Nakamuta et al., https://doi.org/10.1083/jcb.201704157

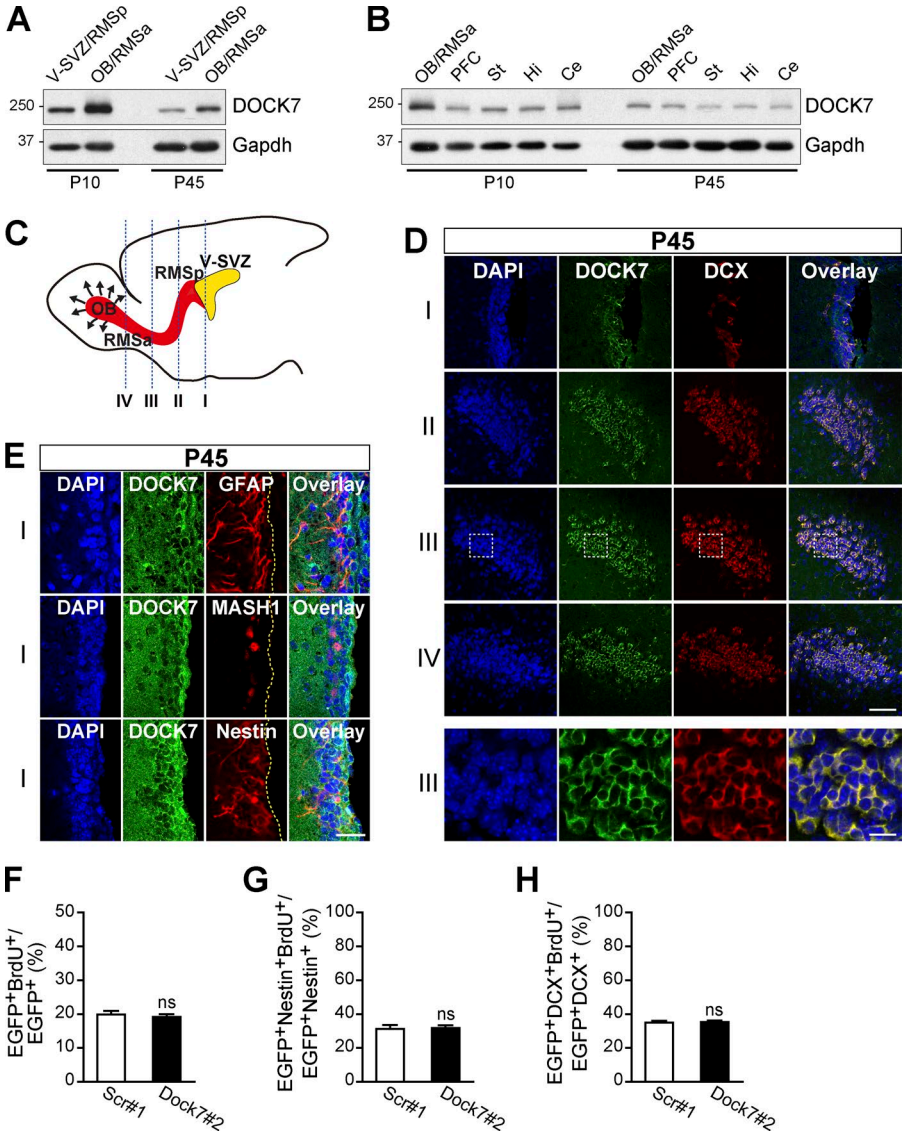


Figure S1. **Expression of DOCK7 in the postnatal/adult mouse forebrain and effect of DOCK7 knockdown on the proliferation of V-SVZ stem/progenitor cells and neuroblasts.** (A and B) Western blots of lysates prepared from indicated brain regions/tissues of P10 and P45 mice probed with antibodies to DOCK7 and GAPDH as a loading control. Ce, cerebellum; Hi, hippocampus; PFC, prefrontal cortex; St, striatum. Molecular masses are given in kilodaltons. (C) A cartoon representation of the V-SVZ–RMS–OB pathway. (D) Coronal sections of forebrains of P45 mice at positions indicated in the cartoon in C immunostained with antibodies to DOCK7 and DCX and then counterstained with DAPI. Boxed regions are enlarged and shown at the bottom. Bars: (top) 50 µm; (bottom) 10 µm. (E) Coronal sections of the V-SVZ region of P45 mice immunostained with antibodies to DOCK7 and GFAP, MASH1, or nestin and counterstained with DAPI. Bar, 25 µm. (F–H) Knockdown of DOCK7 does not affect the proliferation of stem/progenitor cells and neuroblasts. Mouse pups (P3) were electroporated with plasmids expressing EGFP and scr#1 or Dock7#2 shRNA and pulse labeled with BrdU for 2 h at P10. Brain slices were immunostained for GFP and BrdU (F), nestin and BrdU (G), or DCX and BrdU (H). (F) Quantification of $BrdU^+$ transfected cells in the V-SVZ. n = 144–154 cells from at least three animals for each condition. (G) Quantification of $nestin^+$ transfected cells that are $BrdU^+$ in the V-SVZ. n = 117–180 cells from at least three animals for each condition. (H) Quantification of DCX^+ transfected cells that are $BrdU^+$ in the V-SVZ. n = 744–1,199 cells from at least three animals for each condition. Data are shown as means ± SEM. ns, $P > 0.05$; Student's t test.

