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Supplemental Data

SUN1/2 and Syne/Nesprin-1/2 Complexes Connect Centrosome to the Nucleus during Neurogenesis and Neuronal Migration In Mice

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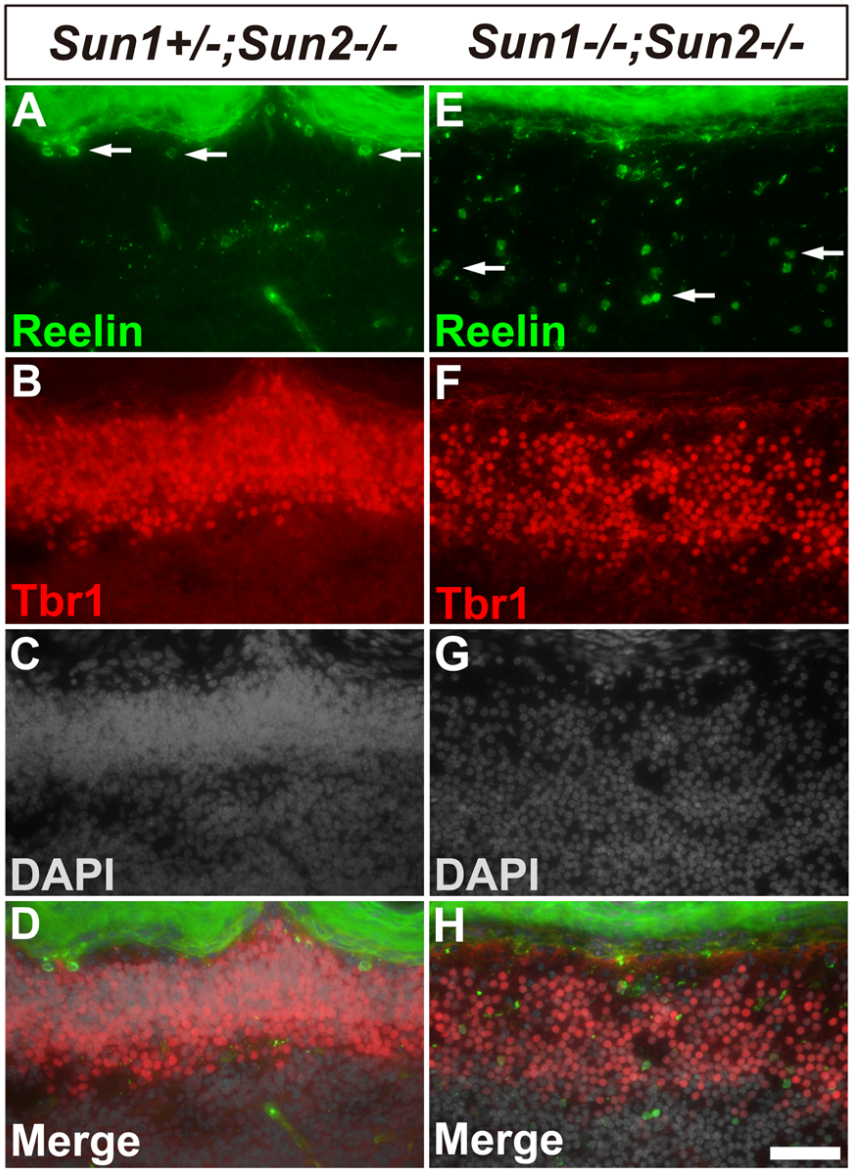

Figure S1. Loss of SUN1 and SUN2 disrupts the development of the marginal zone of the cerebral cortex

Coronal brain sections of E18.5 mouse embryos were stained with anti-Reelin, anti-Tbr1 and DAPI. The Reelin positive cells are localized beneath the pial surface in control (A, white arrows), while this group of cells occupied deeper positions in *Sun1/2* DKO cortex (E, white arrows). Noticeably, the cortical plate was not developed in *Sun1/2* DKO mutants (F, G). Bar, 50μm.

