Supplementary Information to:

Reelin, Rap1 and N-cadherin orient the migration of multipolar neurons in the developing neocortex

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This Supplement contains three Supplementary movies and eleven Supplementary figures.

Supplementary movies:

Movie 1: Movement of multipolar cells in the MMZ of control, Rap1GAP and NCad^{DN} electroporated cortex.
Cortices were electroporated at E14.5 and slices were prepared at E16.5.
One frame per 30 min. Playback speed 7 frames/s. Compressed z-stacks spanning around 90 μm of cortical depth. Height of image is approximately 100 μm.

Movie 2: **Movement of bipolar cells in the RMZ of control, Rap1GAP and NCad^{DN} electroporated cortex.**
Cortices were electroporated at E14.5 and slices were prepared at E16.5.
One frame per 30 min. Playback speed 7 frames/s. Compressed z-stacks spanning around 90 μm of cortical depth. Height of image is approximately 100 μm.

Movie 3: **Movement of control, Rap1GAP and NCad^{DN} neurons migrating in vitro in lattice culture.**
One frame per 5 min. Playback speed 7 frames/s. Superimposed phase contrast and GFP epifluorescence images.

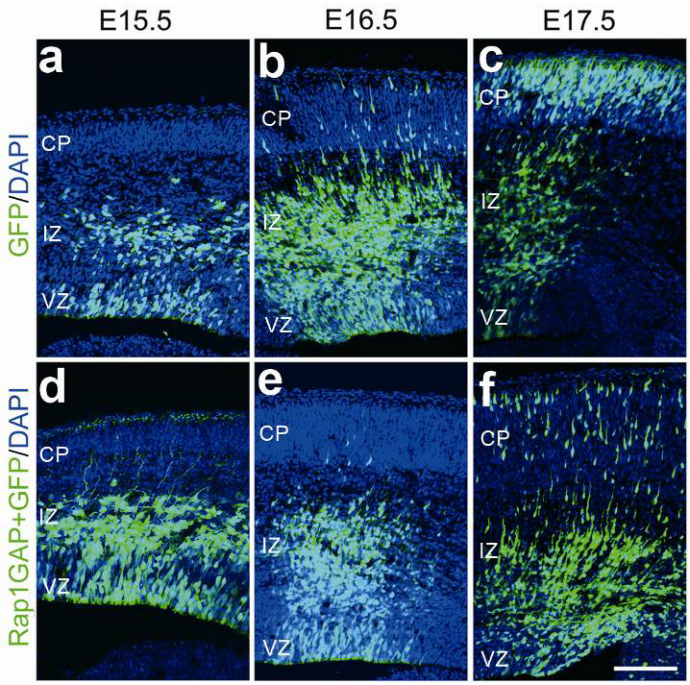

Figure S1. Rap inhibition has no obvious effect on early stages of migration.
(**a-f**) In utero electroporation at embryonic day 14.5 (E14.5) with GFP (**a-c**) or Rap1GAP and GFP (**d-f**). Positions of GFP-positive cells were observed at E15.5 (**a,d**), E16.5 (**b,e**) and E17.5 (**c,f**). CP, cortical plate; IZ, intermediate zone; VZ, ventricular zone. Blue: DAPI. Scale bar, 100 μm.

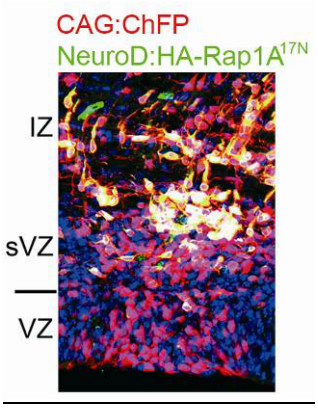

Figure S2. **NeuroD promoter is active in SVZ/IZ neurons but inactive in VZ progenitors.** Co-electroporation of CAG-ChFP with NeuroD-HA-Rap1A^{17N}. The NeuroD promoter is expressed in sVZ and IZ neurons but not VZ progenitors (green), while the CAG promoter is expressed throughout (red). IZ, intermediate zone; sVZ, sub-ventricular zone; VZ, ventricular zone. Blue: DAPI.