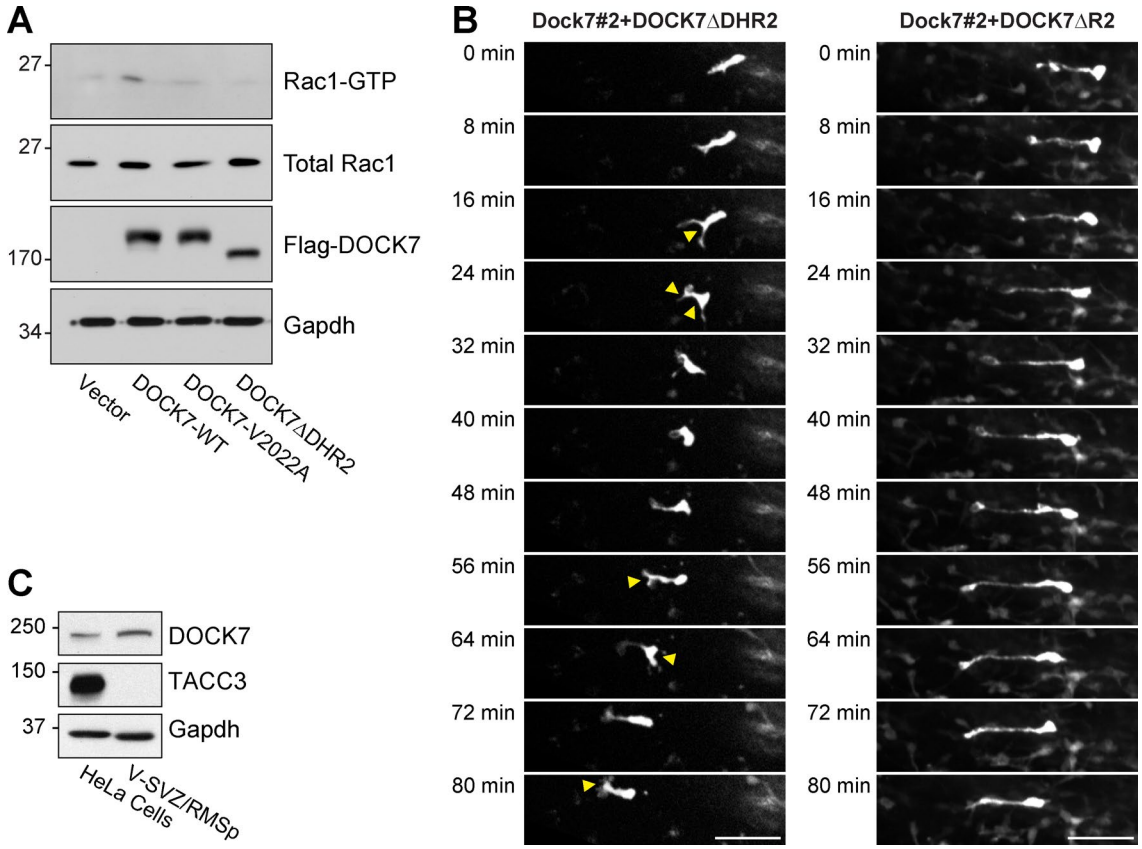


Figure S2. **Branching phenotypes of neuroblasts expressing Dock7#2 shRNA together with DOCK7 mutants defective in Rac activation or lacking the R2 domain and expression of TACC3 in the V-SVZ/RMS.** (A) DOCK7-V2022A and DOCK7ΔDHR2 mutants are deficient in Rac1 activation. Activation of Rac1 measured with p21-activated kinase Rac/Cdc42-binding domain (PBD) pulldown assay. HEK293T cells were transfected with empty control vector, Flag-DOCK7, Flag-DOCK7-V2022A, or Flag-DOCK7ΔDHR2, and GTP-bound Rac1 was precipitated from detergent extracts with GST-PBD. GST-PBD–bound Rac1-GTP, total Rac1, and DOCK7 levels in cell lysates were detected by immunoblotting with anti-Rac1 and anti-Flag antibodies. GAPDH was included as a loading control. Data shown are representative of three independent experiments. (B) DOCK7ΔR2 but not DOCK7ΔDHR2 rescues the branching phenotype induced by DOCK7 RNAi. P2–3 mouse pups were electroporated with a tdTomato-expressing plasmid together with one of the indicated constructs. After 5 d, acute brain slices were prepared, and tdTomato$^+$ transfected neuroblasts were imaged by spinning-disk confocal microscopy for 4 h. Examples of time-lapse series of cells coexpressing Dock7#2 shRNA and DOCK7ΔDHR2 or DOCK7ΔR2 in the lower vertical arm of the RMSp. Arrowheads indicate branching of LP. Bars, 50 µm. (C) TACC3 expression is not detectable in the V-SVZ and RMSp of P10 mice. Western blots of lysates prepared from HeLa cells or the V-SVZ and RMSp regions of P10 mice probed with antibodies to DOCK7 and TACC3 as well as GAPDH as a loading control. Molecular masses are given in kilodaltons.

