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Supplemental Data

Cep120 and TACCs Control Interkinetic Nuclear Migration and the Neural Progenitor Pool

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Supplemental Experimental Procedures

Primers for RT-PCR

The primers used for RT-PCR include: Cep120-2f (5' gtcgaccatactgaccagcc 3') and Cep120-2r (5' cttcctcattcaatactgcaac 3') for Cep120; T1-f (5' cggaattctatggcgttcagtccctgg cagat 3') and T1-r (5' ggggtacc tcagtcggtctttcccagctttg 3') for TACC1; T2A-f (5' gaagatctatgggcaacgagaacagcacct 3') and T2-5'r (5' ggagtgtcaaacataaggtacaag 3') for 5' portion of TACC2A; T2B-f (5' gaagatctatgggagggtcgcagtccctgca 3') and T2-5'r for 5' portion of TACC2B; T2-3'f (5' ttcaccctcagaggagctggacta 3') and T2-3'r (5' cggaattcttagc tttttcccatcttggcgatca 3') for 3' portion of both TACC2A and TACC2B; T3-f (5' cggaattca tgagtctgcatgtcttaaatgac) and T3-r (5' tcccccgggtcagatcttct ccatcttagagat 3') for TACC3.

Plasmids

The mouse Cep120 sequence (mCep120) was derived from the IMAGE clone 6409319 (GenBank accession no.: BU056646). To construct a plasmid for expression of EGFP-Cep120 (pEGFP-Cep120), mCep120 (CDS and partial 3'UTR) was inserted into Bgl II/EcoR I sites of pEGFPc1 in-frame with EGFP. The plasmid for the expression of HA-tagged Cep120 (pCep120-HA) was constructed by inserting mCep120 into Bgl II/EcoR I

sites of pDsRed1-N1 and swapping the EcoR I/Not I fragment containing DsRed1 sequence with a double-HA-tag. To construct a plasmid for the expression of non-tagged Cep120 (pCep120), the Xho I/EcoR I fragment of pCep120-HA was replaced by the Xho I/EcoR I fragment of pEGFP-Cep120 so that a stop codon was placed before the HA-tag. EGFP-TACC plasmids were generated by inserting full-length TACC1s, TACC2B, and TACC3 derived from RT-PCR into EcoR I/Kpn I, Bgl II/EcoR I, and EcoR I/Sma I sites, respectively, of pEGFPc1. To express triple-HA-tagged TACC proteins, the Nhe I/Bgl II fragment (EGFP sequence) of EGFP-TACC plasmids was replaced by an Xba I/Bgl II fragment containing a kozak sequence followed by a triple-HA-tag. For acute brain slice imaging experiments, a plasmid for high-level expression of Venus was constructed by inserting Venus coding sequence into the Xho I/EcoRV sites of pCAGIG. For the expression of an siRNA-resistant silent mutant of Cep120, the Cep120 i2968 targeting sequence "ataaccatgaggaccgcataa" in pCep120 was mutated to "ataatcacgaagatcgtatca" by site-directed mutagenesis. These mutations do not alter amino acid coding.

Antibodies

In addition to Cep120 and Cdk5rap2 antibodies, the following antibodies were used in this study: anti-HA (F-7) (Santa Cruz Biotechnology), anti-pericentrin (BD Transduction Laboratories), anti-α tubulin (Sigma), Tuj1 (Covance), anti-nestin (BD Bioscience); anti-phospho-H3 (Upstate Biotechnology), anti-BrdU (Sigma), anti-Ki67 (Novocastra); rabbit anti-GFP (Molecular Probes), chicken anti-GFP (Aves labs), and goat anti-Brn1 (Santa Cruz). The rabbit anti-GFP antibody was used to detect EGFP-TACC3 in brain sections and to amplify Venus signal in experiments measuring the ratio of nuclear length to width. The chicken anti-GFP antibody was used to identify Venus-expressing cells in

experiments measuring cell cycle exit index and in experiments measuring nucleus-centrosome distance in transfected neural progenitors.

