life Technology) mounting media.

All samples were counterstained with Hoechst 33342 (Sigma). Images were acquired with a Zeiss LSM 700 on an upright Axio Imager M2 equipped with 405, 488, 555 and 639 nm solid-state lasers, a motorized stage and a CCD AxioCam MR or an inverted Zeiss Axio Observer D1.

Antibodies: Active Caspase 3 (Abcam Cat# ab13847, RRID: AB_443014), 1:500, PFA; aPKCζ (Santa Cruz Cat# sc-216, RRID:AB_2300359); Arl13b (Abcam Cat# ab83879, RRID:AB_2060868), 1:500, PFA; ASPM (Santa Cruz Cat# sc-98903, RRID:AB_2258686; Bethyl Laboratories Cat# A300-BL2048, available from Bethyl

upon request, RRID not available), 1:50, MeOH; CENPJ/CPAP (Proteintech Cat# 11517-1-AP, RRID:AB_2244605; Bethyl Cat# A302-986A, RRID:AB_10752588), 1:1000, MeOH; Centrin, clone 20H5 (Millipore Cat# 04-1624, RRID:AB_10563501), 1:1000, MeOH; CEP63 (Millipore Cat# 06-1292, RRID:AB_10918481; Proteintech Cat# 16268-1-AP, RRID:AB_2077079), 1:1000, MeOH; CEP152 (Bethyl Cat# A302-479A, RRID:AB_1966085), 1:2000, MeOH; CEP164 (Santa Cruz Cat# sc-240226, RRID:AB_10841981); Cux1, M-222 (Santa Cruz Cat# sc-13024, RRID:AB_2261231), 1:500, PFA; Ctip2 (Abcam Cat# ab18465, RRID:AB_2064130), 1:250, PFA; Cyclin A, clone H-432 (Santa Cruz Cat# sc-751, RRID:AB_631329), 1:200, MeOH; HA, clone 3F10 (Roche Cat# 11867431001, RRID:AB_390919), 1:500, MeOH; Ki67 (BD Pharmingen Cat# 550609, RRID:AB_393778), 1:1000, PFA; NeuN, clone A60 (Millipore Cat# MAB377, RRID:AB_2298772), 1:250, PFA; Pals1 (Proteintech Cat#17710-1-AP, RRID:AB_2282012); Sox2 (Santa Cruz Cat# sc-17320, RRID:AB_2286684), 1:250, PFA; Tbr1 (Abcam Cat# ab31940, RRID:AB_2200219), 1:500; PFA; Tbr2 (Millipore Cat# AB15894, RRID:AB_10615604, 1:250, PFA; Abcam Cat# ab23345, RRID:AB_778267, 1:500, PFA); α-tubulin (Abcam Cat# ab18251, RRID:AB_2210057), rabbit, 1:600; γ-tubulin (Sigma-Aldrich Cat# T3559, RRID:AB_477575, 1:200, MeOH; Cat# T5192, RRID:AB_261690, 1:250, PFA; clone GTU-88, Cat# T5326, RRID:AB_532292, 1:500, MeOH, *en face*); WDR62 (Bethyl Laboratories Cat# A301-560A, RRID:AB_1040044; Cat# A301-559A, RRID:AB_1040043), 1:50, MeOH. Alexa-conjugated secondary antibodies were obtained from Molecular Probes (Life Technologies) and Jackson ImmunoResearch Laboratories. Hoechst 33342 was obtained from Thermo Fisher Scientific (Cat# 62249).