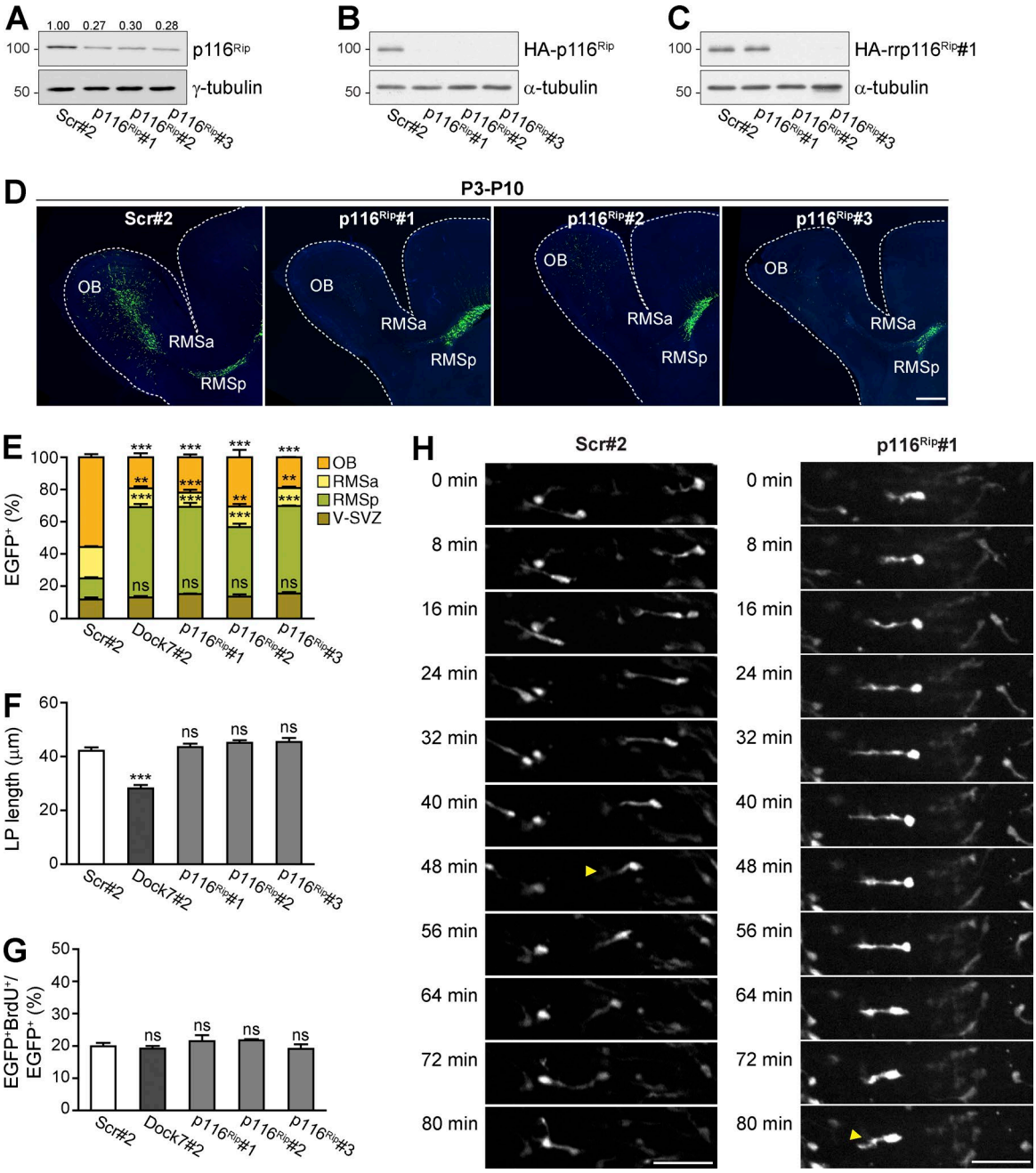


Figure S3. **Effects of p116Rip knockdown on neuroblast migration and V-SVZ stem/progenitor cell proliferation.** (A–C) Knockdown of p116Rip levels using RNAi. (A) Western blot of total lysates from cultured cortical neurons transfected with vectors expressing nontargeting shRNA (scr#2) or p116Rip-targeting shRNAs (p116Rip#1, p116Rip#2, or p116Rip#3) probed with antibodies to p116Rip and γ-tubulin as a loading control. The relative amount of p116Rip protein levels compared with scr#2 control and normalized to γ-tubulin levels are indicated on the top. (B and C) Western blots of lysates prepared from Neuro-2a cells transfected with a plasmid expressing HA-tagged WT p116Rip (B) or RNAi-resistant p116Rip (rrp116Rip#1; C) and one of the indicated constructs, probed with antibodies to HA and α-tubulin as a loading control. Molecular masses are given in kilodaltons. (D–F) p116Rip knockdown impairs neuroblast migration. (D) Composite confocal images of forebrains of mice electroporated at P3 with plasmids expressing EGFP and scr#2 or p116Rip#1, p116Rip#2, or p116Rip#3 shRNA and sacrificed at P10, showing distribution of EGFP+ transfected neuroblasts along the V-SVZ–RMS–OB pathway. Slices were counterstained with DAPI. Dotted lines outline