Supplemental Experimental Procedures

In Situ Hybridization

Sense and anti-sense probes, targeted against nucleotides 1303-1740 of mouse MEKK4 cDNA (Accession #: U85607), were the same as those described recently (Chi et al., 2005). Embryo and adult brains were fixed in DEPC-treated 4% paraformaldehyde in 0.1 M phosphate buffer (4% PFA), digested with proteinase K, and then hybridized to digoxigenin (DIG)-labeled probes (Roche) at 60 °C overnight (o/n). The signal was detected with alkaline phosphatase-conjugated anti-DIG (Roche) and bound antibody was visualized using BM purple-precipitating reagents (NBT/BCIP) as suggested by the manufacturer (Roche).

TUNEL Analysis

For terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling (TUNEL) assays, brains were fixed o/n in 4% PFA, cryoprotected in sucrose and frozen over liquid N_2. Coronal sections (20µm) were processed in accordance with manufacturer's instructions using an ApopTag® Apoptosis Detection Kit (Serological Corporation). Sections were counterstained with TO-PRO-3 iodide (Molecular Probes).

Supplemental Figures and Legends

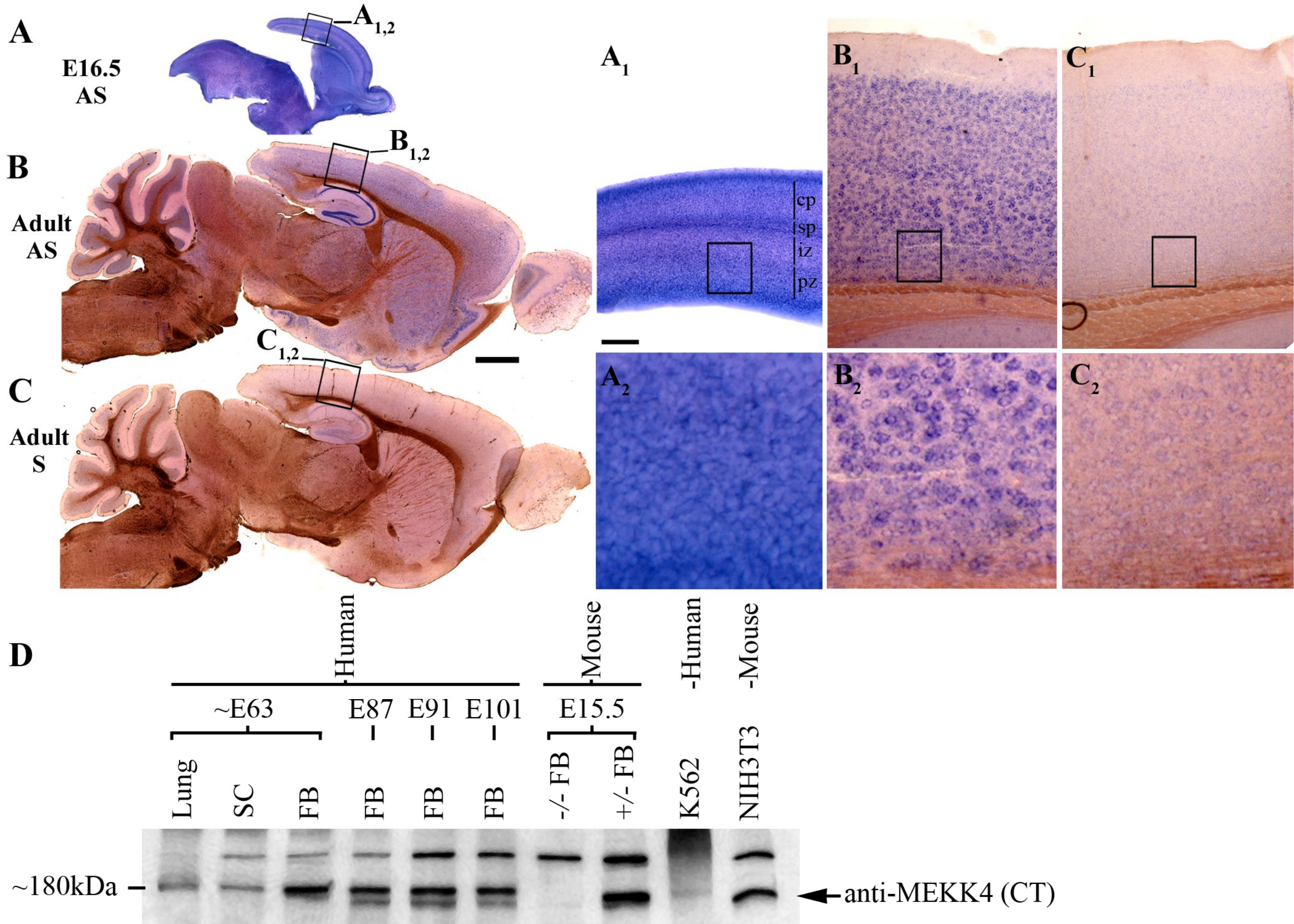

Figure S1. MEKK4 expression in embryonic and adult brain

(**A**) In situ hybridization of an E16.5 sagittal brain section showed MEKK4 antisense (AS) probe localization (blue signal) throughout the brain. Magnified boxed or comparable areas show expression throughout the telencephalic wall (**$A_{1,2}$**). Bar in **A_1**= 100 µm.

(**B**) MEKK4 AS probe in adult sagittal brain sections showed expression throughout the brain (AS signal is purple, brown is staining artifact). Higher magnification of the neocortex (**$B_{1,2}$**) shows expression in all lamina. Bar = 1000µm.

(**C**, **C_1**,, and **C_2**) Sense (S) probe control on adult section.

(**D**) Western blot of human and mouse fetal tissues probed with an anti-MEKK4 antibody. Twenty µg total protein/lane was run for ~E63 lung, spinal cord (SC), forebrain (FB), E87, 91 and 101 FB and E15.5 MEKK4 knockout (-/-) and heterozygous (+/-) FB. A band of approximately 180kDa was absent in *MEKK4-/-* mice. K562 and NIH3T3 were run as positive controls.

