SUPPLEMENTARY INFORMATION FOR:

DOCK7 interacts with TACC3 to regulate interkinetic nuclear migration and cortical neurogenesis

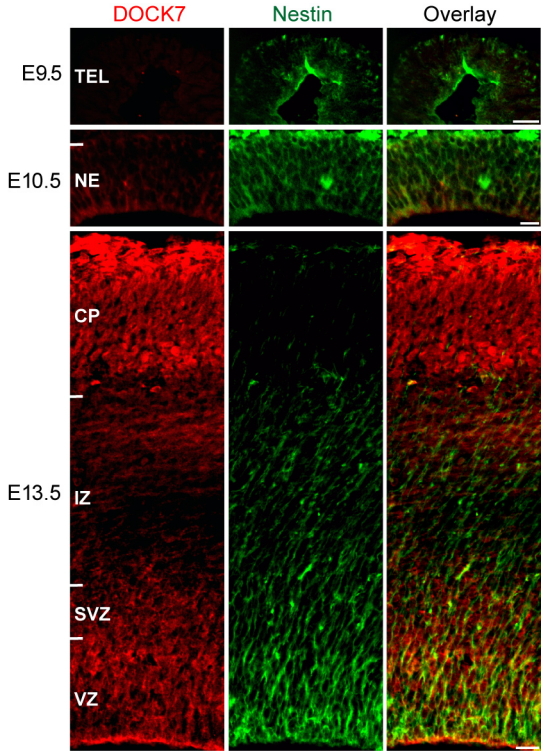
Yu-Ting Yang, Chia-Lin Wang & Linda Van Aelst

Twelve Supplementary Figures

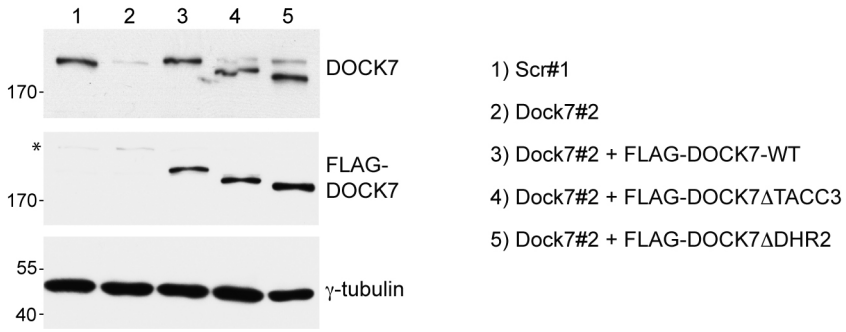
Six Supplementary Videos

Supplemental Information on Data Analyses

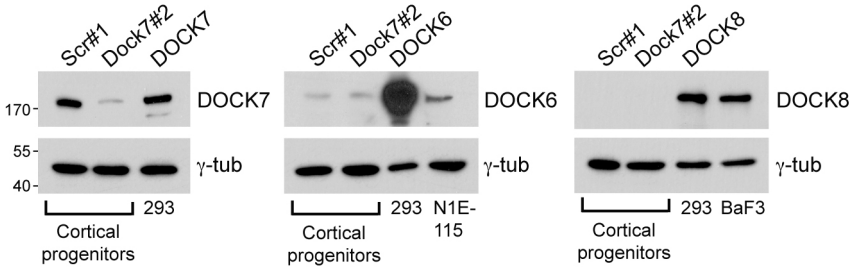
Supplementary Figures



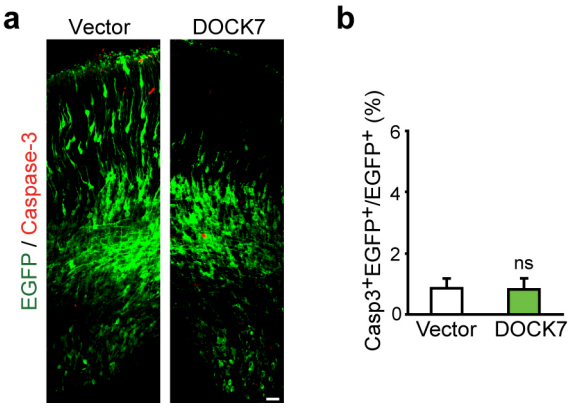
Supplementary Figure 1: DOCK7 expression in the developing neocortex at E9.5, E10.5, and E13.5. Horizontal cryosections of whole mouse embryos (E9.5 and E10.5) or coronal cryosections of mouse brains (E13.5) were immunostained for DOCK7 and neural stem/progenitor marker nestin. Neurogenesis in the neocortex of mice starts at around E10.5. TEL, telencephalon; NE, neuroepithelium; VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate. Scale bars, 20 μm.



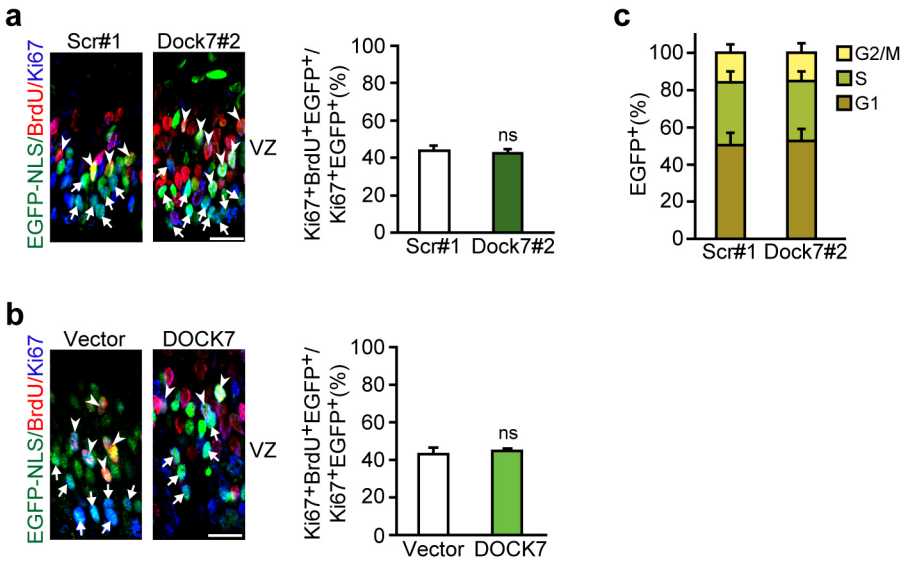
Supplementary Figure 2: Knockdown of DOCK7 levels in cortical progenitors using RNA interference (RNAi). Western blot of total lysates from cultured cortical progenitors isolated from E13.5 mouse cortices transfected with indicated plasmids shows effective knockdown of endogenous DOCK7 levels by Dock7#2 shRNA (Dock7#2), but not control scr#1 shRNA (scr#1), and successful replacement with RNAi-resistant FLAG-DOCK7-WT, FLAG-DOCK7ΔTACC3, or FLAG-DOCK7ΔDHR2 [blotted with anti-DOCK7 antibody (top panel), anti-FLAG antibody (middle panel) and anti-γ-tubulin antibody as a loading control (lower panel)]. Asterisk indicates non-specific band.