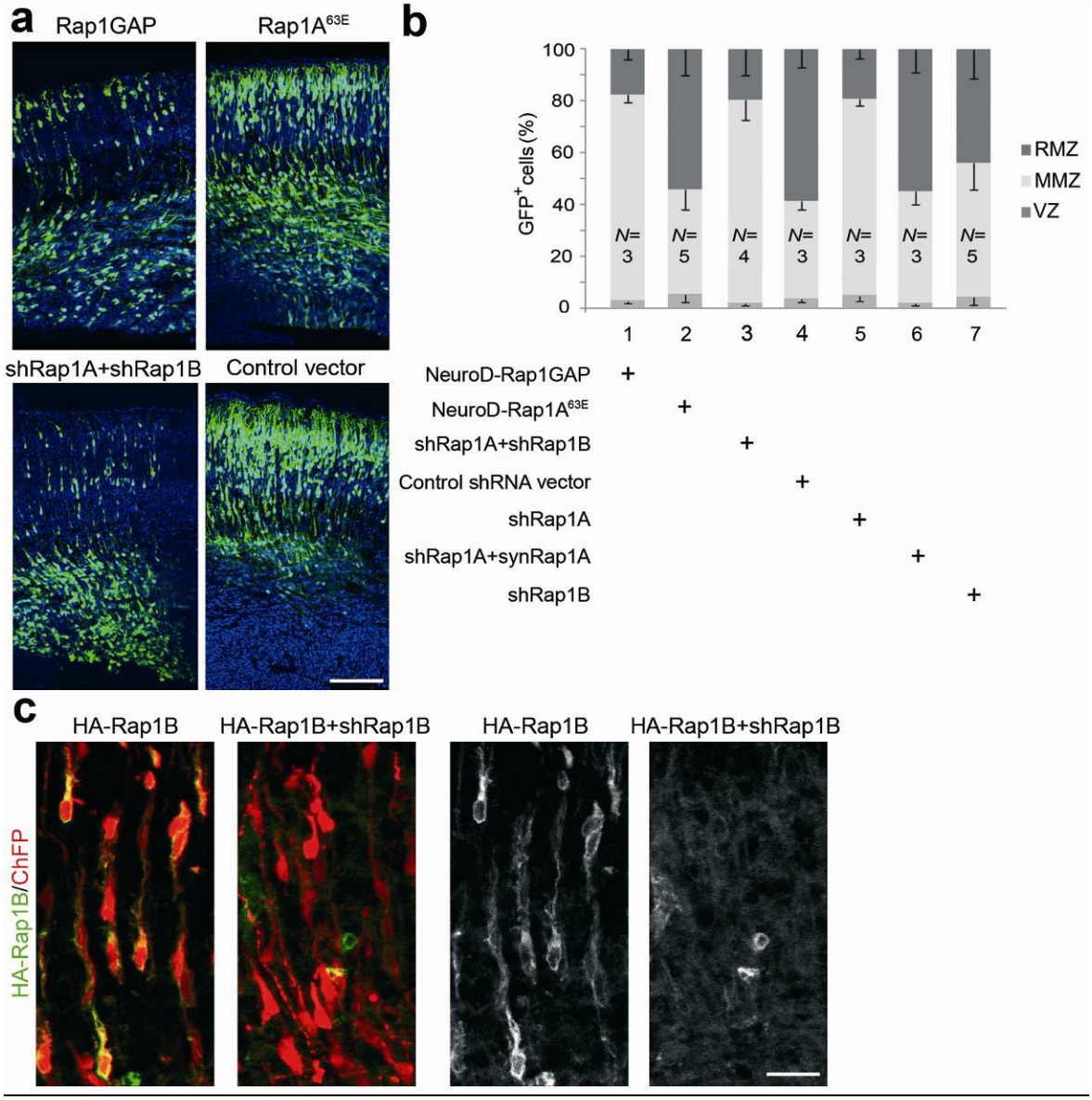

Figure S3. Effects of Rap1 inhibition in post-mitotic neurons and of shRNAs targeting Rap1A and Rap1B.

