Supporting Information

Mandel et al. 10.1073/pnas.1113486108

SI Methods

Immunohistochemistry. Embryonic brains were directly fixed in 4% paraformaldehyde/PBS for 12 h, washed twice, and stored in PBS. Postnatal brains were fixed similarly after transcardial perfusion. Both fetal and postnatal brains were equilibrated in 30% sucrose/PBS solution, sectioned at 40-μm thickness, mounted on gelatin-coated slides, and subjected to immunohistochemistry. Primary antibodies: Chicken anti-GFP 1:500 (Abcam); mouse anti-NeuN 1:200 (Chemicon); mouse anti-Ki67 1:50 (BD Pharmingen); goat anti-DCX 1:100 (Santa Cruz); mouse anti-GFAP 1:500 (Chemicon); rabbit anti GFAP 1:500 (DAKO); mouse anti-MAP2 1:1:200 (Chemicon); rabbit anti-TBR2 1:1,000 (Chemicon); mouse anti-nestin 1:500 (Chemocon); rabbit anti-RESTC 1:1,000 (generated against the last 300 amino acids of the C terminal); rabbit anti-REST p73 1:1,000 (9); rabbit anti-BLBP 1:1,500 (gift from N. Heintz, Rockefeller University, New York, NY); and rabbit anticleaved Caspase3 1:200 (Cell Signaling). Secondary donkey antihost antibodies conjugated to cyanine (Jackson ImmunoResearch) were used as recommended by the manufacturer. Cell nuclei were stained with Hoechst 33258 or ToPro3 (Molecular Probes). Images were collected on a Zeiss confocal laser scanning LSM 510 microscope.

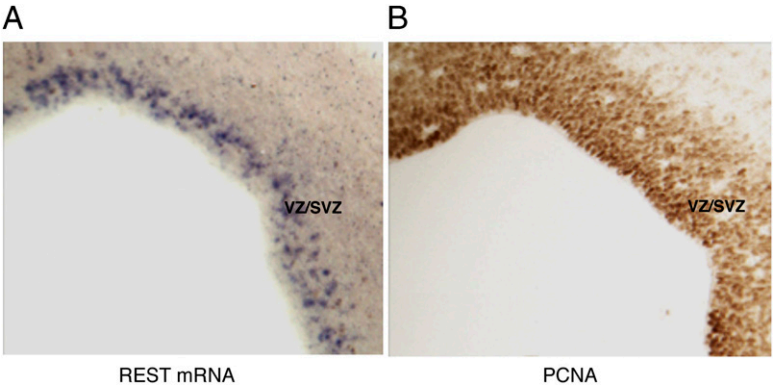

Fig. S1. RE1 silencing transcription factor (REST) mRNA expression is restricted to NS/P cells of the ventricular zone (VZ). Representative images of paraffin-embedded coronal sections of E14 brain. (*A*) In situ hybridization with antisense probe (1) indicating REST mRNA expression in the VZ area. (*B*) immunostaining (DAB staining) for the cell-division marker PCNA showing that REST is expressed in the VZ where progenitors cells are actively dividing.

1. Grimes JA, et al. (2000) The co-repressor mSin3A is a functional component of the REST-CoREST repressor complex. *J Biol Chem* 275:9461–9467.

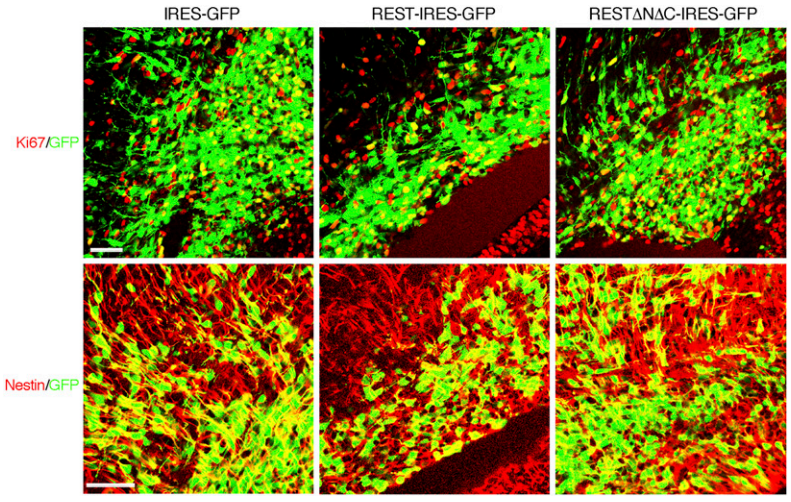

Fig. S2. Cells expressing the different transgenes are mostly at the neural stem/progenitor (NS/P) cell stage, two days post electroporation. Immunostaining of representative coronal sections of E16 brains. Ki67 (red), cell-division marker; nestin (red), NS/P marker. (Scale bars, 50 μm.)