Immunocytochemistry and Immunohistochemistry

Cultured cells were fixed in 4% paraformaldehyde (prepared in PBS) at room temperature for 5 min before immunostaining. To prepare neocortical sections from E13.5 or older embryos, forebrain hemispheres of mouse embryos were dissected out and the hippocampi were partially removed to expose the lateral ventricles. The forebrain hemispheres were then fixed in 4% paraformaldehyde for 20 min at room temperature with occasional rocking. The fixed forebrain hemispheres were soaked in 20% sucrose (prepared in PBS) for 1-2 h at 4°C before embedded in OCT compound. Cryosections of 40-μm were prepared from embryos electroporated with EGFP-TACC3/RFP-CETN2. Cryosections of 12-μm were used in other immunostaining experiments. To detect BrdU, cryosections were treated with hydrochloric acid (2N) for 3 h at room temperature prior to the immunostaining procedure.

Quantification of Tuj1-Positive Cells in Brain Sections

Confocal images were obtained from Tuj1-immunostained cryosections at comparable transfected neocortical regions and analyzed in LSM Image Browser (Zeiss). At high magnification, essentially all Venus-positive cells in the cortical plate and the upper intermediate zone were found to be Tuj1-positive. Thus during quantification we scored all Venus-positive cells in the cortical plate and the upper intermediate zone as Tuj1-positive. We then focus on the cells in the lower intermediate zone and the

subventricular/ventricular zone. To determine whether a cell in these layers is positive for Tuj1, we not only looked for overlapping yellow color, but also turned off either the red (Tuj1) channel or the green (Venus) channel to verify whether there was overlapping signal. The background staining of all the sections used for quantification was adjusted to similar levels.

Supplemental Figures: Figures S1-S10

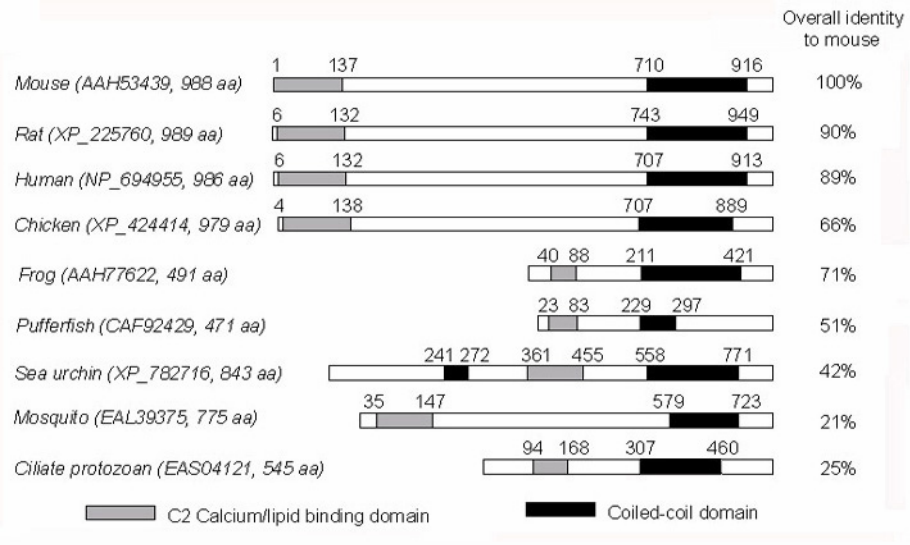

Figure S1. Homology and domain structure of Cep120 orthologs. The C2 calcium/lipid binding domain and the coiled-coil domain are predicted by InterProScan and Lupas's method, respectively.

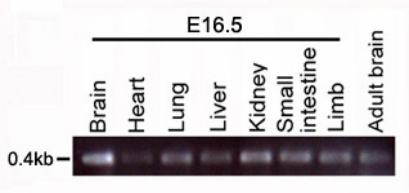

Figure S2. Detection of Cep120 mRNA in different embryonic mouse tissues by RT-PCR. The primers anneal to exon 3 and exon 6 of the Cep120 gene. The expected size of the RT-PCR product is 0.4kb.