(a) In utero electroporation at E14.5 of the indicated genes under the NeuroD promoter and of shRNAs for Rap1A and Rap1B. Brains were analysed 3 days after electroporation. **(b)** Percentage of electroporated cells in each region. N is the number of individual electroporated embryo brains analyzed in a minimum of two independent electroporations. Rescue experiments for shRap1A were performed by co-electroporating a modified cDNA sequence of the Rap1A gene (synRap1A) that is not targeted by the shRNA. **(c)** Efficiency of Rap1B knock down. HA tagged Rap1B (green) was co-electroporated with CherryFP (red) and a control vector or Rap1B shRNA. Blue: DAPI. Scale bars, 100 μm in (a) and 30 μm in (c).

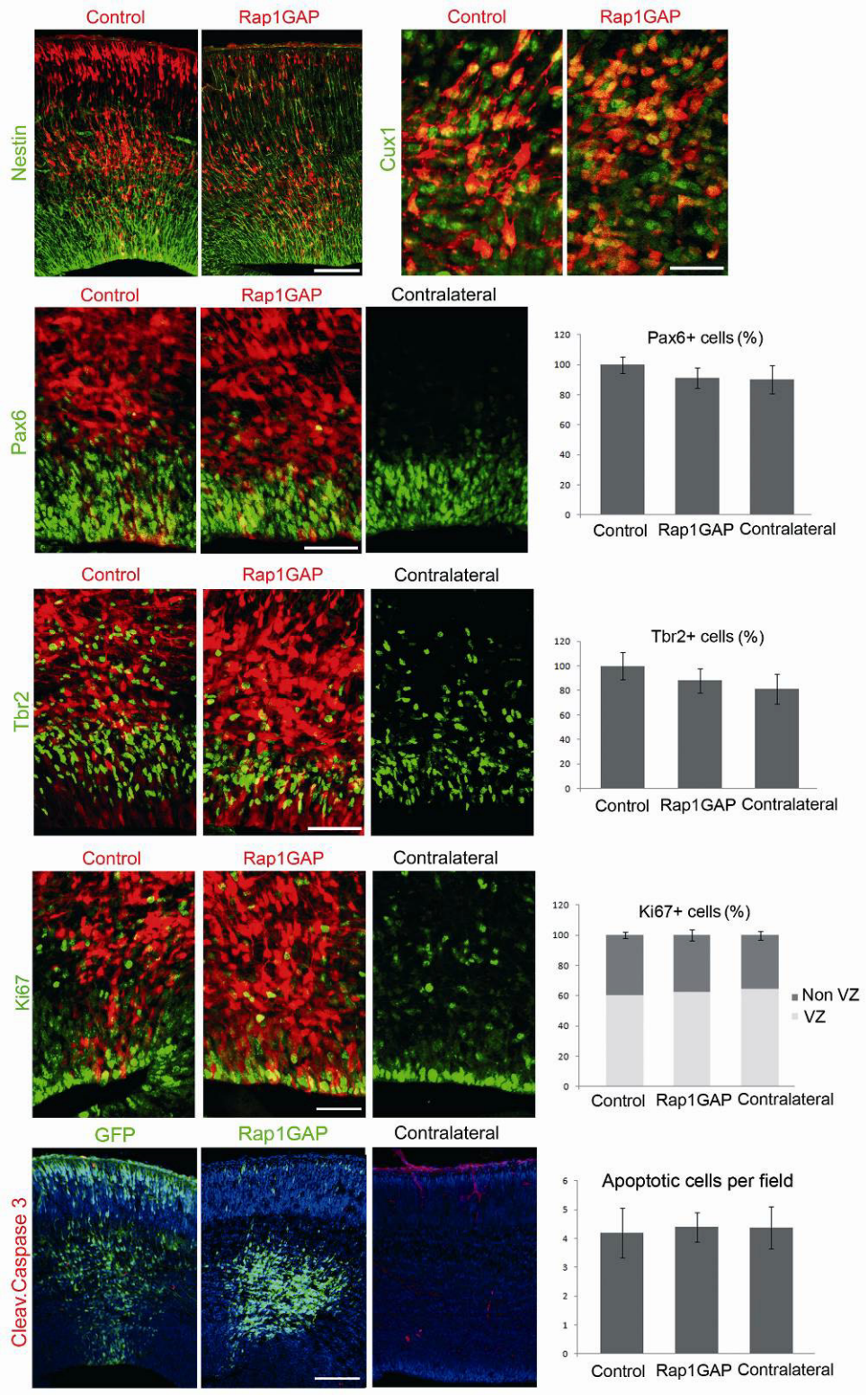

Figure S4. **Inhibition of Rap1 by in utero electroporation did not affect radial glia organization (Nestin), neuronal commitment (Cux1), apical (Pax6) or basal (Tbr2) progenitor cells, cell division (Ki67) or survival (cleaved Caspase-3).**