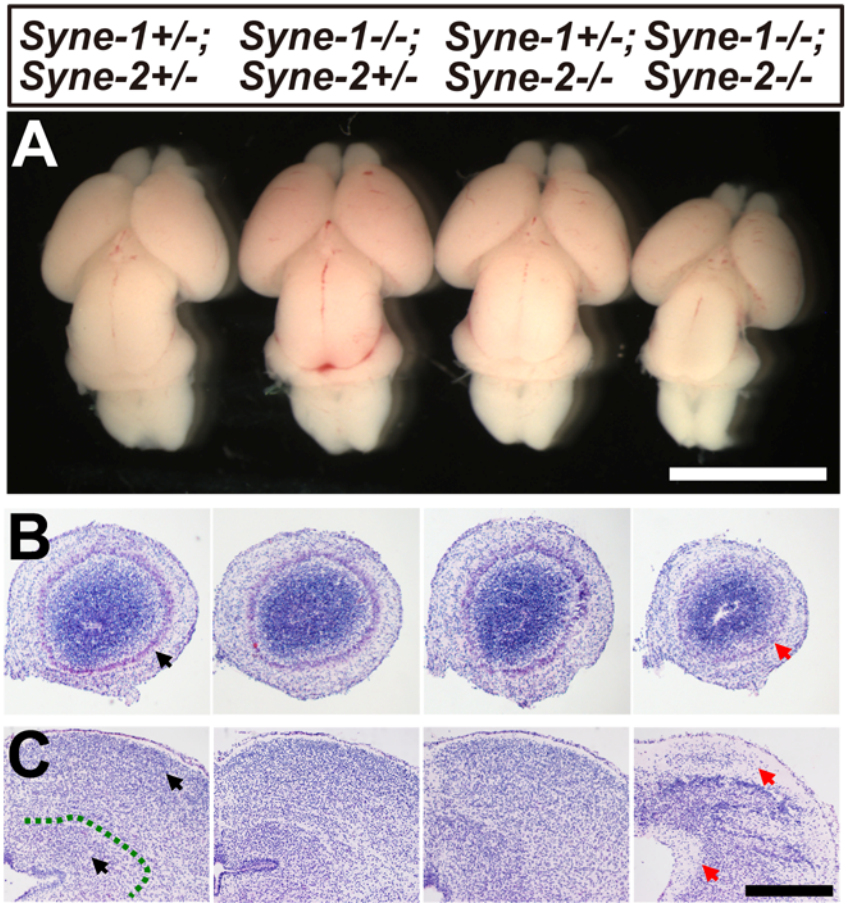

Figure S2. *Syne-1/2* DKD mice display smaller brain and laminary defects in olfactory bulb and midbrain

(A) Dorsal view of *Syne-1/2* mutant brains. The *Syne-1/2* DKD brain was significantly smaller than that of its littermate controls. Bar, 5000μm.

(B-C) Coronal sections of E18.5 mouse brain stained with H&E. (B) The olfactory bulb of *Syne-1/2* DKD mouse displayed a malformed mitral cell layer and a smaller size than its littermate controls. (C) *Syne-1/2* DKD brain displayed severe laminary defects in mid-brain (red arrows). Bar, 500μm.

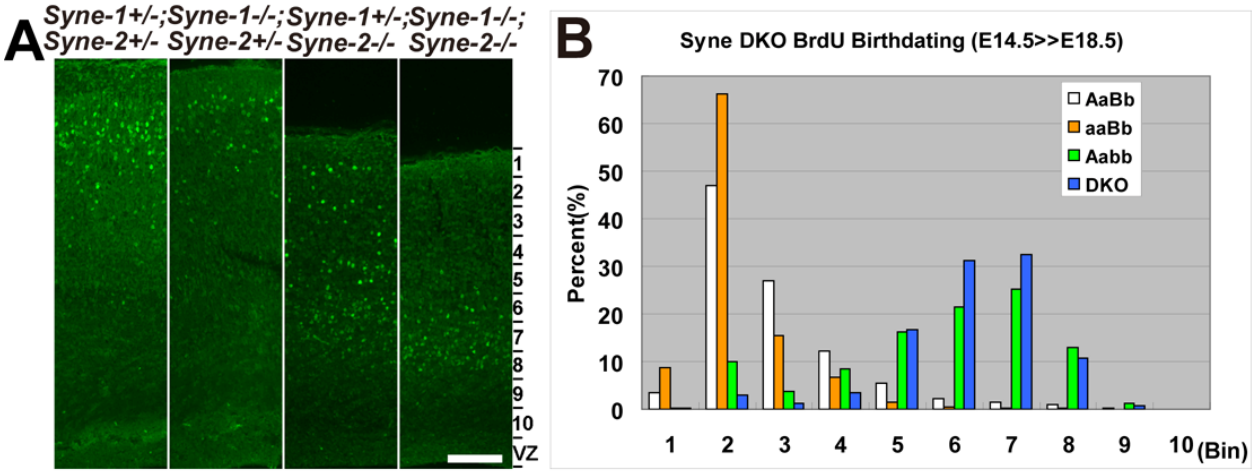

Figure S3. Radial neuronal migration in the neocortex is disrupted in *Syne-2* homozygous knockout mouse brain.