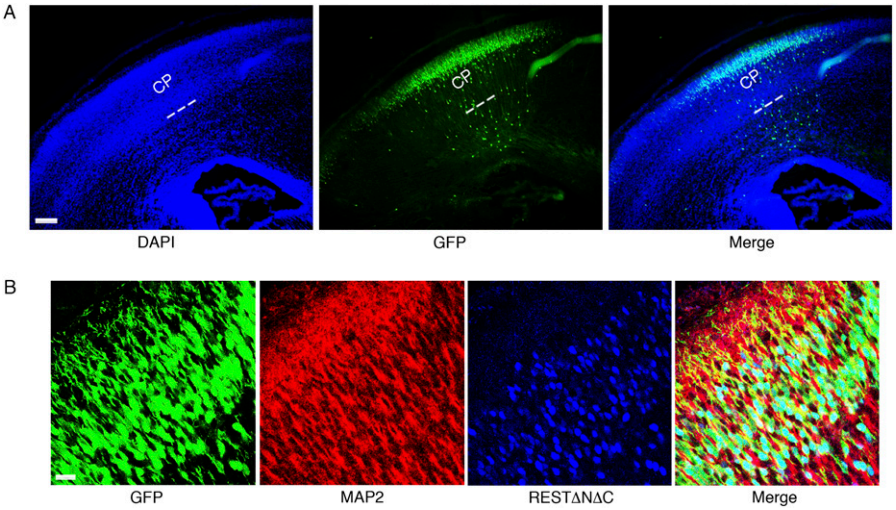

Fig. S3. The RESTΔNΔC-expressing cells migrated to the cortical plate (CP) 4 d postelectroporation. (*A*) Expression of GFP (green) in representative coronal brain sections, 4 d postelectroporation (E14–E18). DAPI represents nuclear staining and serves as a marker for the different cortical layers. (Scale bar, 200 μm.) (*B*) Immunostaining of the RESTΔNΔC-expressing cells for GFP (green), MAP2 (red), and RESTΔNΔC (blue), in representative coronal section of E18 mouse brains. (Scale bar, 20 μm.)