In utero electroporation at E14.5 with Rap1GAP and ChFP or GFP as indicated. Rap1GAP was expressed in progenitors and post-mitotic neurons under the CAG promoter. The results were quantified by counting the number of stained cells in a constant area of each section, and averaged across sections from at least 3 different embryos for each antibody. Immunostaining at E17.5 for the indicated markers Scale bars, 100 μm for Nestin and cleaved Caspase 3, 50 μm for Cux1, Pax6, Tbr2 and Ki67.

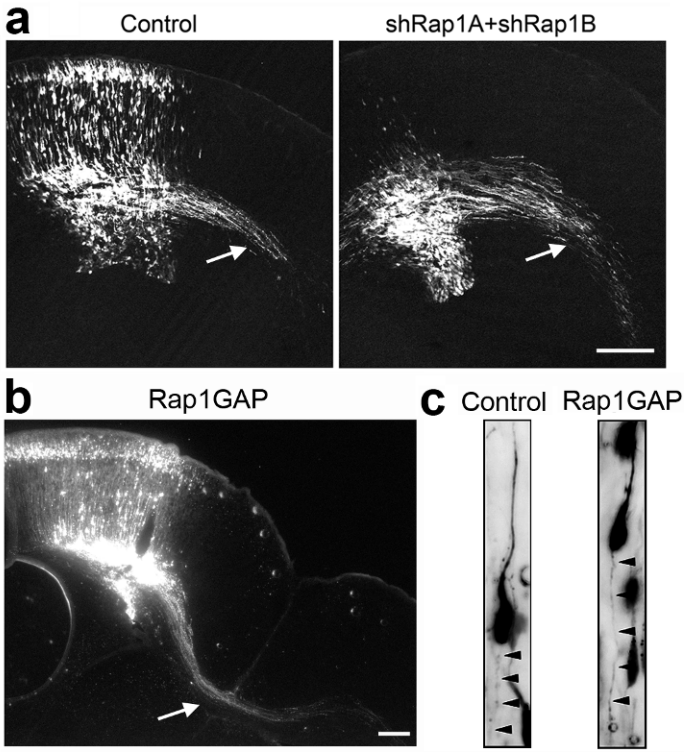

Figure S5. **Axonal growth is not inhibited when Rap is inhibited.**
Brains were electroporated at E14.5 with the indicated plasmids and collected 3 days later at E17.5 **(a)** or 5 days later at E19.5 **(b-c)**.
(a-b) Axons elongate and cross the midline. **(c)** Axons behind migrating bipolar neurons in the CP. Scale bars, 100 μm.