borders of sagittal slices. Bar, 500 μm. (E) Quantification of the distribution of EGFP+ transfected cells along the V-SVZ–RMS–OB pathway. *n* = 652–1,561 cells from at least three animals for each condition. (F) Quantification of the length of the LP of neuroblasts expressing indicated constructs. *n* = 60–216 cells from at least three animals for each condition. (G) p116Rip knockdown does not affect V-SVZ stem/progenitor cell proliferation. Quantification of BrdU+ transfected cells in the V-SVZ of mice electroporated at P3 with indicated plasmids and pulse-labeled with BrdU for 2 h at P10. *n* = 118–190 cells from at least three animals for each condition. Data are shown as means ± SEM. **, P < 0.01; ***, P < 0.001; ns, P > 0.05 compared with scr#2; one-way ANOVA with Dunnett's post hoc test. (H) Knockdown of p116Rip does not affect the branching frequency of V-SVZ neuroblasts' LP. P2–3 mouse pups were electroporated with plasmids expressing tdTomato and scr#2 or p116Rip#1 shRNA. After 5 d, acute brain slices were prepared and tdTomato+ transfected neuroblasts were imaged by spinning-disk confocal microscopy for 4 h. Examples of time-lapse series of cells expressing scr#2 or p116Rip#1 shRNA in the lower vertical arm of the RMSp. Arrowheads indicate branching of LP. Bars, 50 μm.