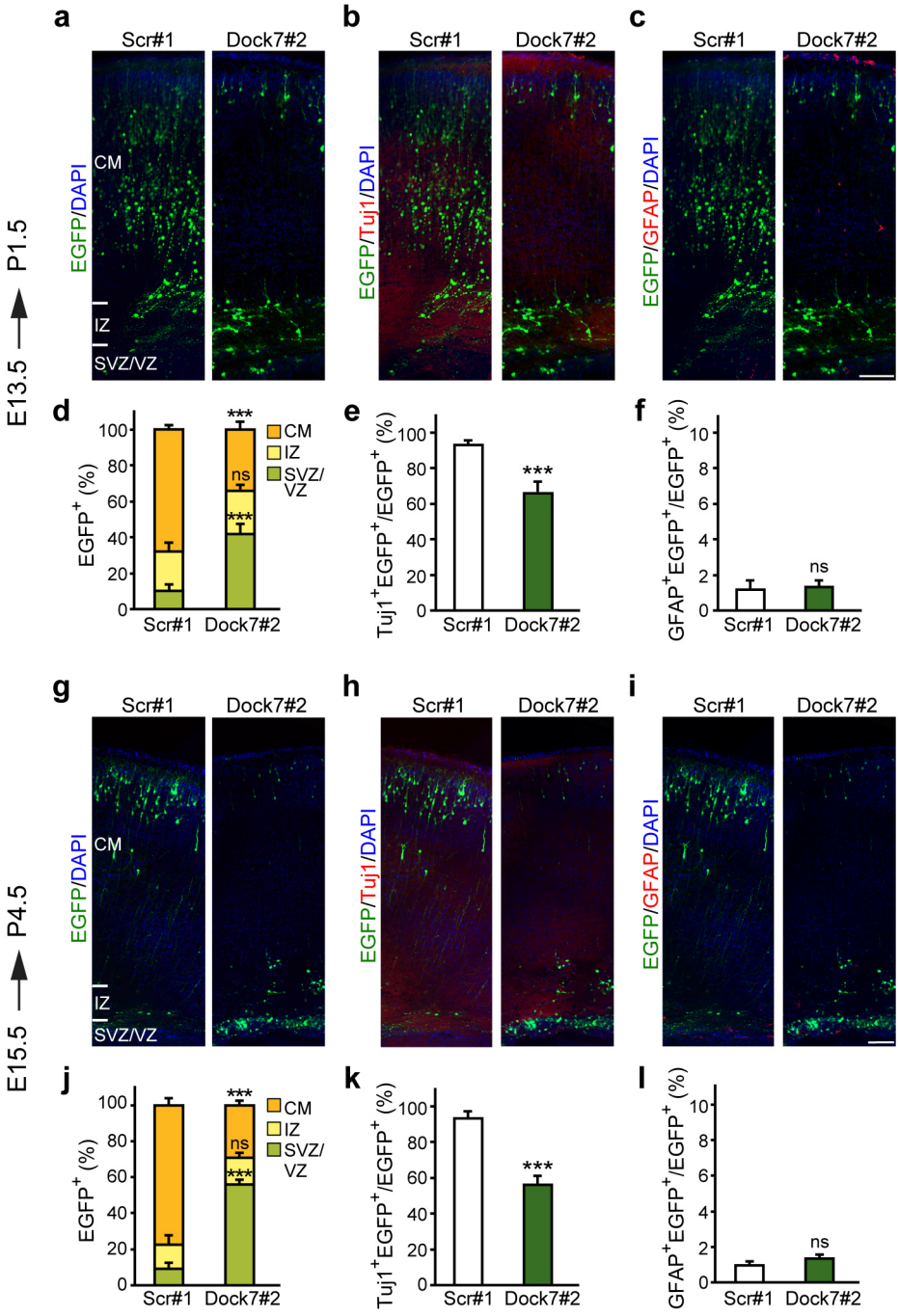
Supplementary Figure 3: Knockdown of DOCK7 does not affect levels of DOCK6 expression, and DOCK8 is not expressed in cortical progenitors. Western blot of total lysates from cultured cortical progenitors isolated from E13.5 mouse cortices (cortical progenitors) transfected with plasmids expressing control scrambled shRNA (scr#1) or Dock7#2 targeting shRNA (Dock7#2), HEK-293 cells (293) ectopically expressing FLAG-DOCK7 (DOCK7), FLAG-DOCK6 (DOCK6) or FLAG-DOCK8 (DOCK8), mouse N1E-115 neuroblastoma cells (N1E-115), or mouse BaF3 pro-B cells (BaF3) were blotted with anti-DOCK7 (left panel), anti-DOCK6 (middle panel) or anti-DOCK8 (right panel) antibody, respectively. Anti-γ-tubulin (γ-tub) blot was included to provide a loading control (lower panels).