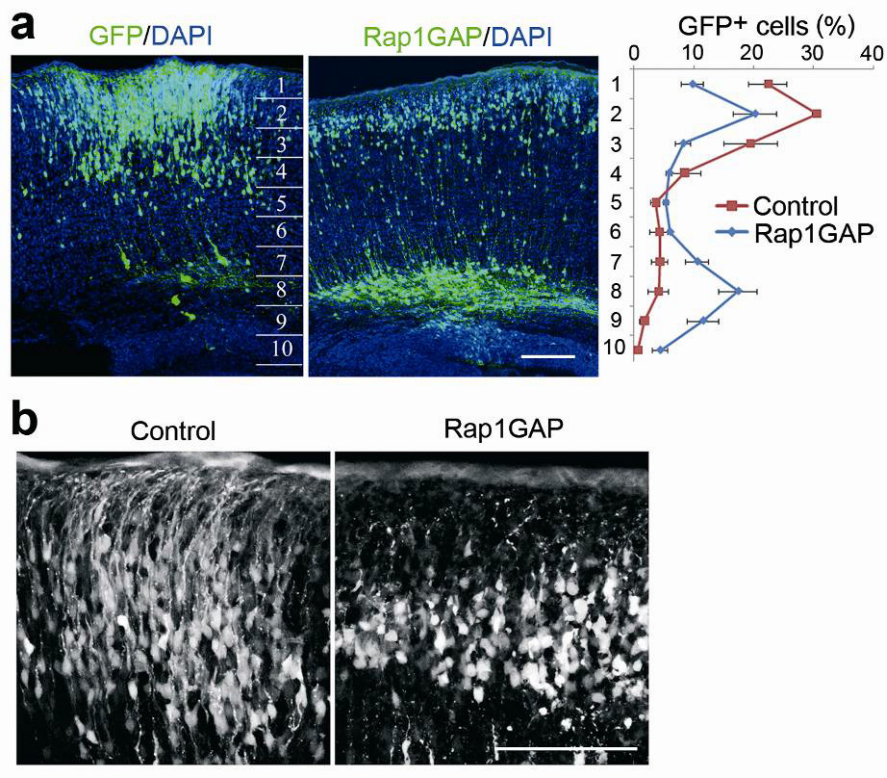

Figure S6. **Rap-inhibited cells that reach the top of the cortical plate exhibit defective apical dendrites**

(a) A population of Rap-inhibited cells reaches the top of the cortical plate at E19.5. The indicated plasmids were electroporated in utero at E14.5, and the distribution of GFP-positive cells examined at E19.5. The cerebral wall was divided into 10 equally sized bins. Percentage of electroporated cells in each bin (mean $\pm$ s.e.m., $N > 3$ brains).

(b) Rap1GAP inhibits apical dendrite formation. In utero electroporation at E14.5 with vector or Rap1GAP and GFP. GFP-expressing cells were observed at E19.5. Arrows indicate apical dendrites in the marginal zone of control but not Rap-inhibited cortex.

Scale bar, 100 μm in (a) and 50 μm in (b).

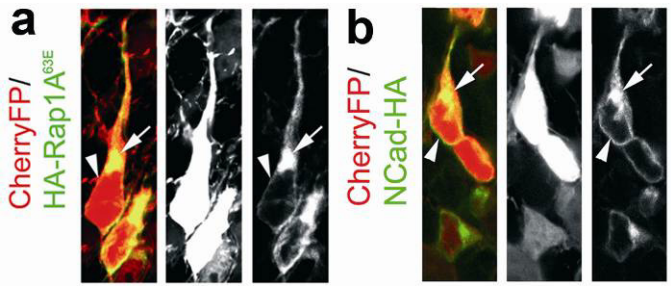

Figure S7. **Localization of Rap1A and NCad in bipolar neurons**.
In utero electroporation of ChFP and HA-Rap1A^{63E} **(a)** or HA-NCad **(b)**, followed by immunofluorescence with anti-HA antibodies (green) and visualization of ChFP (red). Note that both HA-Rap1A^{63E} and NCad-HA are present at the plasma membrane (arrowheads) and in front of the nucleus (arrows) of bipolar neurons in the lower RMZ.