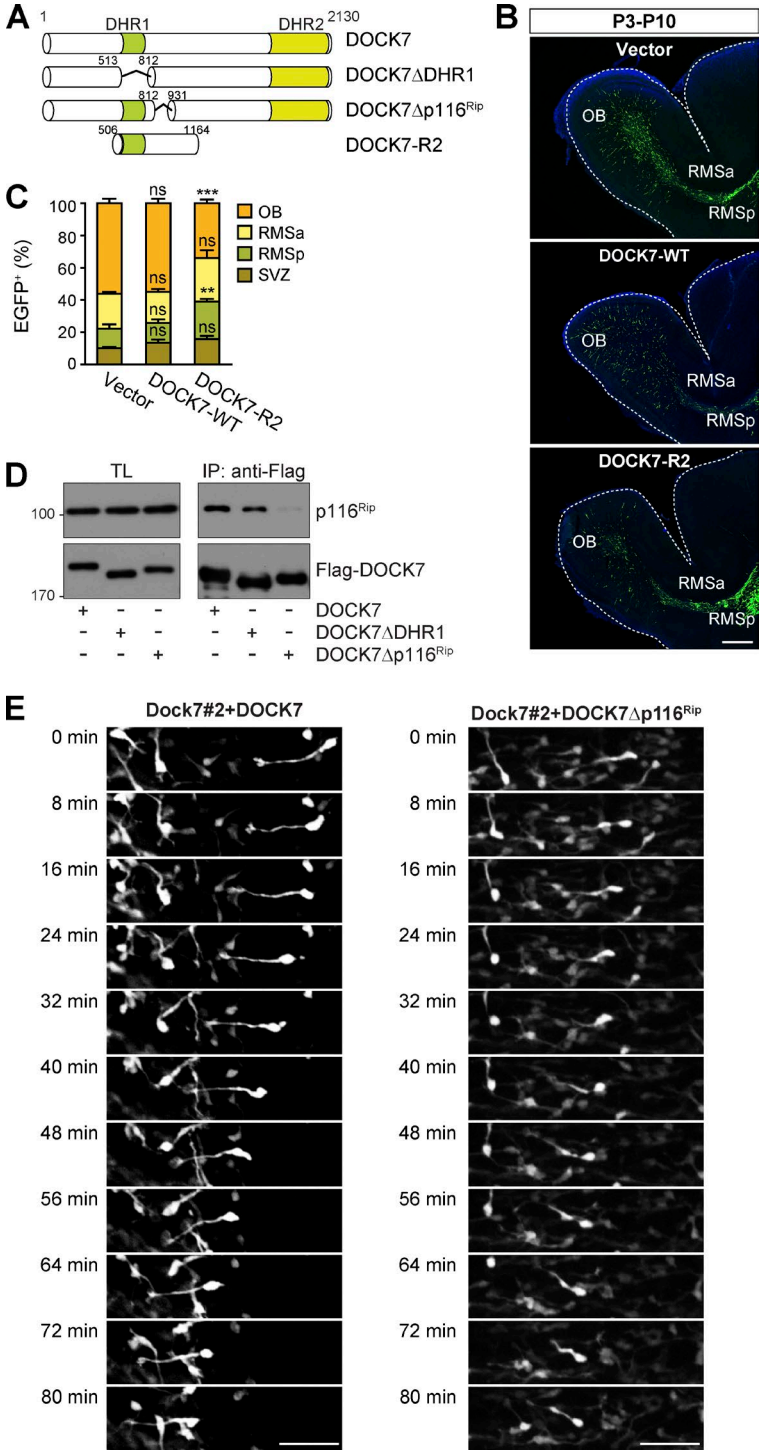


Figure S4. **Effect of ectopic expression of the R2 fragment of DOCK7 on neuroblast migration and the branching phenotypes of migrating neuroblasts expressing Dock7#2 shRNA together with a DOCK7 mutant lacking the minimum p116^{Rip}-binding region (DOCK7Δp116^{Rip}).** (A–C) Ectopic expression of R2 fragment of DOCK7 interferes with V-SVZ–derived neuroblast migration. (A) DOCK7 domain structure and deletion constructs. (B) Composite confocal images of forebrains of mice electroporated at P3 with plasmids expressing EGFP and empty control vector, DOCK7-WT, or DOCK7-R2 and sacrificed at P10, showing distribution of EGFP$^+$ transfected neuroblasts along the V-SVZ–RMS–OB pathway. Slices were counterstained with DAPI. Dotted lines outline borders of sagittal slices. Bar, 500 µm. (C) Quantification of the distribution of EGFP$^+$ transfected cells along the V-SVZ–RMS–OB pathway. *n* = 1,714–4,297 cells from at least three animals for each condition. Data are shown as means ± SEM. **, P < 0.01; ***, P < 0.001; ns, P > 0.05 compared with vector; two-way ANOVA with Dunnett's post hoc test. (D) Mapping of the p116^{Rip}-binding domain in DOCK7. Lysates from HEK293 cells transiently expressing Flag-DOCK7, Flag-DOCK7ΔDHR1, or Flag-DOCK7Δp116^{Rip} were immunoprecipitated (IP) with an antibody to Flag and analyzed by immunoblotting with antibodies to Flag and p116^{Rip}. TL, total lysate. Molecular masses are given in kilodaltons. (E) V-SVZ neuroblasts coexpressing Dock7#2 shRNA and DOCK7Δp116^{Rip} exhibit normal branching frequency. P2–3 mouse pups were electroporated with a tdTomato-expressing plasmid together with one of the indicated constructs. After 5 d, acute brain slices were prepared, and tdTomato$^+$ transfected neuroblasts were imaged by spinning-disk confocal microscopy for 4 h. Examples of time-lapse series of cells coexpressing Dock7#2 shRNA and DOCK7 or DOCK7Δp116^{Rip} in the lower vertical arm of the RMSp. Bars, 50 µm.

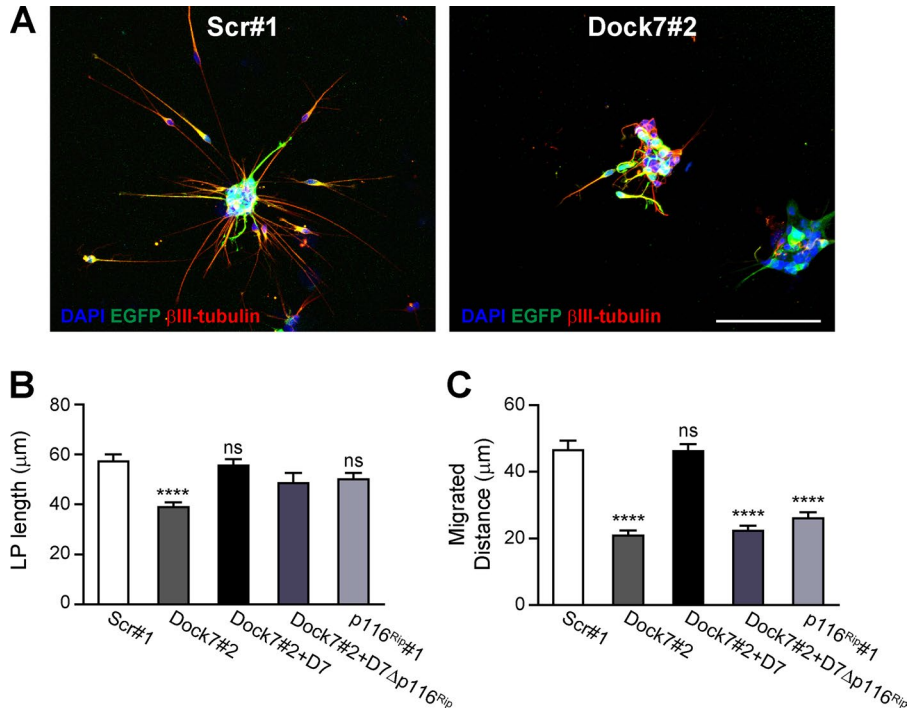
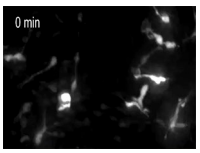
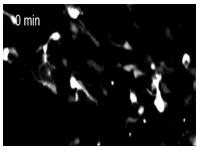


