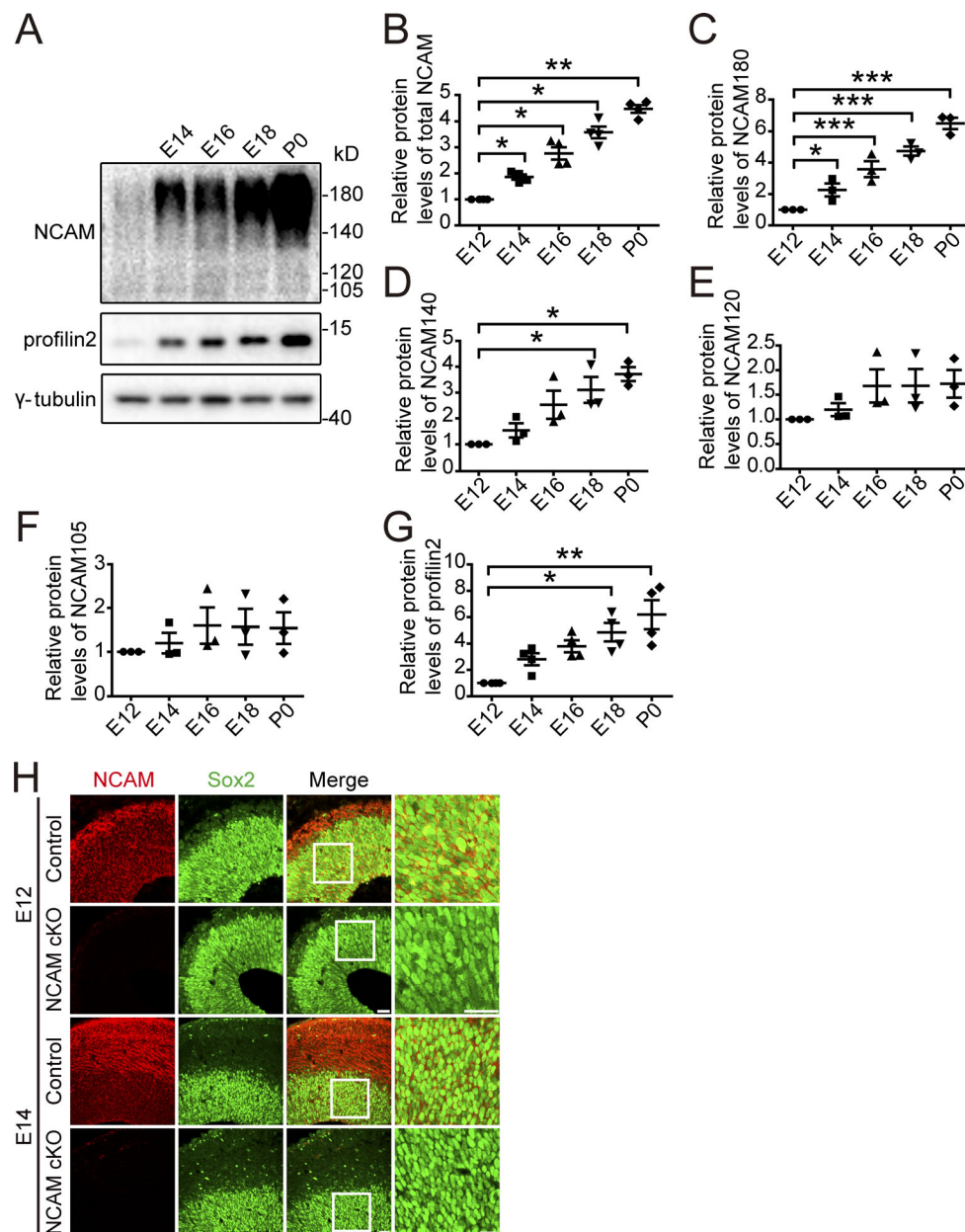# Supplemental material

Figure S1. **Expression of NCAM and profilin2 in the developing cerebral cortex. (A–G)** Western blot analysis of NCAM and profilin2 expression in E12, E14, E16, E18, and P0 mouse cortices. γ-Tubulin served as a control. The protein levels in E14, E16, E18, and P0 mouse cortices were quantified relative to the protein levels in E12 mouse cortices set to 1.0. $n = 3$ or 4 biological replicates (total NCAM and profilin2, respectively). **(H)** Coronal sections of control and NCAM-cKO mouse cortices were coimmunostained for NCAM and Sox2 at E12 and E14. Scale bars, 20 µm. Values represent mean ± SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (two sided). One-way ANOVA with least significant difference correction (C and F), with Dunnett's T3 correction (B, D, and E), or Kruskal-Wallis test with Dunn-Bonferroni post hoc comparison (G).