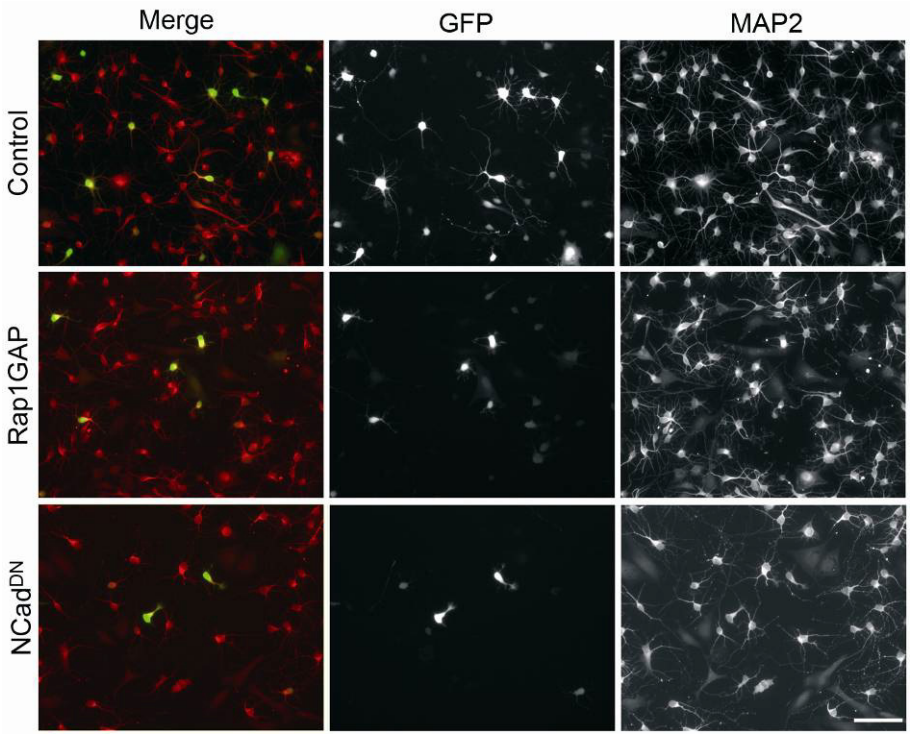

Figure S8. **GFP-expressing cells that bind to NCad-Fc in vitro are MAP2-positive neurons.** Dissociated E15.5 cortical neurons were electroporated with GFP and vector, Rap1GAP or $NCad^{DN}$ and incubated on dishes coated with NCad-Fc. After washing, the attached cells were immunolabeled for MAP2. Note co-expression of GFP and MAP2. Scale bar, 30 μm.