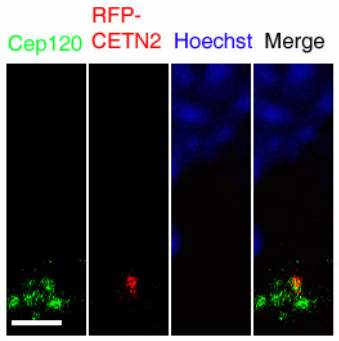

Figure S3. RFP-CETN2, a centrosomal marker, in a transfected neural progenitor co-localizes with Cep120 at the ventricular surface (bottom). A plasmid for the expression of RFP-CETN2 was introduced into neocortical progenitors via in utero electroporation at E11.5 and brains of electroporated embryos were collected at E12.5. Cryosections of the brains were immunostained with the Cep120 antibody and examined under confocal microscopy. Scale bar: 5 μm.

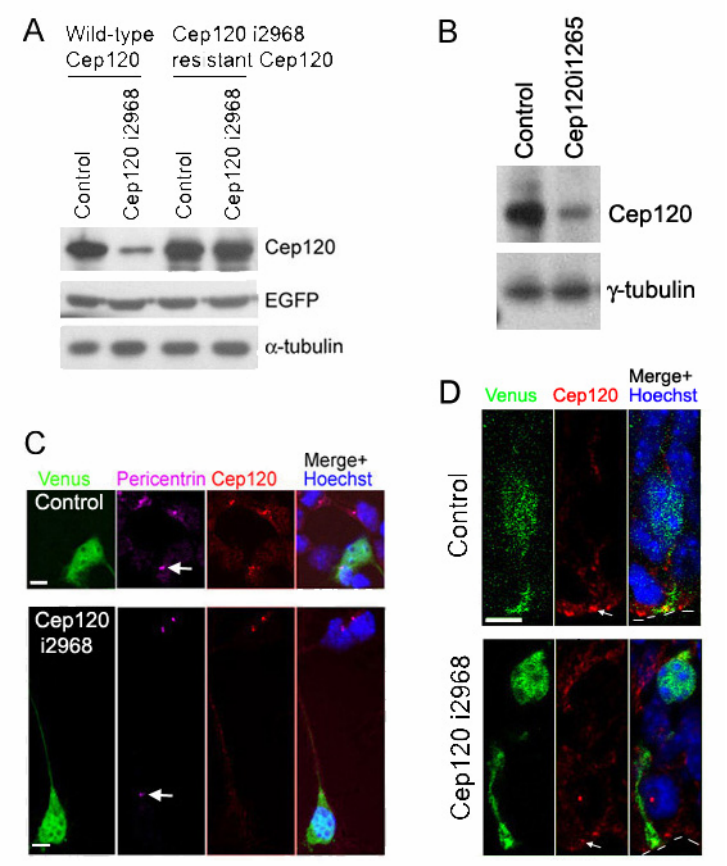

Figure S4. The pSilencer-based Cep120 siRNA plasmids knock down mouse Cep120 *in vitro* (A-C) and *in vivo* (D). (A) Immunoblot of lysates from co-transfected COS7 cells. Cep120 i2968 down-regulates the expression of exogenous wild-type Cep120, but not EGFP or an siRNA-resistant mutant of Cep120. A pSilencer vector containing a random sequence hairpin insert was used as a control for Cep120 i2968. (B) Immunoblot of lysates from CAD cells transfected with Cep120 i1265 or a random sequence control. Lysates were prepared 46 h after transfection. Cep120 i1265 markedly decreased the expression of endogenous Cep120. (C) Cultured neocortical cells were co-transfected with a Venus plasmid and Cep120 i2968 one day after plating and immunostained two days after transfection. Cep120 i2968 generally decreased Cep120 immunostaining of the centrosome. Arrows point to the centrosome labeled by the pericentrin antibody in

transfected cells. (D) Mouse embryos were co-electroporated with a Venus plasmid and Cep120 i2968 at E11.5 and allowed to survive in utero for two days. Cryosections of the brain were then obtained for immunostaining. Cep120 i2968, but not the control siRNA, decreased Cep120 immunostaining of the centrosome (indicated by arrows) at the ventricular surface (indicated by dashed lines). Scale bars: 5μm.