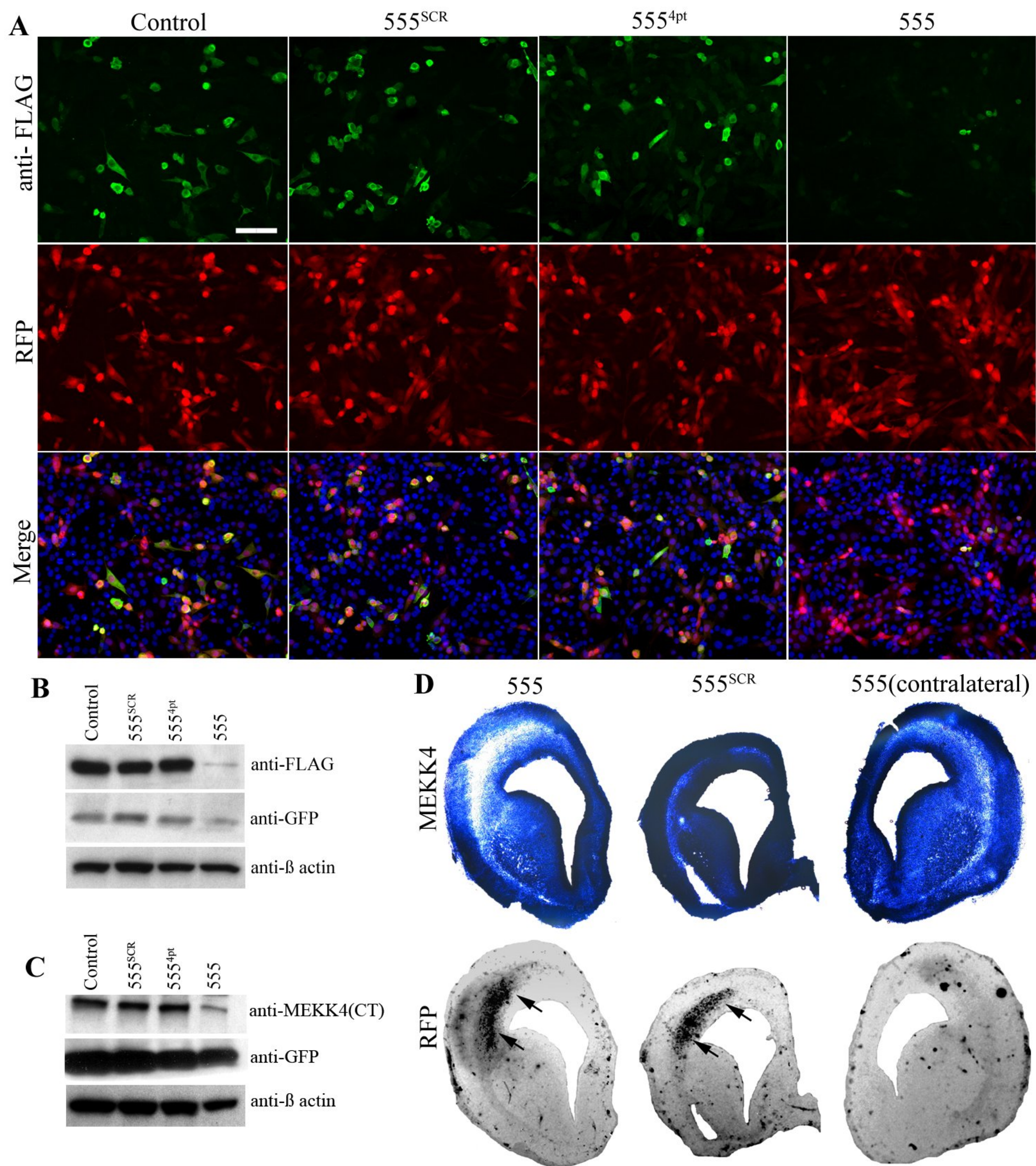

Figure S2. siRNA#555 reduces mouse MEKK4 expression in vitro and in vivo
(**A**) NIH3T3 cells were transfected with plasmids expressing full length FLAG-MEKK4, RFP and either siRNA555^{4pt}, siRNA555^{SCR}, or siRNA555. Cells were fixed 72h post-transfection and immunostained

for FLAG (green). siRNA#555 dramatically reduced FLAG immunostaining compared to FLAG-MEKK4 (control), siRNA#555^{SCR} or siRNA#555^{4pt}. Bar = 50μm.

(**B**) Western blot analysis of similar transfections (GFP was used as a transfection control instead of RFP) confirmed dramatic reduction of FLAG-MEKK4.

(**C**) NIH3T3 were transfected with GFP alone (control) or GFP plus either siRNA555^{4pt}, siRNA555^{SCR}, or siRNA555. Blots were probed for endogenous MEKK4 using an anti-MEKK4 (C-terminus (CT)) antibody. Endogenous MEKK4 was reduced after transfection with siRNA#555. Residual MEKK4 was likely from untransfected cells.

(**D**) E14.5 cerebral cortex was electroporated with siRNA#555^{SCR} and RFP or siRNA#555 and RFP. In situ hybridization for MEKK4 mRNA 48h later showed reduced MEKK4 in the region of siRNA#555 and RFP (arrows; RFP+ cells were converted in Adobe Photoshop 7.0 to black and white) compared to siRNA#555^{SCR} or the contralateral hemisphere (RFP-).