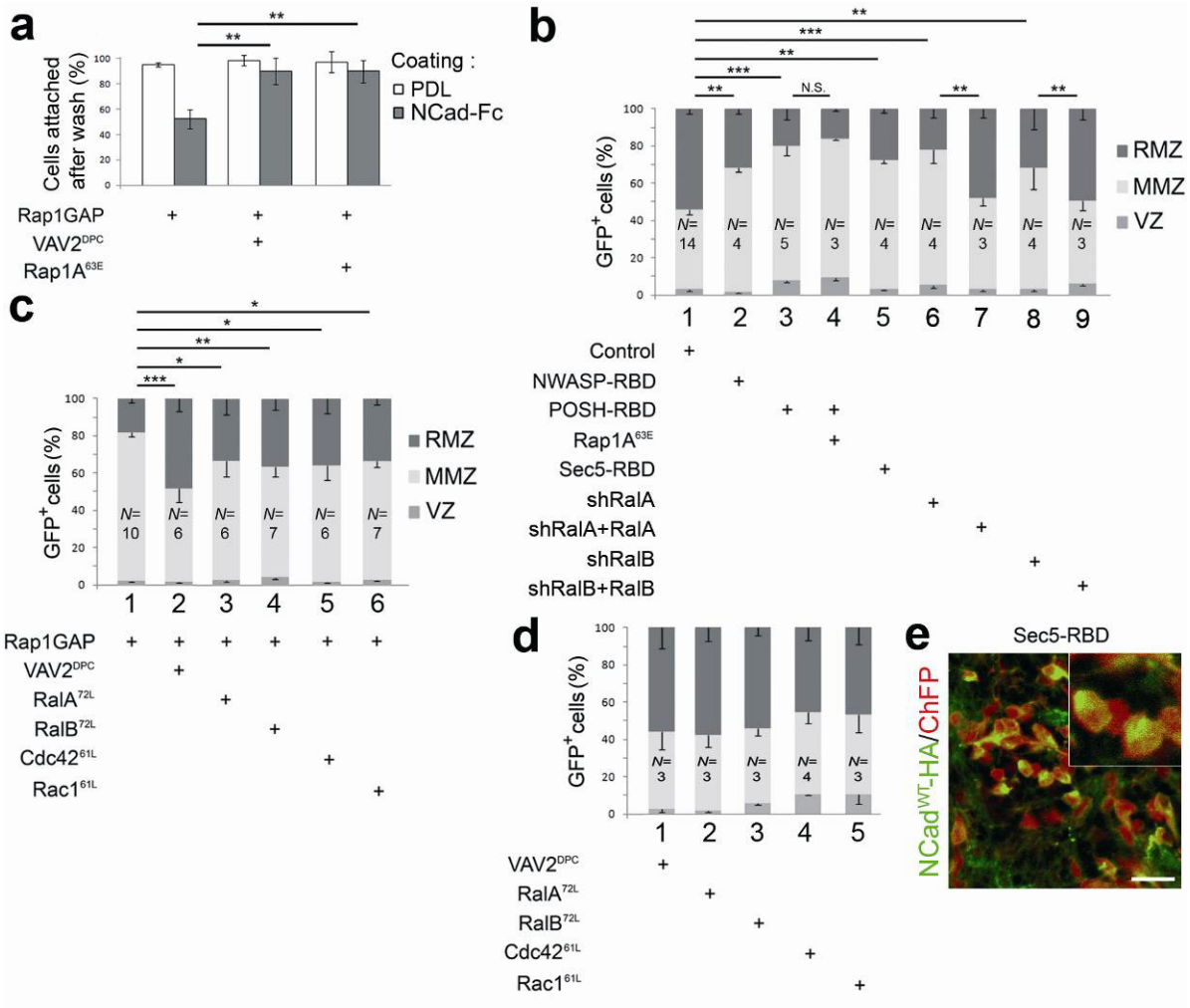

Figure S9. RalA/B, Rac1 and Cdc42 mediate Rap functions in neuron migration.
(a) E15.5 dissociated cortical neurons were electroporated with the indicated plasmids and incubated overnight on dishes coated with NCad-Fc or poly-D-lysine (PDL). GFP-expressing cells were counted before and after washes to determine the percentage of attached cells. **(b-d)** Indicated plasmids were electroporated in utero at E14.5 and the percentage of electroporated cells in each region scored at E17.5 (mean ± s.e.m.). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. N is the number of individual electroporated embryo brains analyzed in a minimum of two independent electroporations **(e)** HA antibody staining (green) of MMZ neurons that had been electroporated in utero with HA-tagged NCad, ChFP and Sec5RBD. Scale bar, 20 μm.