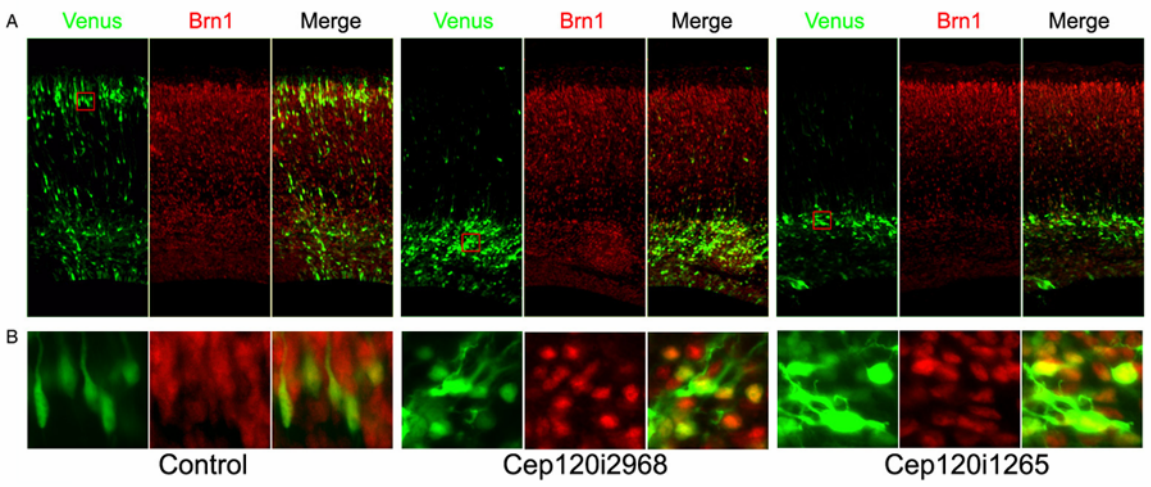

Figure S5. Silencing Cep120 results in mispositioning of neurons. Mouse embryos were electroporated with Venus and siRNA plasmids at E14.5 and sacrificed at E18.5. Cryosections of brains from electroporated embryos were immunostained with an antibody against Brn1, a marker for late born neurons. Boxed areas in (A) were shown at higher magnification in (B). Compared to the control, Cep120 i2968 and Cep120 i1265 groups exhibit marked accumulation of Brn1-positive neurons at deep layers of the neocortex. Similar areas of the neocortex from different embryos were compared. These images are representative of three embryos for each group. Scale bars: 50 μm for (A) and 10 μm for (B).

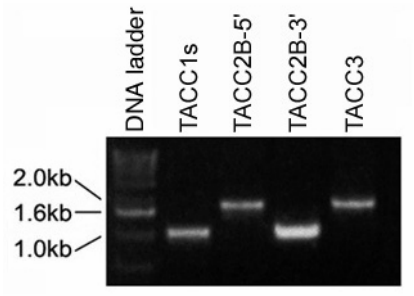

Figure S6. The amplification of TACC1s, TACC2B, and TACC3 from the mouse embryonic brain by RT-PCR.