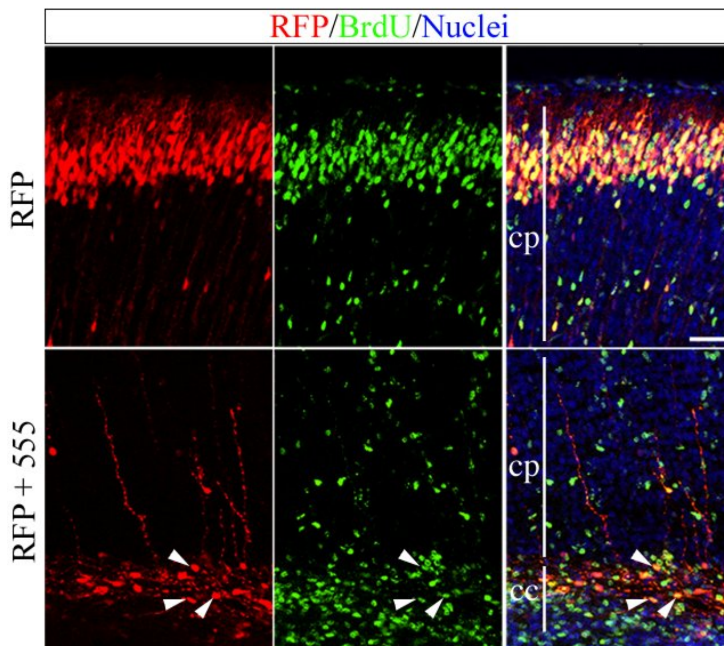

Figure S3. Many RFP+/BrdU+ cells beneath the cortical plate after MEKK4 siRNA.
In utero electroporation of fetuses at E14.5, exposure of dams to BrdU at E15.5, and analysis of electroporated brains at P0. After electroporation of RFP alone (upper panels), most RFP+/BrdU+ cells were in the CP (upper panels). After electroporation with RFP plus siRNA#555 (lower panels) most RFP+ cells in the CC were BrdU+ (arrowheads). Bar = 50μm.

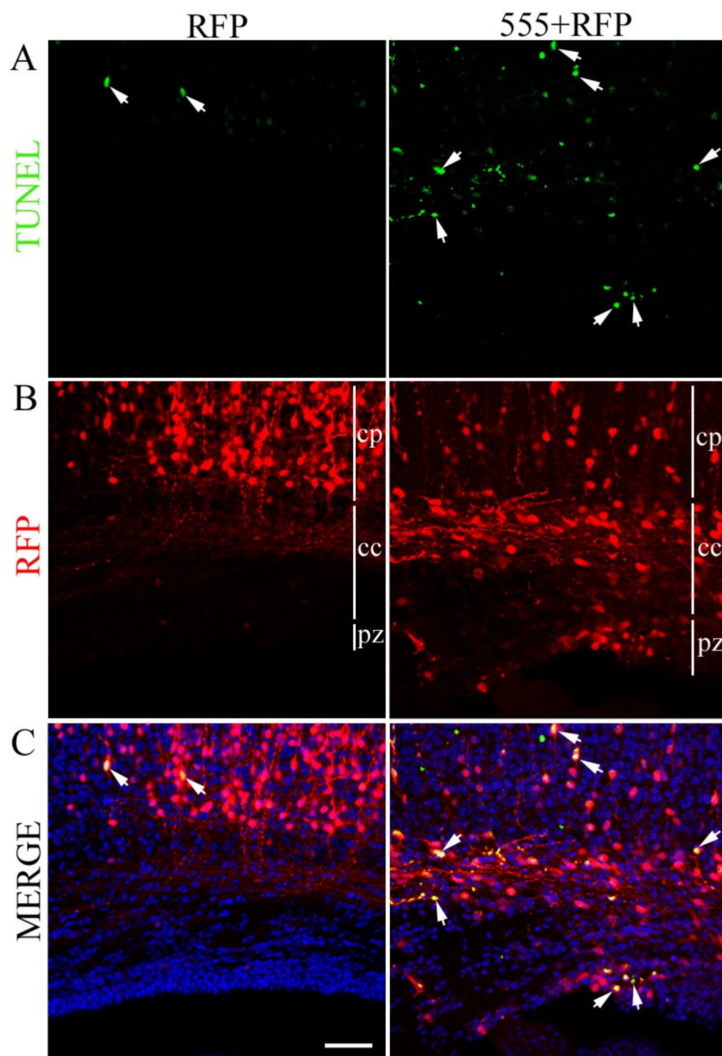

Figure S4. Enhanced apoptosis in the forebrains of P0 mice electroporated with MEKK4 siRNA

(**A**) TUNEL staining revealed increased TUNEL+ cells (arrows) throughout the telencephalic wall after transfection with siRNA#555 compared to RFP alone.

(**B**) RFP+ cells were largely confined to the CP after transfection with RFP alone while siRNA#555 caused many RFP+ cells to get stuck in the CC and PZ.

(**C**) A merged view of TUNEL staining, RFP and nuclei (labeled with TOPRO (blue)) shows numerous TUNEL+ cells were located within the PZ, CC and CP compared to TUNEL+ cells in RFP which were in the CP. Bar = 50μm.