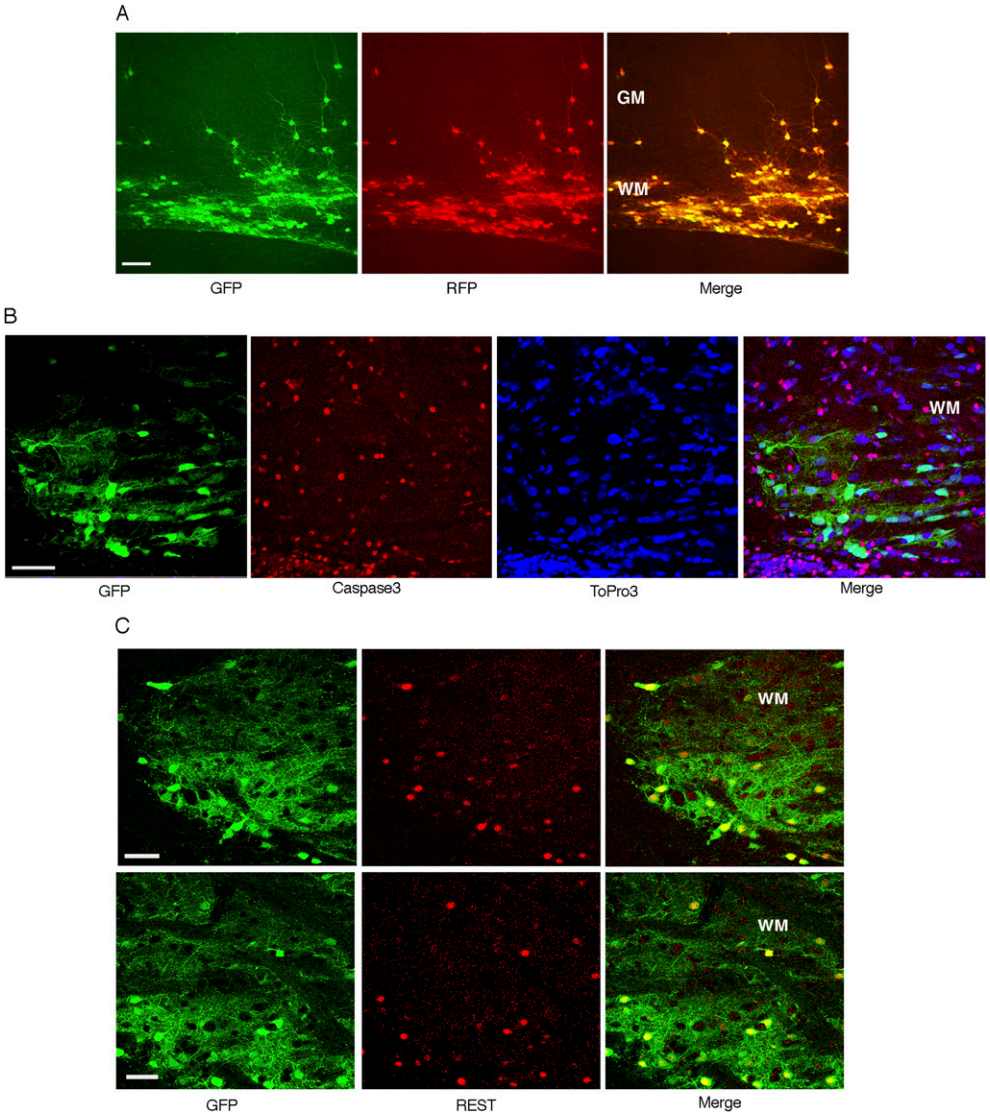

Fig. S4. At postnatal stages, the electroporated cells still express REST and are not apoptotic. (*A*) Coexpression of mRFP and GFP at P14. Images of coronal sections from P14 brains coelectroporated at E14 with mRFP and REST-IRES-GFP cDNAs. Note the colocalization of mRFP and GFP in the merged image. (Scale bar, 100 μm.) (*B*) Cells expressing REST-IRES-GFP are not apoptotic. Images of coronal brain sections for the apoptotic marker Caspase3. ToPro3 represents nuclear staining. Brains were electroporated at E14 with REST-IRES-GFP cDNA and harvested at P23. (Scale bar, 50 μm.) (*C*) The electroporated cells still express REST at P23. Images of immunostaining of coronal brain sections for REST and GFP. Brains were electroporated at E14 and harvested at P23. (Scale bars, 50 μm.) WM, white matter; GM, gray matter.

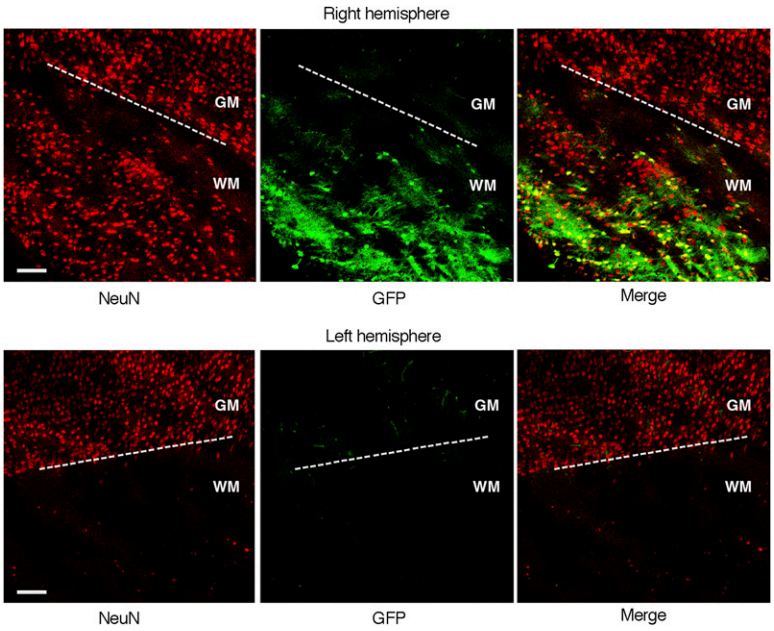

Fig. S5. A noncell-autonomous effect of the arrested REST/GFP-expressing cells. Brains were electroporated with REST-IRES-GFP at E14 and harvested at P21. Representative brain sections immunostained with anti-GFP (green) and anti-NeuN (red) antibodies. Dotted lines represent the border between the white matter (WM) and gray matter (GM). Note the cells in the WM of the electroporated right hemisphere (*Upper*) that are $NeuN^+$ but GFP^-, but the WM of the noninjected left hemisphere (*Lower*) lacks $NeuN^+$ cells. (Scale bars, 100 μm.)