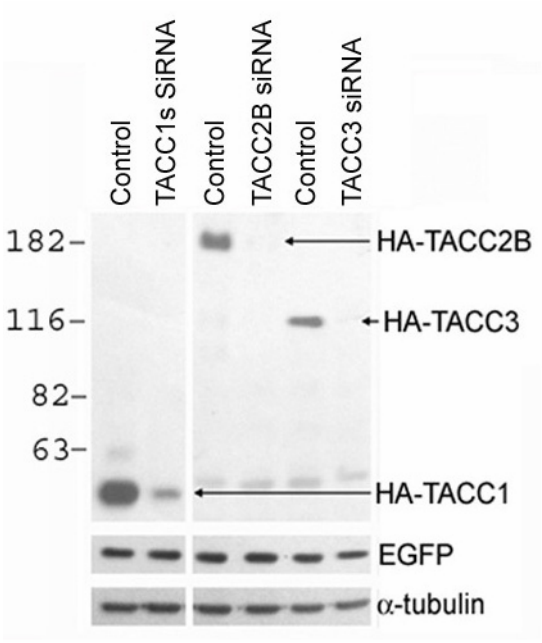

Figure S7. The expression of HA-tagged TACCs and the inhibition of their expression by pSilencer-based siRNA plasmids. Lysates from transfected COS7 cells were resolved by SDS-PAGE and subjected to immunoblot analysis. TACC siRNA plasmids inhibit the expression of HA-tagged TACCs, but not EGFP.

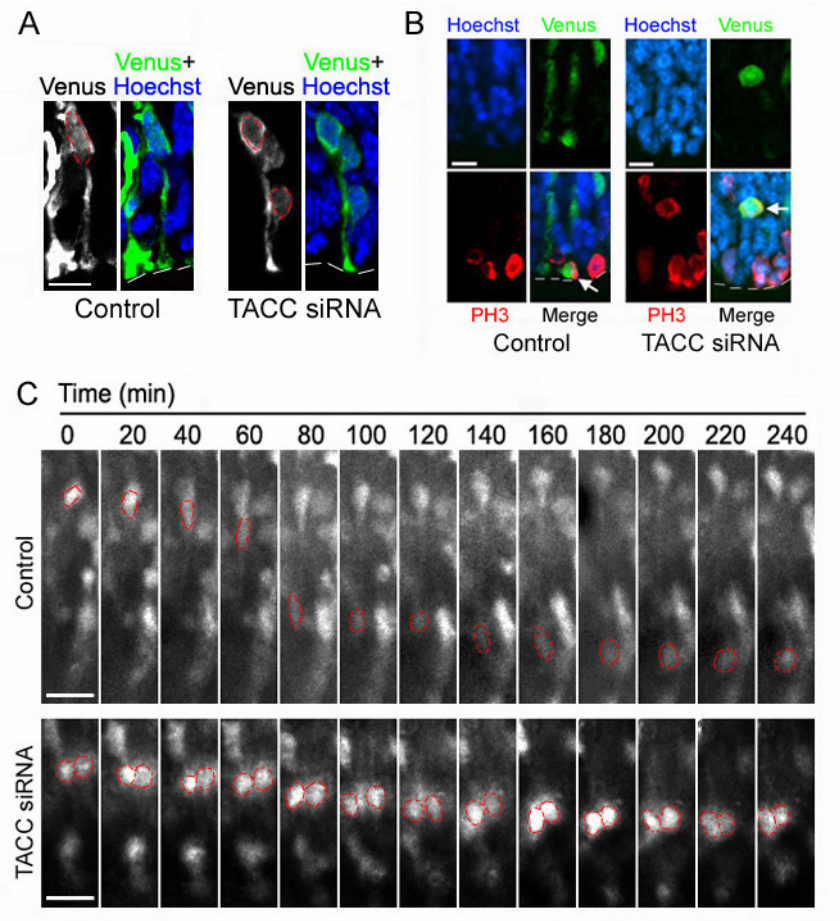

Figure S8. TACC siRNA impairs INM. (A) Representative images showing less elongated nuclei in TACC siRNA-expressing neural progenitors compared to the control. Mouse embryos were electroporated at E11.5 and sacrificed at E14.5, and the nuclei (outlined by red dashed lines) in transfected cells were visualized by Hoechst and anti-GFP staining. The ventricular surface is indicated by white dashed lines. (B) Representative images showing an ectopic PH3-positive nucleus (indicated by an arrow) in a neural progenitor expressing TACC siRNA. Mouse embryos were electroporated at E11.5 and sacrificed at E13.5. The ventricular surface is indicated by white dashed lines. (C) Representative image series showing that TACC siRNA impairs INM in brain slice preparations. Mouse embryos were electroporated at E11.5 and acute brain slices of 150-

μm were prepared at E13.5 for imaging. Examples of nuclei migrating toward the ventricle (bottom of the images) were outlined by red dashed lines. Scale bars: 10 μm in (A) and (B), 20 μm in (C).