(A) Coronal sections showing BrdU positive cells (green) at E18.5 in a BrdU birth-dating assay begin from E14.5. Most E14.5 born neurons have successfully migrated to layer2-3 in *Syne-1*$^{+/-}$*; Syne-2*$^{+/-}$ (AaBb) and *Syne-1*$^{-/-}$*; Syne-2*$^{+/-}$ (aaBb) cortex, while neurons in *Syne-1*$^{+/-}$*; Syne-2*$^{-/-}$ (Aabb) and *Syne-1/2* DKD (DKO) cortex failed to migrate to correct positions. Bar, 100μm.

(B) Statistical analysis showing the E12.5 labeled BrdU positive neurons of *Syne-1*$^{+/-}$*; Syne-2*$^{-/-}$ (Aabb) and *Syne-1/2* DKD (DKO) mice occupied inverted position in the cortex compared to *Syne-1*$^{+/-}$*; Syne-2*$^{+/-}$ (AaBb) and *Syne-1*$^{-/-}$*; Syne-2*$^{+/-}$ (aaBb) controls.

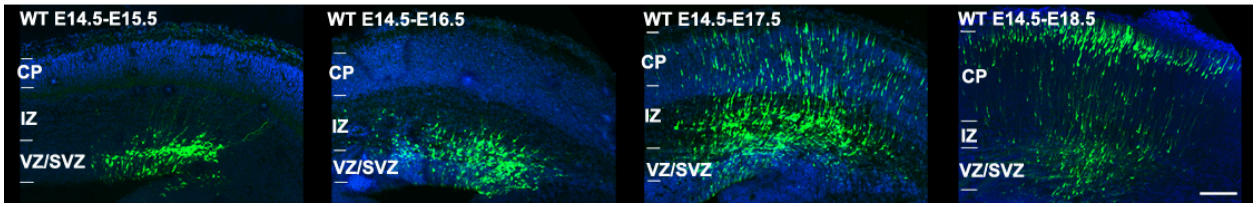

Figure S4. Radial neuronal migration in the wild-type mouse brain

Plasmid expressing EYFP was introduced into the lateral ventricles of E14.5 wild-type mouse embryos by *in utero* electroporation, and EYFP positive cells were examined on coronal sections at E15.5, E16.5, E17.5 and E18.5. Noticeably, most EYFP positive cells had migrated to layer 2-3 at E18.5. CP, cortical plate; IZ: intermediate zone; VZ/SVZ, ventricular zone/subventricular zone. Bar, 252 μm.