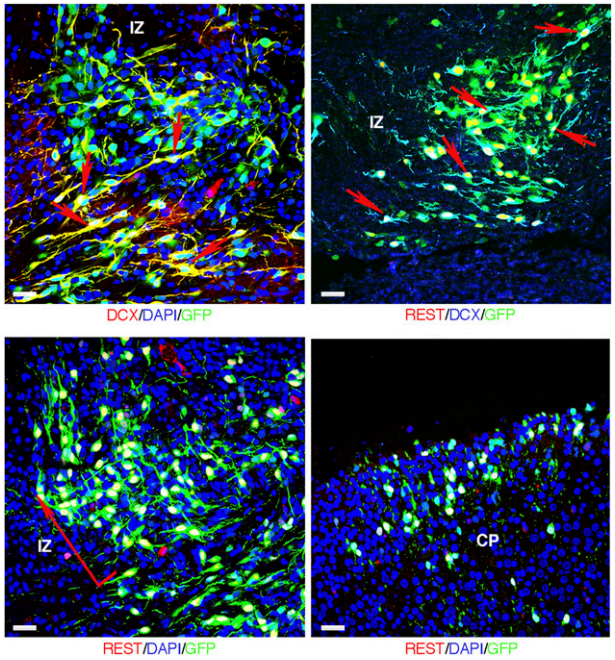

Fig. S6. The radially migrating cells in the intermediate zone (IZ) and the neurons in the CP, which were rescued by doublecortin (DCX), still express REST with GFP. Brains were coelectroporated with REST-IRES-GFP and DCX at E14 and harvested at E19. Representative brain sections immunostained for GFP DCX or REST, as indicated. DAPI represents nuclear staining. (*Upper*) Red arrows show examples of GFP/DCX-expressing cells, which are bipolar. (*Lower Left*) Red arrow shows radial migration. (Scale bars, 50 μm.)

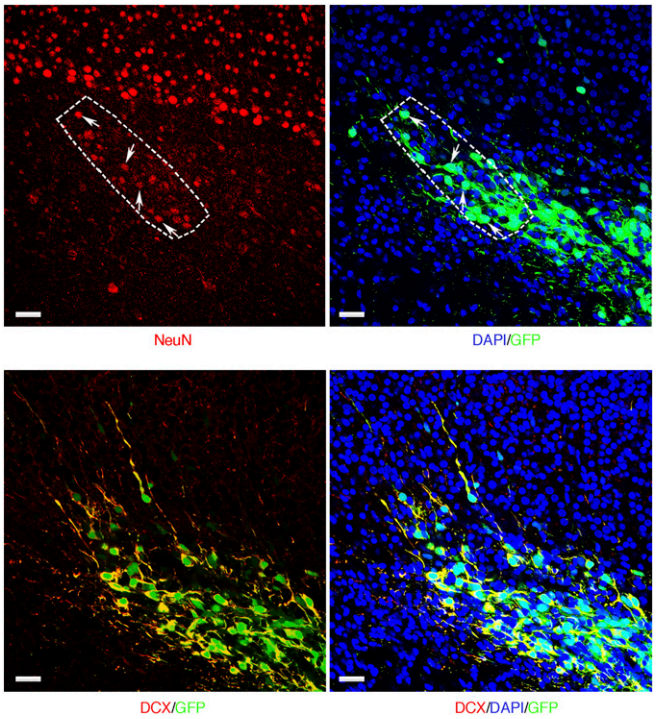

Fig. S7. The migrating REST- and DCX-expressing cells differentiate into neurons. Brains were coelectroporated with REST-IRES-GFP and DCX at E14 and harvested at E19. Representative brain sections immunostained for GFP (green) and DCX or NeuN (red), as indicated. DAPI represents nuclear staining. Dotted oval lines show the migrating cells that are $NeuN^+$. Arrows show examples of cells that are both GFP^+ and $NeuN^+$. (Scale bars, 50 μm.)