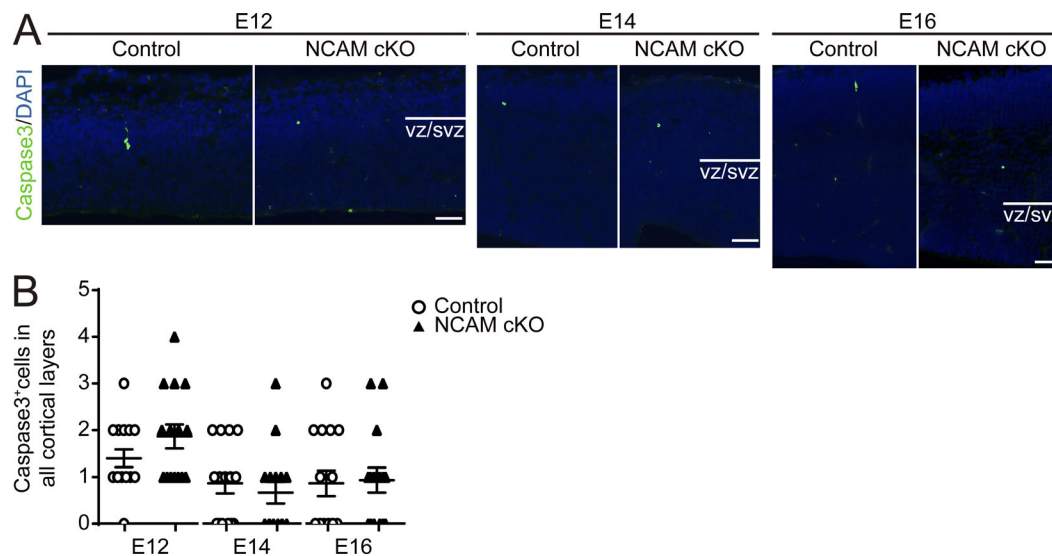
Figure S2. **NCAM deficiency does not lead to increased NPC apoptosis during embryonic development. (A)** Coronal sections of E12, E14, and E16 control and NCAM-cKO cortices were immunostained for activated, cleaved caspase3 and counterstained with DAPI. **(B)** Numbers of caspase3$^+$ cells in the entire hemitelencephalon cortex. Mean ± SEM values. n = 15 brain slices from three mice. Mann-Whitney test did not reveal a statistically significant differences between groups. Scale bars, 50 µm.