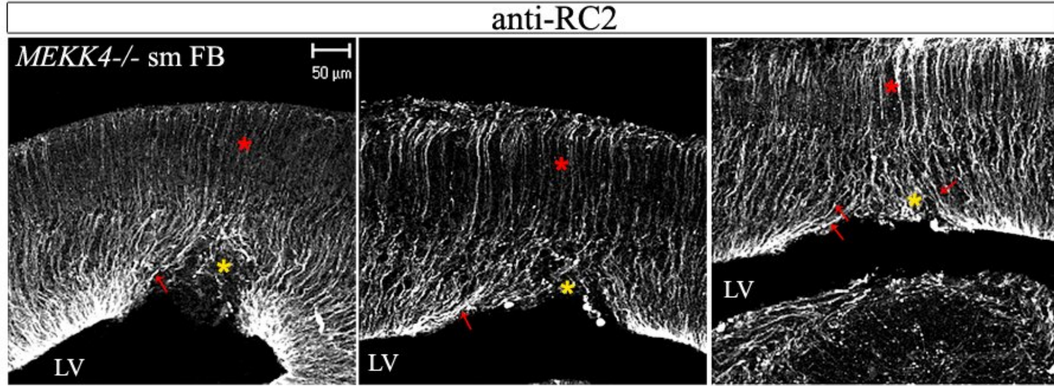

Figure S5. Gross defects in the radial glial fiber pattern at the *MEKK4-/-* ventricular surface but not in overlying cortical plate

Examples of RC2-immunostaining in several regions of E18.5 *MEKK4-/-* small FB. Breaks in the lining of the ventricular surface were associated with disrupted radial glia (yellow asterisks). Adjacent regions showed RC2+ fibers (red arrows) that navigated around disrupted areas to establish an organized pattern of radial glial fibers in the cortical plate (red asterisks).

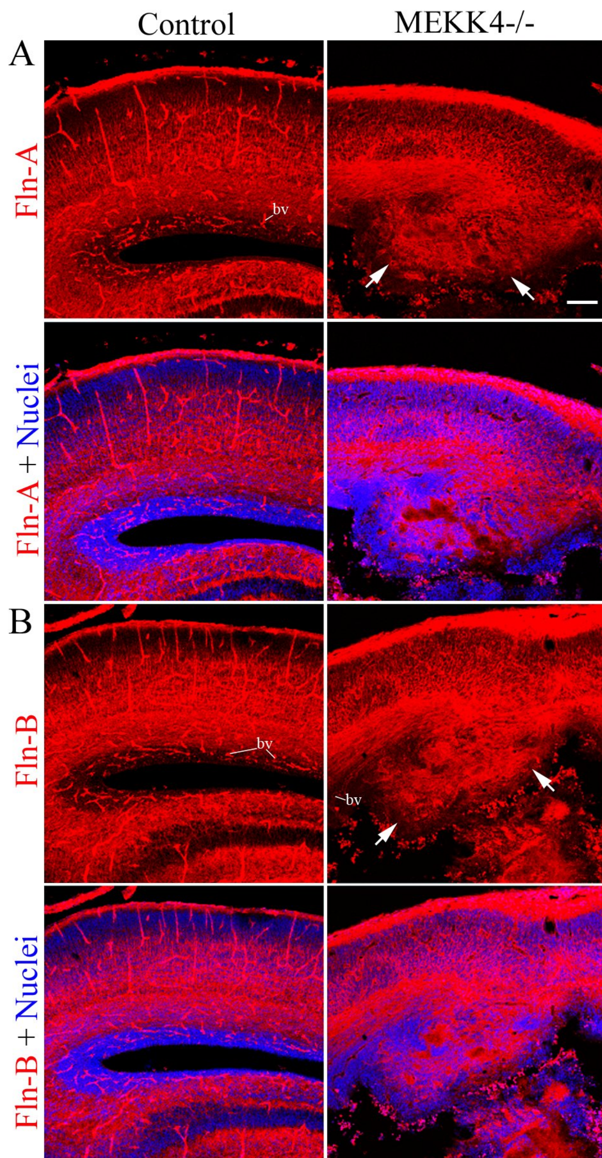

Figure S6. Expression of Fln-A and Fln-B in PVH of E17.5 *MEKK4-/-* mice

(**A**) Anti-FLN-A immunostaining (mAb(4-4) showed presence of Fln-A largely in the CC and CP in control but persisted into PVH (arrows) of *MEKK4-/-* mice. Blood vessels (BV) were also detected with this antibody. Lower panels show counterstaining of nuclei with TO-PRO-3.

(**B**) Anti-Fln-B immunoreactivity showed a similar pattern to Fln-A in controls and also persisted into PVH (arrows) of *MEKK4-/-* mice. Bar= 100µm.