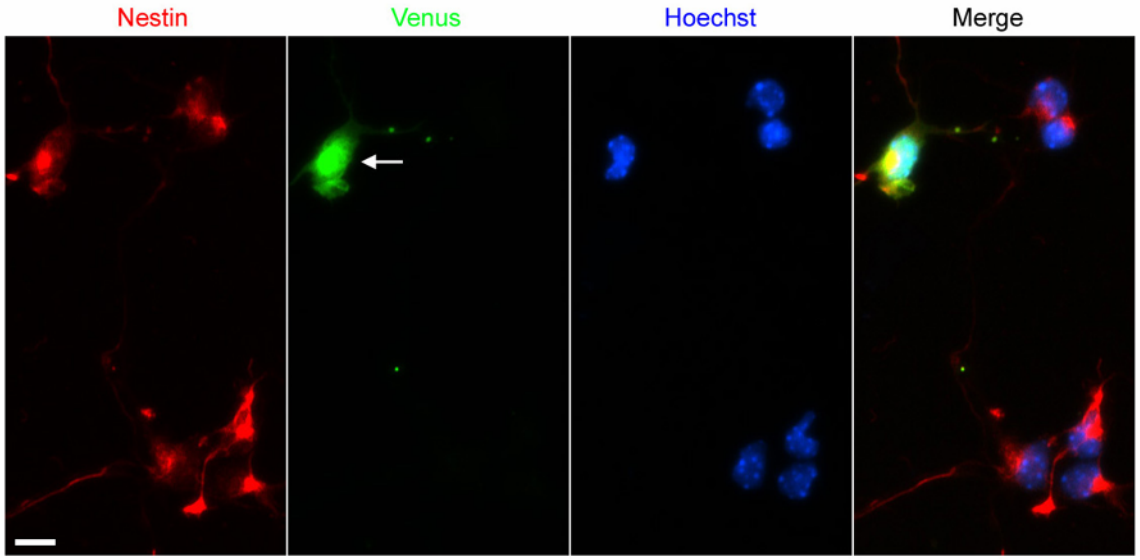

Figure S9. Immunostaining of dissociated neocortical cells using an anti-nestin antibody. Neocortical cells from electroporated mouse embryos were maintained in explant cultures for two days and then dissociated and cultured overnight before fixation (see Experimental Procedures). These cells are generally positive for Nestin. The arrow indicates a transfected cell expressing Venus. Scale bar: 10 μm.

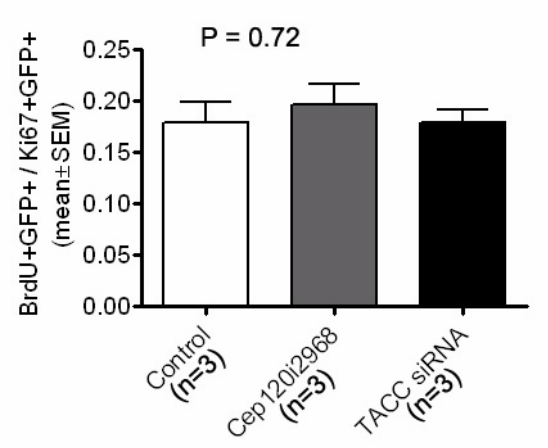

Figure S10. Cep120 i2968 and TACC siRNA do not affect cell cycle length in neural progenitors. Mouse embryos were electroporated at E11.5. BrdU was injected into the dams intraperitoneally at E13.5 and embryos were sacrificed 2 h after BrdU injection. The ratio of the number of BrdU-positive transfected cells to the number of Ki67-positive transfected cells was used to compare cell cycle length. This ratio is not significantly different among control, Cep120 i2968, and TACC siRNA groups (P=0.72, one-way ANOVA).