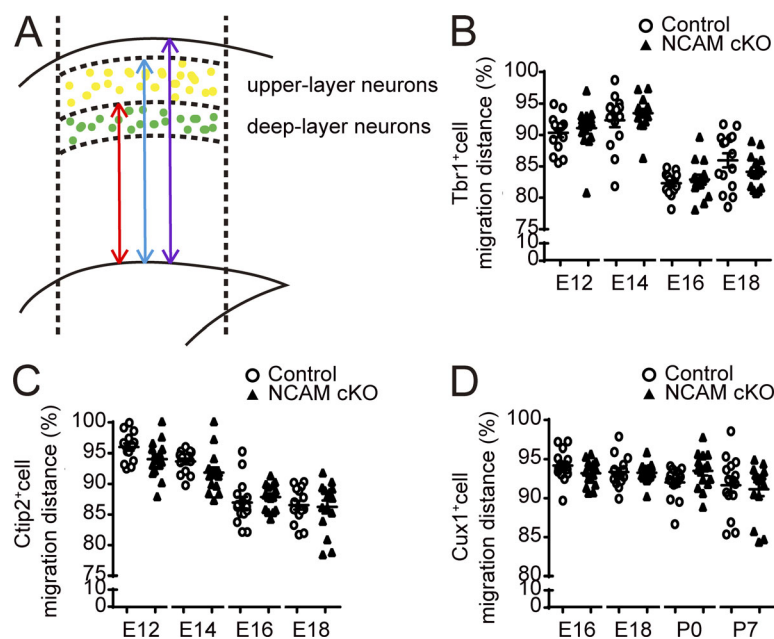

Figure S3. **NCAM deficiency does not affect the distribution of neonatal cortical neurons in the coronal plane. (A)** The cortical neuron distribution was analyzed by the maximum migration distance of deep-layer (red arrow) or upper-layer (blue arrow) neurons from VZ to cortical surface/total cortical length (purple arrow). **(B–D)** Percentages of the maximum migration distance of Tbr1$^+$ (B), Ctip2$^+$ (C), and Cux1$^+$ (D) neurons in total cortical length. Mean ± SEM values. n = 15 brain slices from three mice. Student's t test or Mann-Whitney test (E12 and E14 in B and E18 in D).
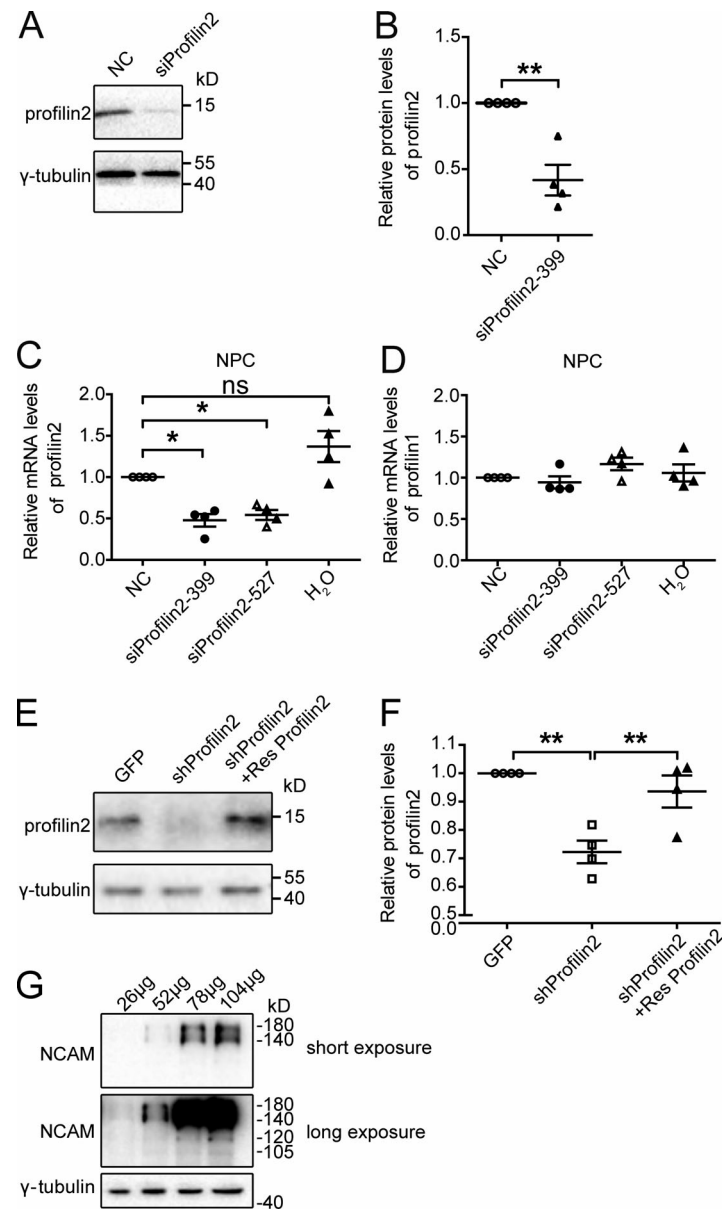
Figure S4. **Profilin2 expression is downregulated specifically by profilin2 RNAi. (A)** Western blot analysis of profilin2 levels in Neuro-2a cells transfected with either siProfilin2 or NC. **(B)** Levels of profilin2 in siProfilin2-transfected cells relative to those in NC-transfected cells, which were set to 1.0. **(C and D)** Quantitative PCR analysis of the levels of profilin2 (C) or profilin1 (D) mRNA in cultured NPCs transfected with either siProfilin2 (399 or 527) or NC. The mRNA levels of profilin2/1 in NC-transfected NPCs were set to 1.0. **(E and F)** Western blot analysis of profilin2 levels in Neuro-2a cells transfected with scrambled shRNA (GFP) or profilin2 shRNA (shProfilin2) only or cotransfected