Electron Microscopy

Cells were grown on Aclar coverslips and fixed overnight at 4°C in a 1:1 dilution of culture media to 5% glutaraldehyde, 2.5% paraformaldehyde, 0.06 % picric acid in 0.2M sodium cacodylate buffer (pH 7.4). Fixed cells were washed 3x in 0.1M cacodylate buffer, then post-fixed in 1% osmium tetroxide (OsO4)/1.5% potassium ferrocyanide (KFeCN6) for 30 min. Cells were then washed in water 3x and incubated in 1% aqueous uranyl acetate for 30 min, followed by 2 washes in water and subsequent dehydration in grades of alcohol (5 min each; 50%, 70%, 95%, 2x 100%). Cells were embedded in TAAB Epon (Marivac Canada Inc. St. Laurent, Canada) and polymerized at 60°C for 48 hrs. After polymerization, the Aclar coverslip was peeled off and 1mm squares of the embedded monolayer were glued onto an empty Epon block for sectioning.

To do transmission electron microscopy (TEM) for morphology, mice were perfused with 2.5% glutaraldehyde/2%PFA in 0.1M sodium cacodylate buffer pH 7.4 (Electron Microscopy Sciences); the brains were dissected and post-fixed in the same fixative overnight. Fixed brains underwent vibratome sectioning at a thickness of 100 µm. Sections were washed in 0.1 M cacodylate buffer and postfixed with 1% osmium tetroxide (OsO4)/1.5% potassium ferrocyanide (KFeCN6) for 1 hour. Then sections were washed in water 3x and incubated in 1% aqueous uranyl acetate for 1 hour, followed by 2 washes in water and subsequent dehydration in grades of alcohol (10 min each; 50%, 70%, 90%, 2x 10 min 100%). The samples were then infiltrated for 15 min in a 1:1 mixture of propylene oxide and TAAB Epon (Marivac Canada Inc. St. Laurent, Canada). The samples were embedded in drops of TAAB Epon between 2 sheets of Aclar plastic (Electron Microscopy Sciences) and polymerized at 60°C for 48 hours.

Ultrathin sections (about 80 nm) were cut on a Reichert Ultracut-S microtome, placed onto copper grids, stained with lead citrate (along with uranyl acetate for brain sections) and examined in a JEOL 1200EX transmission electron microscope. Images were recorded with an AMT 2k CCD camera at 2000x and 10000x.

Statistical Analyses

All analyses were performed using at least 3 different animals of each genotype, or 3 replicates for cell lines. Data are represented as mean ± SEM. Two-tailed p-values are generally reported. Fisher's exact test was used for categorical data (i.e., the % of cells with <4 centrosomes versus 4 centrosomes) in Figure 3. Images were processed with Adobe Photoshop software (https://www.adobe.com/products/photoshop.html?promoid=KLXLS, RRID:SCR_014199), and quantified using ImageJ software (http://rsb.info.nih.gov/ij/index.html, RRID:SCR_003070) where applicable. All figures were prepared using Adobe Illustrator software (http://www.adobe.com/products/illustrator.html, RRID:SCR_010279).

For Arl13b / γ-tubulin immunostaining in E12.5 brains, we adhered to the data analysis in Paridaen et al., *Cell* (2013) as faithfully as possible. We only counted mitotic apical progenitors and used 3D projections (max intensity projections of z-stacks at 60X magnification) to ensure that centrosomes in different focal planes were included.

Mitotic spindle orientation (*en face*) analysis was carried out in 3D using a previously developed algorithm to calculate mitotic spindle angles from confocal image stacks (Juschke et al., 2014). While this angle would conventionally be defined in relation to the ventricular surface of the developing mouse neocortex, this surface is not

perfectly flat, so we calculated a reference plane from the experimental data by choosing 5 points on the original surface in the confocal stack and determining the plane of best fit by regression analysis. We used Imaris software (Bitplane, http://www.bitplane.com/imaris/imaris, RRID:SCR_007370) to automate this process, using the "3D Crop" tool to make a 3D reconstruction, the "Ortho Slicer" tool to scroll up and down the z-axis and visualize all the mitotic centrosomes within the stack, the "Spots" tool to mark anaphase centrosomes, and the "statistics" tool to save the 3D coordinates of the centrosomes into Excel, where the spindle angle for each cell can be calculated relative to the reference plane.

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