Supplementary Figure 4: Ectopic DOCK7 expression does not affect cortical progenitor cell survival. Mouse embryos were co-electroporated at E13.5 with plasmids expressing EGFP marker protein and FLAG-DOCK7 (DOCK7) or empty control vector (vector), and sacrificed at E15.5. Coronal brain slices were co-immunostained for Caspase-3 (red) and EGFP (green). (**a**) Confocal images of neocortices. Scale bar, 25 μm. (**b**) Quantification of the transfected cells that are Caspase-3 positive ($Casp3^+$) (vector, n = 3031 cells; DOCK7, n = 2961 cells; from 3 animals for each condition). Data are shown as mean ± s.e.m. P = 0.605; ns, not significant; Student's t-test.

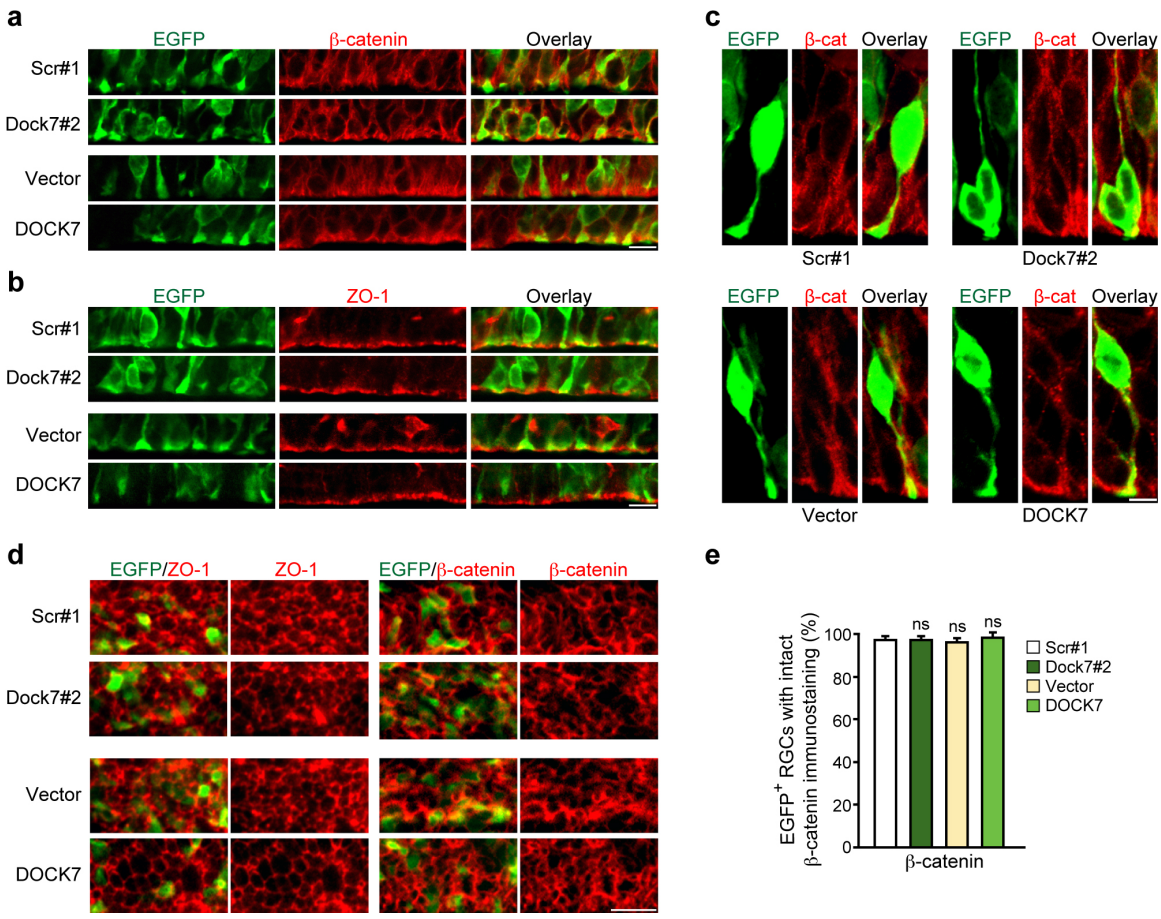


Supplementary Figure 5: DOCK7 does not affect cell cycle duration. (**a,b)** Mouse embryos were co-electroporated at E13.5 with plasmids expressing EGFP-NLS marker protein and non-targeting shRNA (scr#1) or Dock7 targeting shRNA (Dock7#2) (a), or empty control vector (vector) or FLAG-DOCK7 (DOCK7) (b). BrdU was administrated at E15.5, and embryos were sacrificed 30 min after BrdU injection. Coronal brain slices were co-immunostained for BrdU (red), Ki67 (blue), and EGFP (green). Left panels: Confocal images of the VZ of neocortices. Arrows indicate transfected cells that are $Ki67^+$ and $BrdU^-$. Arrowheads indicate transfected cells that are $BrdU^+$ and $Ki67^+$. Scale bars, 20 μm. Right panels: The ratio of the number of transfected cells that are $BrdU^+$ and $Ki67^+$ to the number of transfected cells that are $Ki67^+$ was used to estimate cell cycle duration. This ratio is not significantly different between scr#1 and Dock7#2 shRNA groups, or between control vector and FLAG-DOCK7 groups. Data are shown as mean ± s.e.m.; scr#1, n = 593 cells; Dock7#2, n = 629 cells; vector, n = 451 cells; DOCK7, n = 391 cells; from 3 animals for each condition. P = 0.23 for Dock7#2 (a), and P = 0.45 for DOCK7 (b); ns, not significant; Student's t-test. (**c**) Neuro-2A cells were infected with lentiviral vectors co-expressing EGFP and Dock7#2 shRNA (Dock7#2) or scr#1 shRNA (scr#1). Cells were harvested 3-4 days post-infection, fixed with 0.5% PFA and 75 % cold ethanol, stained with propidium iodide (50 μg/ml), and DNA content profiles were determined by flow cytometry. The number of infected cells in each phase of the cell cycle was quantified. Data are shown as mean ± s.e.m.; n = 3 independent experiments. P > 0.288 for each phase of the cell cycle; Student's t-test.