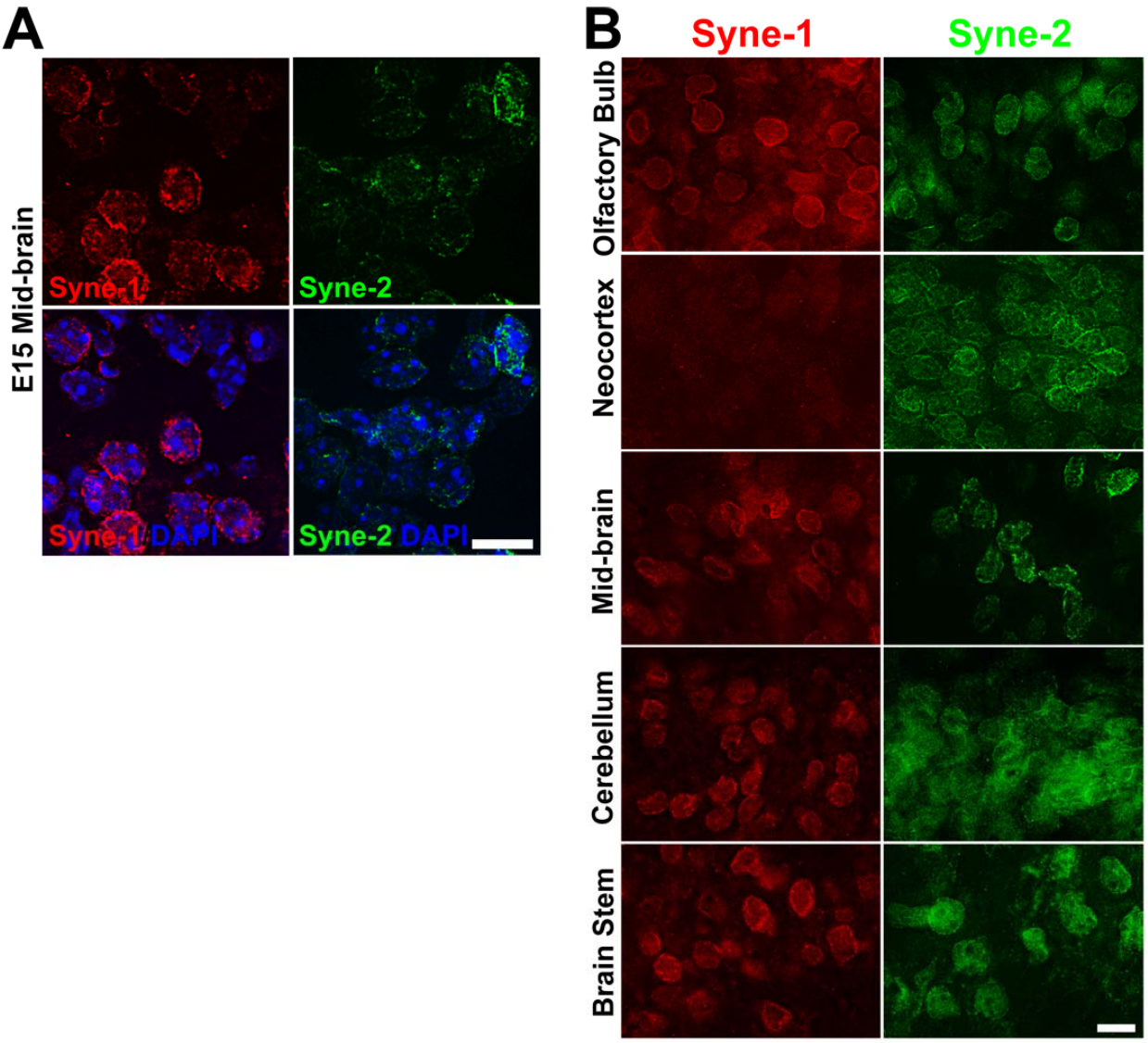

Figure S5. Subcellular localization of Syne-1 and Syne-2 in several brain regions

(A) Both Syne-1 and Syne-2 were localized at the NE in mouse mid-brain at E15.5. Bar, 10 μm.

(B) Immunofluorescence staining of sagittal sections of Syne-1 and Syne-2 in P1 mouse brain. Syne-2 was localized to the NE in all indicated brain regions, and Syne-1 was localized to the NE in all indicated brain regions except the cerebral cortex. Bar, 10 μm.