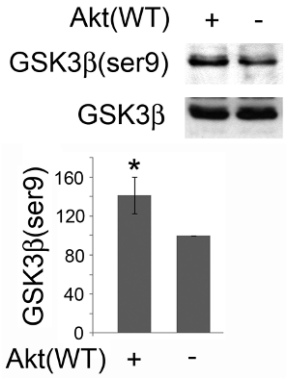

Figure S10. **Over-expression of Akt^{WT} increases Akt signalling.**
293T cells were transfected with a plasmid expressing Akt^{WT} under the CAG promoter, as used for in utero electroporations. Cells lysates were processed for Western blotting using antibodies for phosphorylated GSK3β(Ser 9) and GSK3β. The graph shows that over-expression of Akt^{WT} significantly increases the phosphorylation of GSK3β (mean $\pm$ s.e.m.). *, $P < 0.05$.

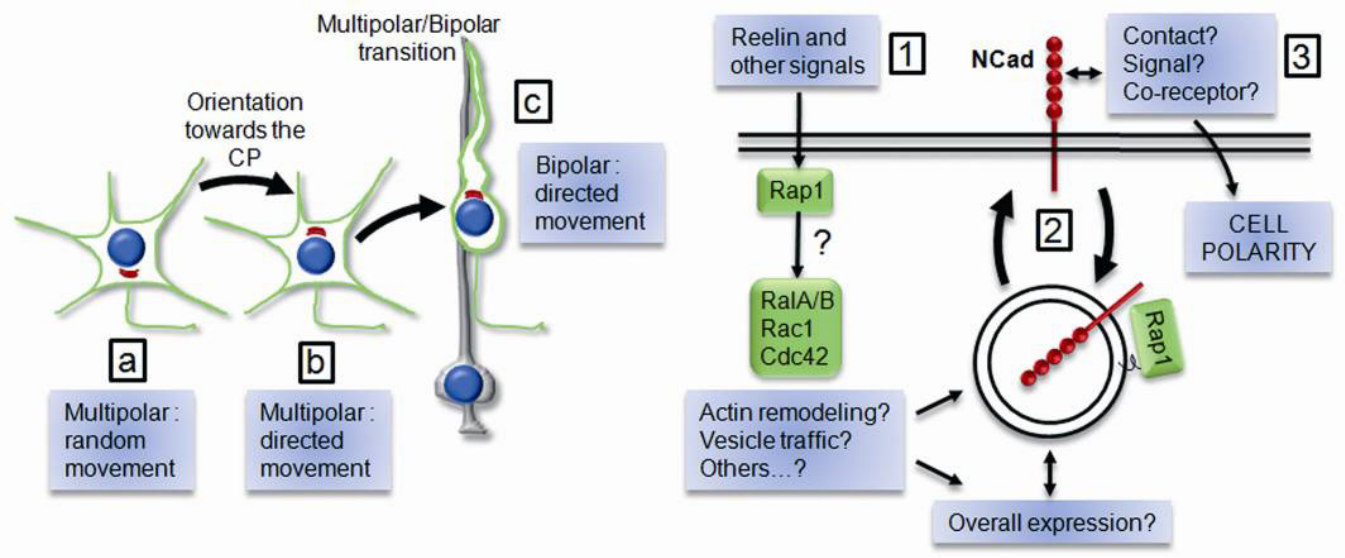

Figure S11. Model for orienting multipolar neurons.

(Left) Cellular events. (a) Most of the multipolar neurons in the MMZ (sVZ and lower IZ) move randomly. **(b)** When they encounter Reelin (and possibly other signals), they orient their Golgi apparatus and their direction of migration towards the cortical plate. **(c)** Later, they transition to bipolar morphology and commence locomotion along radial glia.
(Right) Molecular events. (1) Reelin (and possibly other signals) activate Rap1 and its target GTPases RalA/B, Rac1 and Cdc42. (2) NCad levels increase on the surface and decrease internally, likely due to changes in membrane traffic. (3) NCad on the cell surface helps sense directional cues in the environment that orient the Golgi apparatus and the direction of migration towards the cortical plate. Subsequent locomotion along radial glia appears to be largely independent of Rap and NCad. See text for discussion.