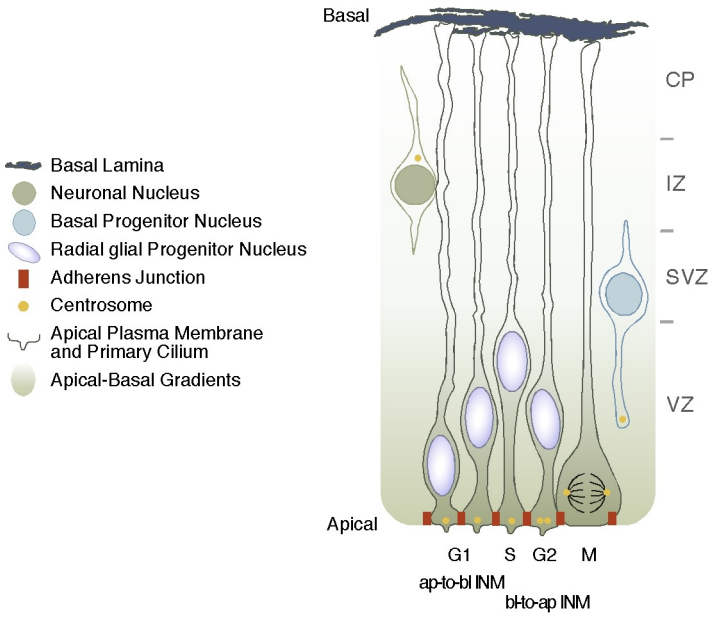


Supplementary Figure 6: DOCK7 knockdown causes persistent perturbations in neurogenesis. Mouse embryos were co-electroporated at E13.5 or E15.5 with plasmids expressing EGFP marker protein and non-targeting shRNA (scr#1) or Dock7 targeting shRNA (Dock7#2), and sacrificed at P1.5 (E13.5→P1.5, a-f) and P4.5 (E15.5→P4.5, g-l), respectively. Coronal brain slices were co-immunostained for EGFP (green) and Tuj1 or GFAP (red), and counterstained with DAPI (blue). (**a,g**) Confocal images of neocortices showing distribution of $EGFP^+$ transfected cells across neocortical layers.

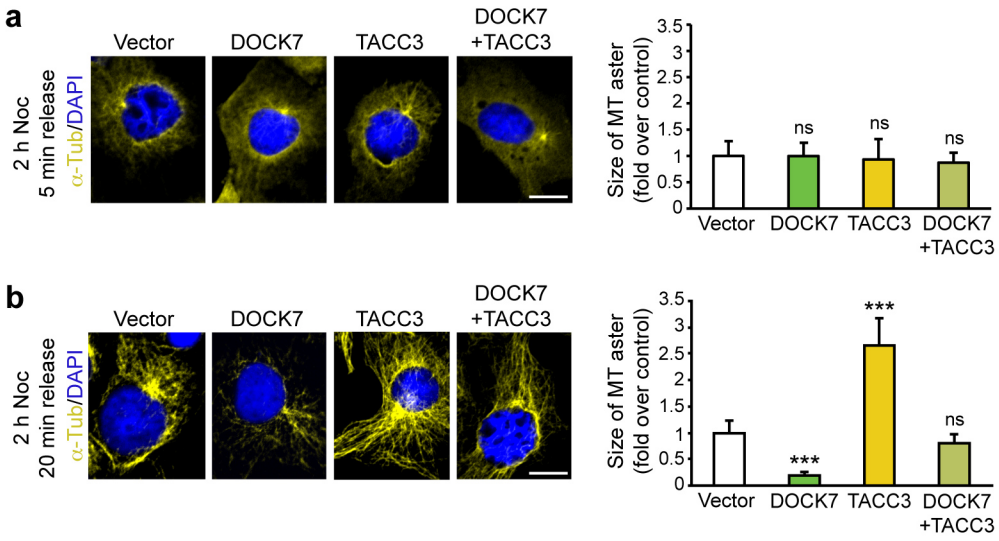
(**d,j**) Quantification of the distribution of $EGFP^+$ transfected cells in entire neocortex. Data are shown as mean ± s.e.m.; scr#1, n = 2546 cells in d and 2051 cells in j; Dock7#2, n = 2445 cells in d and 2077 cells in j; from 3 animals for each condition. ***$P < 0.001$; $P = 0.232$ (d) and 0.106 (j) for Dock7#2 in IZ; ns, not significant; Student's t-test. (**b,h**) Confocal images of neocortices showing $EGFP^+$ and $Tuj1^+$ cells. (**e,k**) Quantification of the transfected cells that are $Tuj1^+$. Data are mean ± s.e.m.; scr#1, n = 2089 cells in e and 1879 cells in k; Dock7#2, n = 2108 cells in e and 1986 cells in k; from 3 animals for each condition. ***$P < 0.001$, Student's t-test. (**c,i**) Confocal images of neocortices showing $EGFP^+$ and $GFAP^+$ cells. (**f,l**) Quantification of the transfected cells that are $GFAP^+$. Data are mean ± s.e.m.; scr#1, n = 2275 cells in f and 1963 cells in l; Dock7#2, n = 2189 cells in f and 2012 cells in l; from 3 animals for each condition. $P = 0.588$ (f) and 0.107 (l) for Dock7#2; ns, not significant; Student's t-test. CM, cortical mantle; IZ, intermediate zone; SVZ/VZ, subventricular zone/ventricular zone. Scale bars, 100 μm.