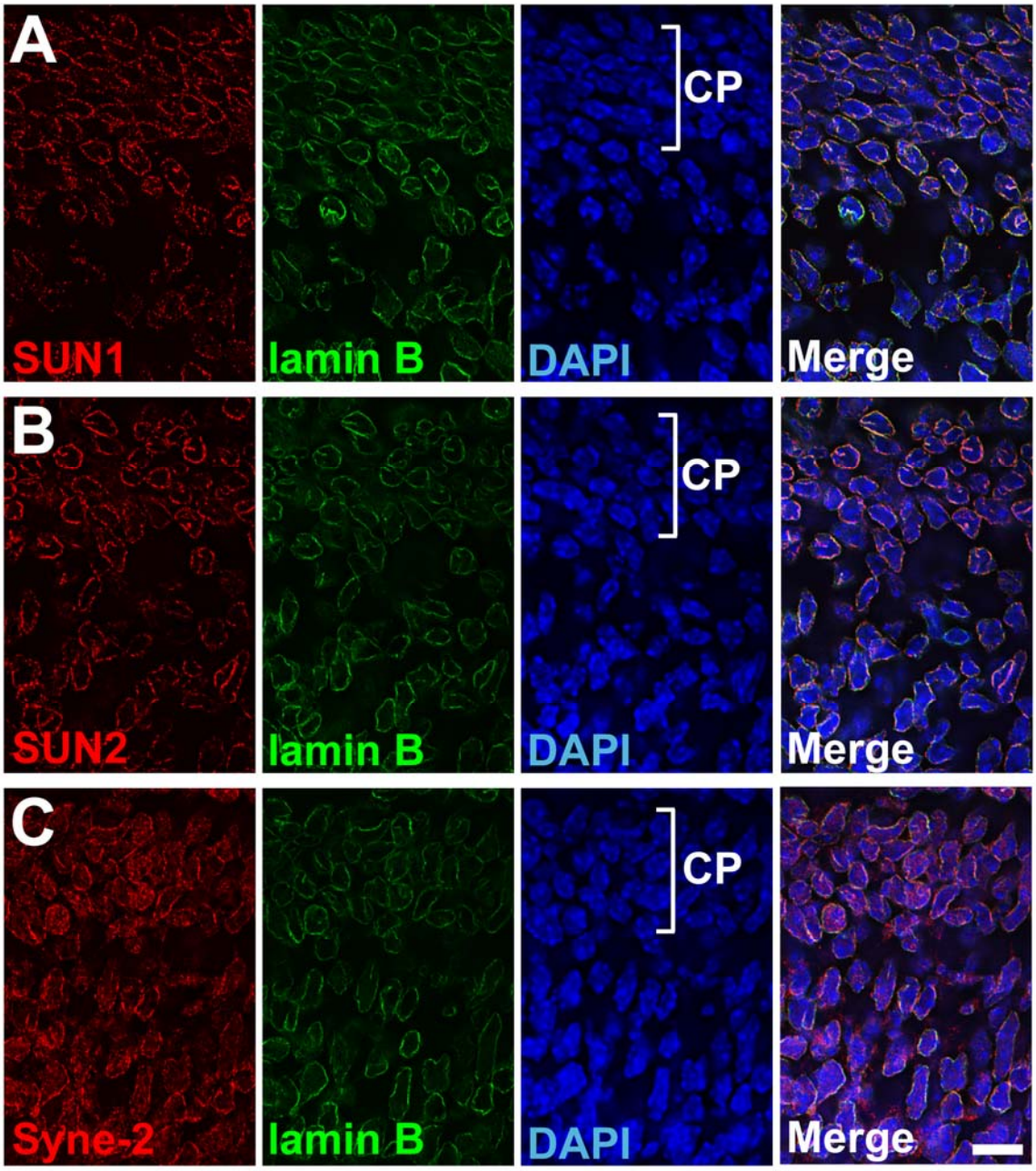

Figure S6. SUN1, SUN2 and Syne-2 co-localize with lamin B at the nuclear envelope in the mouse cerebral cortex.

Coronal sections of E13.5 wild-type mouse embryo were stained with anti-lamin B (green) and anti-SUN1 (A), anti-SUN2 (B) or anti-Syne-2 (C). All three proteins co-localized with lamin B at the nuclear envelope. CP, cortical plate. Bar, 10 μm.