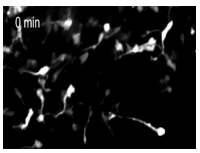
Figure S5. **DOCK7/p116^{Rip} signaling is essential for V-SVZ neuroblast migration in Matrigel cultures.** (A–C) Reaggregated neuroblasts isolated from V-SVZ tissue of P1–3 mice were embedded in Matrigel 48 h after nucleofection of an EGFP-expressing vector and one of the indicated constructs and were allowed to migrate for 30 h before immunostaining for GFP and βIII-tubulin. Nuclei were visualized by DAPI staining. (A) Representative images of neuroblasts expressing scr#1 or Dock7#2 shRNA migrating from the edge of the aggregates. Bar, 100 μm. (B) Quantification of the length of the LP of neuroblasts expressing indicated constructs. *n* = 127–160 cells from at least three animals for each condition. (C) Quantification of the migrated distance of neuroblasts expressing indicated constructs. *n* = 94–185 cells from at least three animals for each condition. Data are shown as means ± SEM. ****, P < 0.0001; ns, P > 0.05 compared with scr#1; one-way ANOVA with Dunnett's post hoc test.



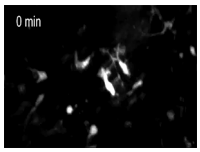
Video 1. **Example of migrating V-SVZ neuroblasts expressing control scr#1 shRNA in the RMS.** Time-lapse imaging was performed on acute sagittal mouse brain slices 5 d after electroporation of plasmids expressing scr#1 shRNA and tdTomato fluorescent marker. Videos were made from the lower vertical arm of the RMSp, with the OB toward the left. Images were acquired at 4-min intervals for 80 min.



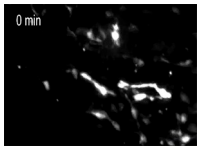
Video 2. **Example of migrating V-SVZ neuroblasts expressing Dock7#2 shRNA in the RMS.** Time-lapse imaging was performed on acute sagittal mouse brain slices 5 d after electroporation of plasmids expressing Dock7#2 shRNA and tdTomato fluorescent marker. Videos were made from the lower vertical arm of the RMSp, with the OB toward the left. Images were acquired at 4-min intervals for 80 min.



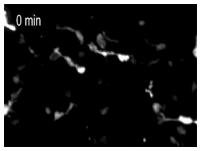
Video 3. **Example of migrating V-SVZ neuroblasts expressing Dock7#2 shRNA and DOCK7 in the RMS.** Time-lapse imaging was performed on acute sagittal mouse brain slices 5 d after electroporation of plasmids expressing Dock7#2 shRNA, DOCK7, and tdTomato fluorescent marker. Videos were made from the lower vertical arm of the RMSp, with the OB toward the left. Images were acquired at 4-min intervals for 80 min.



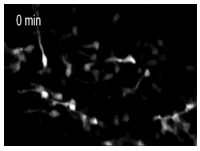
Video 4. **Example of migrating V-SVZ neuroblasts expressing Dock7#2 shRNA and DOCK7ΔDHR2 in the RMS.** Time-lapse imaging was performed on acute sagittal mouse brain slices 5 d after electroporation of plasmids expressing Dock7#2 shRNA, DOCK7ΔDHR2, and tdTomato fluorescent marker. Videos were made from the lower vertical arm of the RMSp, with the OB toward the left. Images were acquired at 4-min intervals for 80 min.



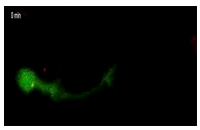
Video 5. **Example of migrating V-SVZ neuroblasts expressing Dock7#2 shRNA and DOCK7ΔR2 in the RMS.** Time-lapse imaging was performed on acute sagittal mouse brain slices 5 d after electroporation of plasmids expressing Dock7#2 shRNA, DOCK7ΔR2, and tdTomato fluorescent marker. Videos were made from the lower vertical arm of the RMSp, with the OB toward the left. Images were acquired at 4-min intervals for 80 min.