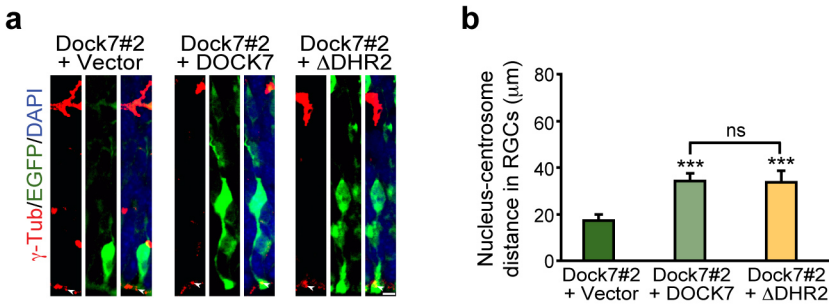
Supplementary Figure 7: DOCK7 does not affect radial glial cell (RGC) adhesion and polarity. Mouse embryos were co-electroporated at E13.5 with plasmids expressing EGFP marker protein and non-targeting shRNA (scr#1), Dock7 targeting shRNA (Dock7#2), empty control vector (vector) or FLAG-DOCK7 (DOCK7), and sacrificed at E15.5. Coronal brain slices were co-immunostained for EGFP (green) and β-catenin (a,c,d; red) or ZO-1 (b,d; red), markers for adherens junctions (AJs). (**a**,**b**) Confocal images of apical endfeet of RGCs in electroporated regions. AJs, visible as a straight line in the X-Y axis by β-catenin (a) or ZO-1 (b) immunostainings, are intact in $EGFP^+$ transfected RGCs of all groups. Scale bars, 10 µm. (**c**) Enlarged views of the cell body and apical process of RGCs are shown. Scale bar, 5 µm. β-cat, β-catenin. (**d**) *En face* view of the ventricular surface (X-Z axis) showing that AJs, visible as honeycomb structures by ZO-1 (left panels) or β-catenin (right panels) immunostainings, are intact in $EGFP^+$ transfected RGCs of all groups. Scale bar, 10 µm. (**e**) Quantification of $EGFP^+$ transfected RGCs displaying intact β-catenin immunostaining. Data are shown as mean ± s.e.m.; scr#1, $n = 495$ cells; Dock7#2, $n = 612$ cells; vector, $n = 461$ cells; DOCK7, $n = 312$ cells; from 3 animals for each condition. $P = 0.883$ for Dock7#2, 0.571 for vector and 0.649 for DOCK7; ns, not significant; one-way ANOVA. Similar results were obtained for quantification of $EGFP^+$ transfected RGCs with intact ZO-1 immunostaining (data not shown).



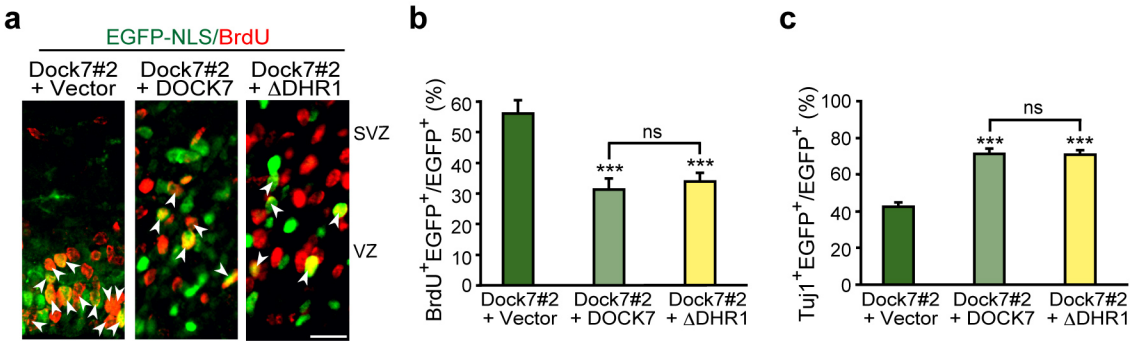
Supplementary Figure 8: Interkinetic nuclear migration (INM) of RGCs. RGCs possess an apical-basal polarity and span the entire cortical wall from the basal lamina to the ventricular (apical) surface. They are coupled by adherens junctions at the apical side and contact the basement membrane at the basal side. The nuclei of RGCs occupy different locations along the apical-basal axis depending on their cell cycle position. After undergoing mitosis at the apical side of the neuroepithelium, the nuclei translocate basally, away from the ventricle surface, as they pass through G1, and replicate their DNA (S-phase) in the basal part of the ventricular zone (VZ). After S-phase completion, G2 phase nuclei migrate to the apical side and then undergo mitosis close to the ventricular surface.