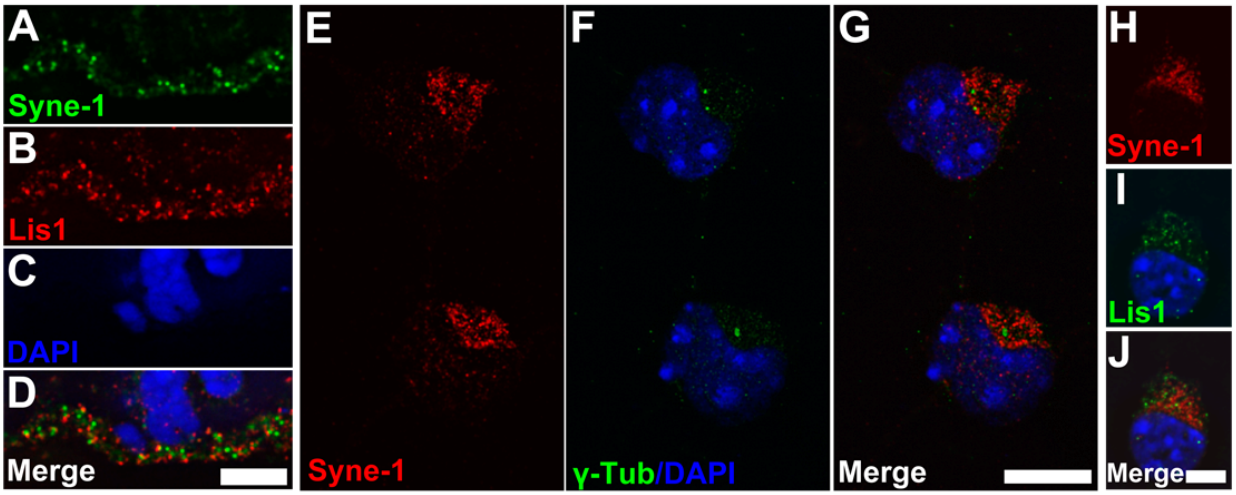

Figure S7. Subcellular localization Syne-1 in cortical neurons

(A-D) Immuno-staining images showing the partial co-localization of Syne-1(green) with Lis1 (red) at the apical surface of the VZ of E15.5 mouse brain. Bar, 5 μm.

(E-G) Images showing Syne-1 (red) was localized around the centrosome (green, labeled by anti-γ-tubulin antibody staining) in primary cultured cortical neurons. Bar, 10 μm.

(H-J) Immuno-staining images showing the punctate localization of Syne-1 (red) and Lis1 (green) in primary cultured cortical neurons. Bar, 10 μm.

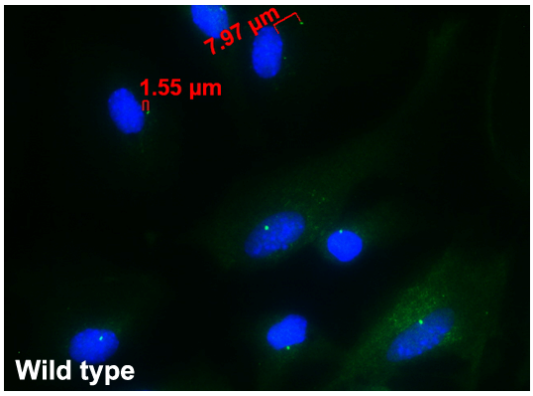

Figure S8. Localization of the centrosome and nucleus in wild-type glial cells

Glial cells isolated from E15.5 C57BL/6J embryos were stained with anti-γ-tubulin (green) and DAPI (blue). The centrosome-nucleus distance was measured by AxioVision (Carl Zeiss).

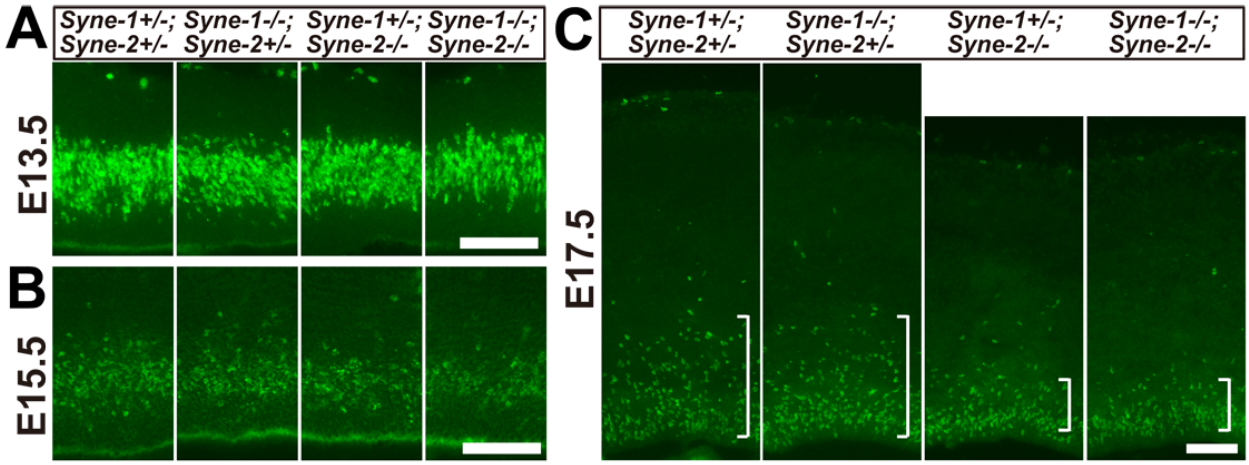

Figure S9. Loss of Syne-2 results in proliferation defects at late embryonic stages

BrdU pulse labeling was carried out in *Syne-1/2* mutants at indicated time points. At E17.5, the number of BrdU positive neurons was decreased in the VZ of *Syne-1*$^{+/-}$*; Syne-2*$^{-/-}$ (Aabb) and *Syne-1/2* DKD (DKO) embryos (brackets). Bar, 100 μm.