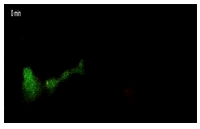
Video 6. **Example of migrating V-SVZ neuroblasts expressing p116^{Rip}#1 shRNA in the RMS.** Time-lapse imaging was performed on acute sagittal mouse brain slices 5 d after electroporation of plasmids expressing p116^{Rip}#1 shRNA and tdTomato fluorescent marker. Videos were made from the lower vertical arm of the RMSp, with the OB toward the left. Images were acquired at 4-min intervals for 80 min.



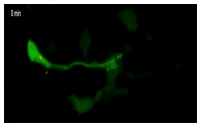
Video 7. **Example of migrating V-SVZ neuroblasts expressing Dock7#2 shRNA and DOCK7Δp116^{Rip} in the RMS.** Time-lapse imaging was performed on acute sagittal mouse brain slices 5 d after electroporation of plasmids expressing Dock7#2 shRNA, DOCK7Δp116^{Rip}, and tdTomato fluorescent marker. Videos were made from the lower vertical arm of the RMSp, with the OB toward the left. Images were acquired at 4-min intervals for 80 min.



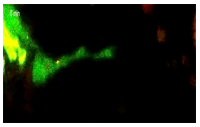
Video 8. **Time-lapse images of a V-SVZ neuroblast expressing control scr#2 shRNA during somal translocation in a Matrigel culture.** Neuroblasts dissociated from the V-SVZ of P1–3 mice were coelectroporated with a scr#2 shRNA-expressing plasmid and a plasmid coexpressing EGFP and PACT-mKO1, reaggregated and embedded in Matrigel, and then subjected to live-cell imaging. Images were acquired at 3-min intervals for 90 min. Signals of EGFP and PACT-mKO1 are shown in green and red, respectively.



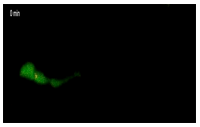
Video 9. **Time-lapse images of a V-SVZ neuroblast expressing p116$^{Rip\#1}$ shRNA during somal translocation in a Matrigel culture.** Neuroblasts dissociated from the V-SVZ of P1–3 mice were coelectroporated with a p116$^{Rip\#1}$ shRNA-expressing plasmid and a plasmid coexpressing EGFP and PACT-mKO1, reaggregated and embedded in Matrigel, and then subjected to live-cell imaging. Images were acquired at 3-min intervals for 90 min. Signals of EGFP and PACT-mKO1 are shown in green and red, respectively.



Video 10. **Time-lapse images of a V-SVZ neuroblast expressing Dock7#2 shRNA and DOCK7 during somal translocation in a Matrigel culture.** Neuroblasts dissociated from the V-SVZ of P1–3 mice were coelectroporated with plasmids expressing Dock7#2 shRNA and DOCK7 as well as a plasmid coexpressing EGFP and PACT-mKO1, reaggregated and embedded in Matrigel, and then subjected to live-cell imaging. Images were acquired at 3-min intervals for 90 min. Signals of EGFP and PACT-mKO1 are shown in green and red, respectively.



Video 11. **Time-lapse images of a V-SVZ neuroblast expressing Dock7#2 shRNA and DOCK7Δp116^{Rip} during somal translocation in a Matrigel culture.** Neuroblasts dissociated from the V-SVZ of P1–3 mice were coelectroporated with plasmids expressing Dock7#2 shRNA and DOCK7Δp116^{Rip} as well as a plasmid coexpressing EGFP and PACT-mKO1, reaggregated and embedded in Matrigel, and then subjected to live-cell imaging. Images were acquired at 3-min intervals for 90 min. Signals of EGFP and PACT-mKO1 are shown in green and red, respectively.



Video 12. **Time-lapse images of a V-SVZ neuroblast expressing Dock7#2 shRNA during somal translocation in a Matrigel culture.** Neuroblasts dissociated from the V-SVZ of P1–3 mice were coelectroporated with a Dock7#2 shRNA-expressing plasmid and a plasmid coexpressing EGFP and PACT-mKO1, reaggregated and embedded in Matrigel, and then subjected to live-cell imaging. Images were acquired at 3-min intervals for 90 min. Signals of EGFP and PACT-mKO1 are shown in green and red, respectively.