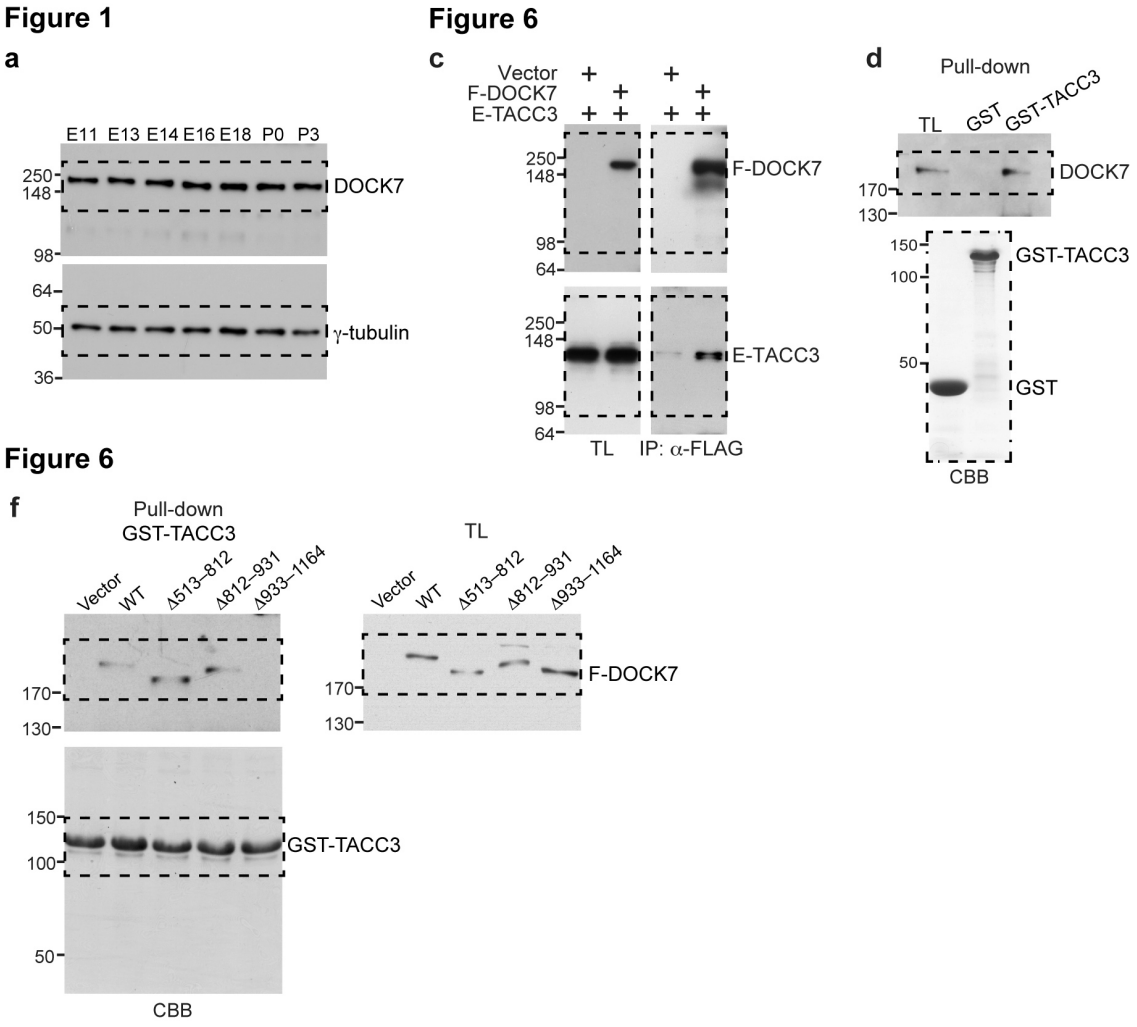
Supplementary Figure 9: Neither DOCK7 nor TACC3 affects centrosomal microtubule nucleation. (**a,b**) COS7 cells were transfected with empty control vector (vector) or plasmids expressing FLAG-DOCK7 (DOCK7) and EGFP-TACC3 (TACC3) alone, or in combination. 3 d post-transfection, cells were treated with 5 μg/ml nocodazole (noc) for 2 h. Following treatment, cells were washed with PBS and allowed to recover in nocodazole-free medium for 5 min (a) or 20 min (b). Left panels: α-tubulin immunostaining (yellow) of transfected COS7. Cells were also co-immunostained for FLAG and EGFP (not shown), and counterstained with DAPI (blue). Scale bars, 10 μm. Right panels: Measurement of the microtubule (MT) aster size relative to that of control vector, which was set as 1. Data are shown as mean ± s.e.m. (a: vector, n = 127 cells; DOCK7, n = 76 cells; TACC3, n = 81 cells; DOCK7 + TACC3, n = 63 cells; b: vector, n = 105 cells; DOCK7, n = 88 cells; TACC3, n = 78 cells; DOCK7 + TACC3, n = 59 cells; from 3 independent experiments for each condition). ***$P < 0.001$; ns, not significant; one-way ANOVA.



Supplementary Figure 10: DOCK7ΔDHR2 rescues the decrease in nucleus-centrosome distance caused by DOCK7 knockdown in RGCs *in vivo*. Mouse embryos were electroporated at E13.5 with a plasmid co-expressing EGFP marker protein and Dock7 targeting shRNA (Dock7#2), together with an empty control vector (vector), a FLAG-DOCK7 WT (DOCK7) or a FLAG-DOCK7ΔDHR2 (ΔDHR2)-expressing plasmid, and sacrificed at E15.5. Coronal brain slices were co-immunostained for γ-tubulin (red) and EGFP (green), and counterstained with DAPI (blue). (**a**) Examples of transfected RGCs. Arrowheads indicate centrosomes of transfected cells. Scale bar, 5 μm. (**b**) Quantification of nucleus-centrosome distance. Data are mean ± s.e.m.; Dock7#2 + vector, $n = 323$ cells; Dock7#2 + DOCK7, $n = 257$ cells; Dock7#2 + ΔDHR2, $n = 238$ cells; from 3 animals for each condition. ***$P < 0.001$; $P = 0.185$ between Dock7#2 + DOCK7 and Dock7#2 + DOCK7ΔDHR2; ns, not significant; one-way ANOVA.



Supplementary Figure 11: The DHR1 domain of DOCK7 is not required for its role in INM and neurogenesis. Mouse embryos were co-electroporated at E13.5 with plasmids expressing EGFP-NLS (a,b) or EGFP (c) marker protein and plasmids expressing Dock7 targeting shRNA (Dock7#2) and empty control vector (vector), FLAG-DOCK7 WT (DOCK7) or FLAG-DOCK7ΔDHR1 (ΔDHR1), pulse labeled with BrdU for 2 h (a,b) at E15.5, or not labeled (c), and sacrificed. Coronal brain slices were co-immunostained for EGFP and BrdU (a,b) or Tuj1 (c). (**a**) Confocal images of the VZ/SVZ of electroporated neocortices co-immunostained for EGFP and BrdU. Arrowheads indicate transfected cells that are $BrdU^+$. Scale bar, 20 μm. (**b**) Quantification of the percentage of transfected cells that are $BrdU^+$ in VZ. Data are mean ± s.e.m.; Dock7#2 + vector, n = 1137 cells; Dock7#2 + DOCK7, n = 1189 cells; Dock7#2 + ΔDHR1, n= 1159 cells; from 3 animals for each condition. ***$P < 0.001$; $P = 0.205$ between Dock7#2 + DOCK7 and Dock7#2 + DOCK7ΔDHR1; ns, not significant; one-way ANOVA. (**c**) Quantification of the percentage of transfected cells that are $Tuj1^+$ in entire neocortex. Data are mean ± s.e.m.; Dock7#2 + vector, n = 2192 cells; Dock7#2 + DOCK7, n = 2098 cells; Dock7#2 + ΔDHR1, n = 2211 cells; from 3 animals for each condition. ***$P < 0.001$; $P = 0.576$ between Dock7#2 + DOCK7 and Dock7#2 + DOCK7ΔDHR1; ns, not significant; one-way ANOVA.