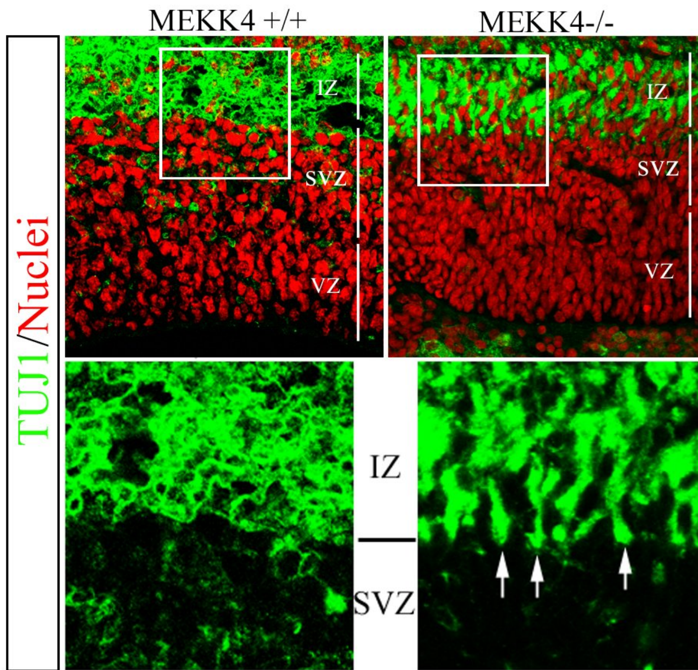

Figure S7. Radially-oriented TUJ1+ fibers in *MEKK4-/-* intermediate zone
TUJ1 immunoreactivity (green) and propidium iodide-labeled nuclei (red) in E18.5 *MEKK4*+/+ and -/- forebrain (upper panels). TUJ1 was strongly expressed in the IZ of both mice, however, the morphology of fibers at the SVZ/IZ transition appeared more radially-oriented in -/- IZ. Higher magnification of the boxed areas (lower panels) showed processes that appeared more bipolar and thickened (arrows) than +/+ fibers.

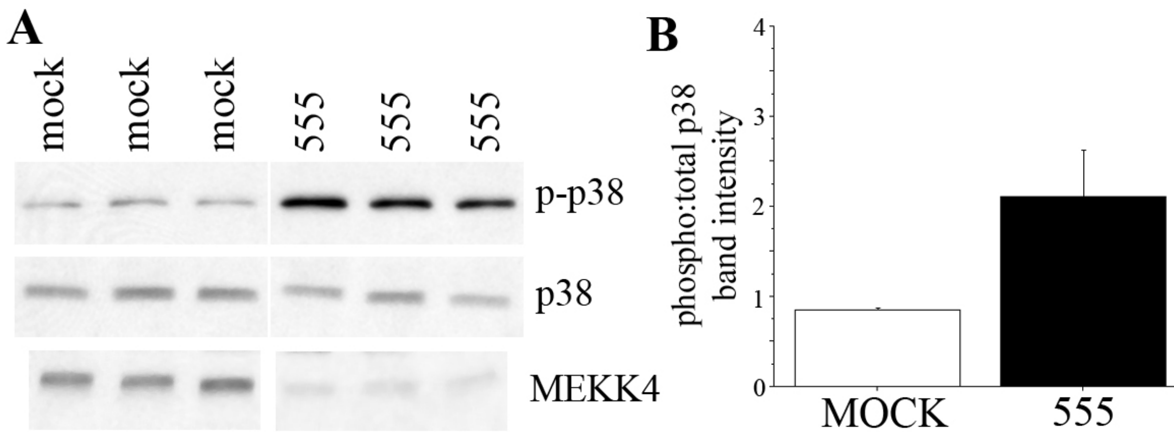

Figure S8. Enhanced p38 phosphorylation after MEKK4 RNAi.
(**A**) NIH3T3 cells were mock or siRNA#555-transfected and cultured for 96h. Lysates (20µg/lane) from different mock and siRNA#555 (555)-transfected wells were western blotted and probed with phospho-p38 (upper blot), total p38 (middle blot) and MEKK4 (lower blot) and antibodies. Increased phosphorylation of p38 is observed after transfection with siRNA#555.
(**B**) The relative phospho- to total p38 band intensity was quantified for five separate transfections/groups which showed increased p38 phosphorylation in siRNA#555-transfected cultures.