with shProfilin2 and shRNA-resistant profilin2 (Res Profilin2). The levels of profilin2 protein were quantified relative to those in GFP-transfected cells set to 1.0. **(G)** NCAM levels in brain homogenates loaded in different quantities (26, 53, 78, and 104 µg). Values represent mean ± SEM. $n = 4$ biological replicates. *, $P < 0.05$; **, $P < 0.01$ (two sided); ns, not statistically significant. Paired t test (B), one-way ANOVA with Dunnett's T3 correction (C and D), or least significant difference correction (F).
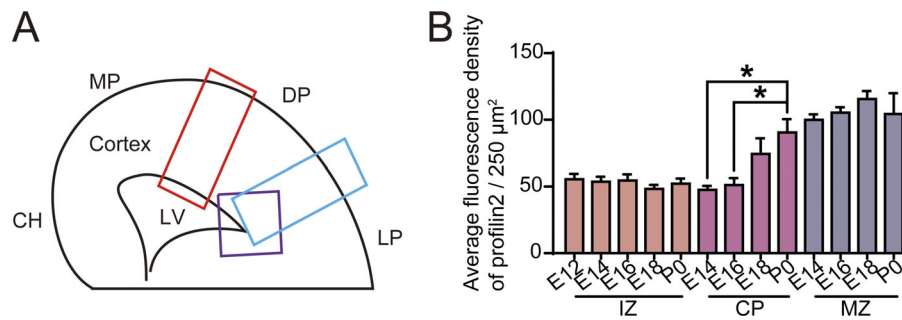
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Figure S5. **Schematic diagram showing areas chosen for quantification of cells in imaging analysis. (A)** Red rectangle indicates the 100 × 250-μm area of interest in the dorsal pallium (DP) perpendicular to the VZ. Blue rectangle indicates the 100 × 250-μm areas of interest in the DP. Purple square indicates the 150 × 150-μm area of interest in the DP adjacent to VZ. CH, cortical hem; MP, medial pallium; LP, lateral pallium; LV, lateral ventricle (see Materials and methods for details). **(B)** Average immunofluorescence density of profilin2 in each cortical layer. n = 9 brain slices from three mice. Values represent mean ± SEM. *, $P < 0.05$ (two sided). Kruskal-Wallis test with Dunn-Bonferroni post hoc test (CP) and one-way ANOVA with Bonferroni correction (IZ and MZ).