Supplementary Figure 12: Full-length Western blots and Coomassie Brilliant Blue (CBB) stainings for Figures 1 and 6. Samples were loaded on an 8% (Figures 1a, 6c,d) or 6% (Figure 6f) gel and immunoblotted with the indicated antibodies or stained with CBB. Dotted lines indicate the bands used for inclusion in Figures 1 and 6.

Supplementary Videos

Supplementary Video 1: Example of a control vector expressing RGC (top left cell) undergoing bl-to-ap INM in VZ of neocortex. Time-lapse imaging was carried out on acute cortical slices 2 d after in utero electroporation of plasmids expressing empty control vector, and EGFP (green) and mKO2-F (red) fluorescent markers (at E15.5). Images acquired over an 8 h time period are shown; time (in min) is indicated on the top. Note: the cell body of the RGC migrates steadily toward the ventricle. After about 5 h, it reaches the ventricular (apical) surface, and the cell subsequently divides at the ventricular surface.

Supplementary Video 2: Example 1 of a DOCK7 overexpressing RGC (cell in the middle) undergoing bl-to-ap INM in VZ of neocortex. Time-lapse imaging was carried out on acute cortical slices 2 d after in utero electroporation of plasmids expressing FLAG-DOCK7, and EGFP (green) and mKO2-F (red) fluorescent markers (at E15.5). Images acquired over an 8 h time period are shown; time (in min) is indicated on the top. Note: the cell body of the RGC remains at its original basal position for about 5 h, which is followed by cell division away from the ventricular surface.

Supplementary Video 3: Example 2 of a DOCK7 overexpressing RGC (cell in upper middle) undergoing bl-to-ap INM in VZ of neocortex. Time-lapse imaging was carried out on acute cortical slices 2 d after in utero electroporation of plasmids expressing FLAG-DOCK7, and EGFP (green) and mKO2-F (red) fluorescent markers (at E15.5). Images acquired over an 8 h time period are shown; time (in min) is indicated on the top. Note: the cell body of the RGC remains at its basal position for about 4.5 h and then moves over a very short distance toward the ventricle, which is followed by cell division away from the ventricular surface.

Supplementary Video 4: Example of a control scr#1 shRNA expressing RGC (cell in the middle) undergoing bl-to-ap INM in VZ of neocortex. Time-lapse imaging was carried out on acute cortical slices 2 d after in utero electroporation of plasmids expressing scr#1 shRNA, and EGFP (green) and mKO2-F (red) fluorescent markers (at E15.5). Images acquired over an 8 h time period are shown; time (in min) is indicated on the top. Note: the cell body of the RGC migrates steadily toward the ventricle. After about 5 h, it reaches the ventricular (apical) surface and the cell subsequently divides at the ventricular surface.

Supplementary Video 5: Example 1 of a Dock7#2 shRNA expressing RGC (cell in the middle) undergoing bl-to-ap INM in VZ of neocortex. Time-lapse imaging was carried out on acute cortical slices 2 d after in utero electroporation of plasmids expressing Dock7#2 shRNA, and EGFP (green) and mKO2-F (red) fluorescent markers (at E15.5). Images acquired over an 8 h time period are shown; time (in min) is indicated on the top. Note: the cell body of the RGC migrates considerably faster to the ventricle surface than that of the control scr#1 shRNA expressing RGC, where it then remains for about 3 h before the cell undergoes apical mitosis.

Supplementary Video 6: Example 2 of a Dock7#2 shRNA expressing RGC (cell in the middle) undergoing bl-to-ap INM in VZ of neocortex. Time-lapse imaging was carried

out on acute cortical slices 2 d after in utero electroporation of plasmids expressing Dock7#2 shRNA, and EGFP (green) and mKO2-F (red) fluorescent markers (at E15.5). Images acquired over an 8 h time period are shown; time (in min) is indicated on the top. Note: the cell body of the RGC migrates considerably faster to the ventricle surface than that of the control scr#1 shRNA expressing RGC, where it then remains for > 4 h before the cell undergoes apical mitosis.

Supplemental Information on Data Analyses

Figure 2: DOCK7 modulates the VZ progenitor pool size. (**c**) scr#1, *n* = 1389 cells; Dock7#2, *n* = 1897 cells; rescue, *n* = 1238 cells; from 3 animals for each condition. (**d**) scr#1, *n* =1087 cells; Dock7#2, *n* = 1320 cells; rescue, *n* = 921 cells; from 3 animals for each condition. (**f**) vector, *n* = 1243 cells; DOCK7, *n* = 1738 cells; from 3 animals for each condition. (**h**) vector, *n* = 1096 cells; DOCK7, *n* = 1565 cells; from 3 animals for each condition. (**j**, **k**) scr#1, *n* = 867 cells; Dock7#2, *n* = 1199 cells; from 3 animals for each condition. (**m**, **n**) vector, *n* = 1292 cells; DOCK7, *n* = 962 cells; from 3 animals for each condition.

Figure 3: DOCK7 is required for the transition of RGCs to BPs and genesis of neurons. (**c**) scr#1, *n* = 853 cells; Dock7#2, *n* = 941 cells; from 3 animals for each condition. (**d**) scr#1, *n* = 1141 cells; Dock7#2, *n* = 1268 cells; from 3 animals for each condition. (**g**) vector, *n* = 937 cells; DOCK7, *n* = 1164 cells; from 3 animals for each condition. (**h**) vector, *n* = 1049 cells; DOCK7, *n* = 1034 cells; from 3 animals for each condition. (**j**) scr#1, *n* = 1983 cells; Dock7#2, *n* = 2053 cells; from 3 animals for each condition. (**k**) scr#1, *n* = 1593 cells; Dock7#2, *n* = 1318 cells; from 3 animals for each condition. (**m**) vector, *n* = 2305 cells; DOCK7, *n* = 2453 cells; from 3 animals for each condition. (**n**) vector, *n* = 2086 cells; DOCK7, *n* = 2331 cells; from 3 animals for each condition.

Figure 4: DOCK7 controls basal-to-apical INM of RGCs. (**a**) scr#1 (15 min), *n* = 418 cells; (2 h), *n* = 372 cells; (4 h), *n* = 406 cells; (6 h), *n* = 477 cells; Dock7#2 (15 min), *n* = 398 cells; (2 h), *n* = 483 cells; (4 h), *n* = 480 cells; (6 h), *n* = 538 cells; from 3 animals for each condition. (**b**) vector (15 min), *n* = 457 cells; (2 h), *n* = 584 cells; (4 h), *n* = 476 cells; (6 h), *n* = 383 cells; DOCK7 (15 min), *n* = 530 cells; (2 h), *n* = 591 cells; (4 h), *n* = 589 cells; (6 h), *n* = 601 cells; from 3 animals for each condition. (**c**) scr#1 (2 h), *n* = 973 cells; (6 h), *n* = 901 cells; Dock7#2 (2 h), *n* = 956 cells; (6 h), *n* = 887 cells; from 3 animals for each condition. (**d**) vector (2 h), *n* = 844 cells; (6 h), *n* = 1009 cells; DOCK7 (2 h), *n* = 881 cells; (6 h), *n* = 754 cells; from 3 animals for each condition.

Figure 5: Altered DOCK7 expression affects apically directed INM of RGCs in acute cortical slices. (**e**) vector, *n* = 19 cells from 3 experiments; DOCK7, *n* = 37 cells from 7 experiments; scr#1, *n* = 24 cells from 4 experiments; Dock7#2, *n* = 43 cells from 6 experiments.

Figure 7: DOCK7 antagonizes TACC3 function during cortical neurogenesis. (**b**) scr#2, *n* = 937 cells; Tacc3#1, *n* = 850 cells; from 3 animals for each condition. (**c**) scr#2, *n* = 903 cells; Tacc3#1, *n* = 1173 cells; from 3 animals for each condition. (**d**) scr#2, *n* = 871 cells; Tacc3#1, *n* = 793 cells; from 3 animals for each condition. (**e**) scr#2, *n* = 2491 cells; Tacc3#1, *n* = 2498 cells; from 3 animals for each condition. (**g**) scr#1 + scr#2, n= 995 cells; Dock#2 + scr#2, *n* = 1138 cells; scr#1 + Tacc3#1, *n* = 893 cells; Dock7#2 + Tacc3#1, *n* = 790 cells; from 3 animals for each condition. (**h**) scr#1 + scr#2, *n* = 972 cells; Dock#2 + scr#2, *n* = 952 cells; scr#1 + Tacc3#1, *n* = 1038 cells; Dock7#2 + Tacc3#1, *n* = 937 cells; from 3 animals for each condition. (**i**) scr#1 + scr#2, *n* = 905 cells; Dock#2 + scr#2, *n* = 1017 cells; scr#1 + Tacc3#1, *n* = 820 cells; Dock7#2 + Tacc3#1, *n* = 739 cells; from 3 animals for each condition. (**j**) scr#1 + scr#2, *n* = 1839 cells; Dock#2 + scr#2, *n* = 2031 cells; scr#1 + Tacc3#1, *n* = 1868 cells; Dock7#2 +

Tacc3#1, *n* = 1859 cells; from 3 animals for each condition. (**l**) Dock7#2 + vector, *n* = 1063 cells; Dock#2 + DOCK7, *n* = 909 cells; Dock7#2 + DOCK7ΔTACC3, *n* = 835 cells; Dock7#2 + DOCK7ΔDHR2, *n* = 972 cells; from 3 animals for each condition. (**m**) Dock7#2 + vector, *n* = 1137 cells; Dock#2 + DOCK7, *n* = 1189 cells; Dock7#2 + DOCK7ΔTACC3, *n* = 1116 cells; Dock7#2 + DOCK7ΔDHR2, *n* = 1056 cells; from 3 animals for each condition**.** (**n**) Dock7#2 + vector, *n* = 973 cells; Dock#2 + DOCK7, *n* = 891 cells; Dock7#2 + DOCK7ΔTACC3, *n* = 792 cells; Dock7#2 + DOCK7ΔDHR2, *n* = 925 cells; from 3 animals for each condition**.** (**o**) Dock7#2 + vector, *n* = 2192 cells; Dock#2 + DOCK7, *n* = 2098 cells; Dock7#2 + DOCK7ΔTACC3, *n* = 2329 cells; Dock7#2 + DOCK7ΔDHR2, *n* = 2321 cells; from 3 animals for each condition.

Figure 8: DOCK7 antagonizes the microtubule growth promoting/stabilizing function of TACC3. (**b**) vector, *n* = 115 cells; DOCK7, *n* = 74 cells; TACC3, *n* = 79 cells; DOCK7 + TACC3, *n* = 69 cells; from 3 experiments for each condition. (**d**) scr#1, *n* = 103 cells; Dock7#2, *n* = 83 cells; Tacc3#1, *n* = 90 cells; Dock7#2 + Tacc3#1, *n* = 77 cells; from 3 experiments for each condition. (**f**) scr#1, *n* = 258 cells; Dock7#2, *n* = 371 cells; Tacc3#1, *n* = 239 cells; Dock7#2 + Tacc3#1, *n* = 215 cells; from 3 animals for each condition.