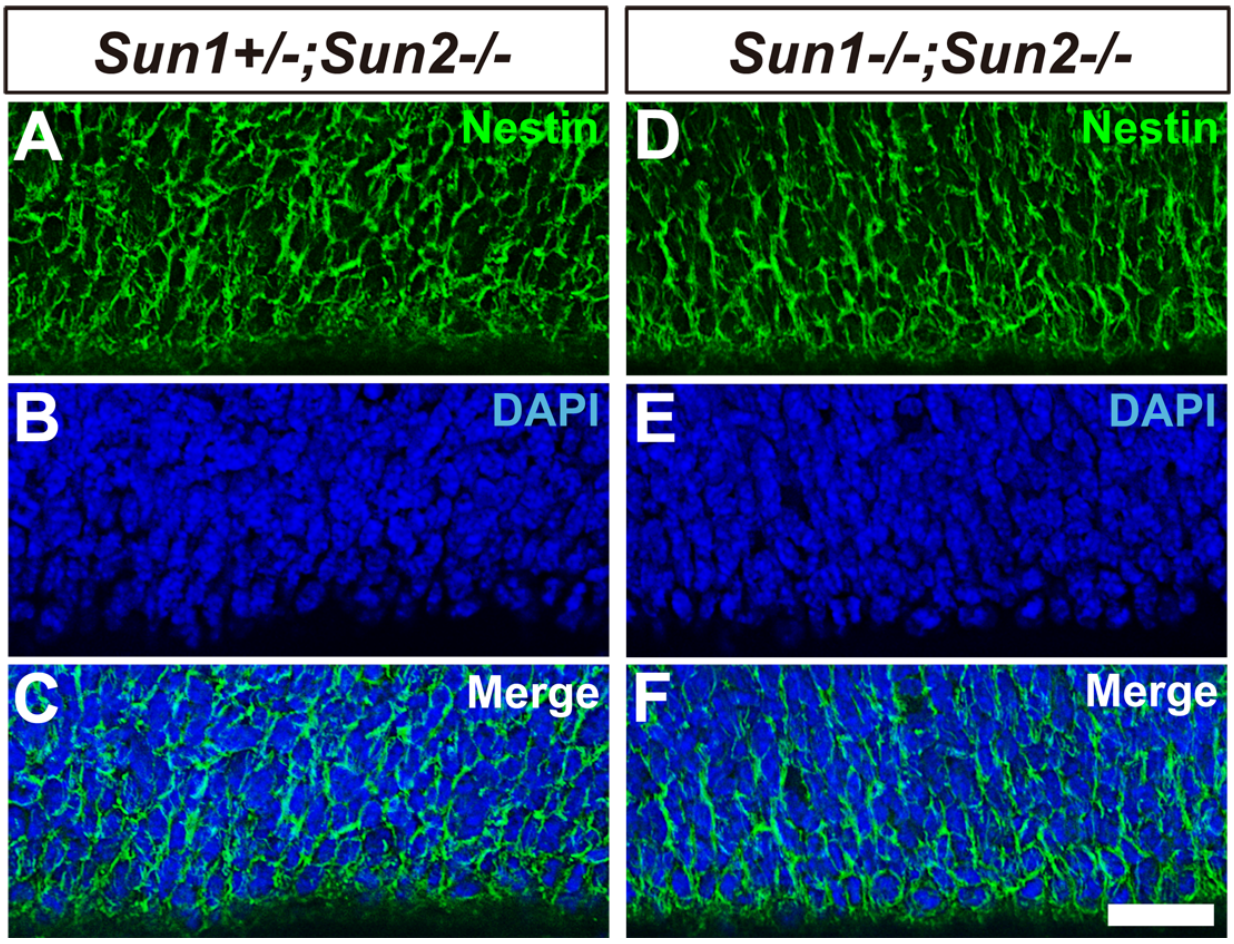

Figure S10. Identification of neural progenitor cells in *Sun1/2* double mutants

Immuno-staining images showing the ventricular zone of E15.5 *Sun1/2* double mutants stained with anti-Nestin (green) and DAPI (blue). Nuclei that are surrounded by round green circles indicate neural progenitors. Bar, 25 μm.

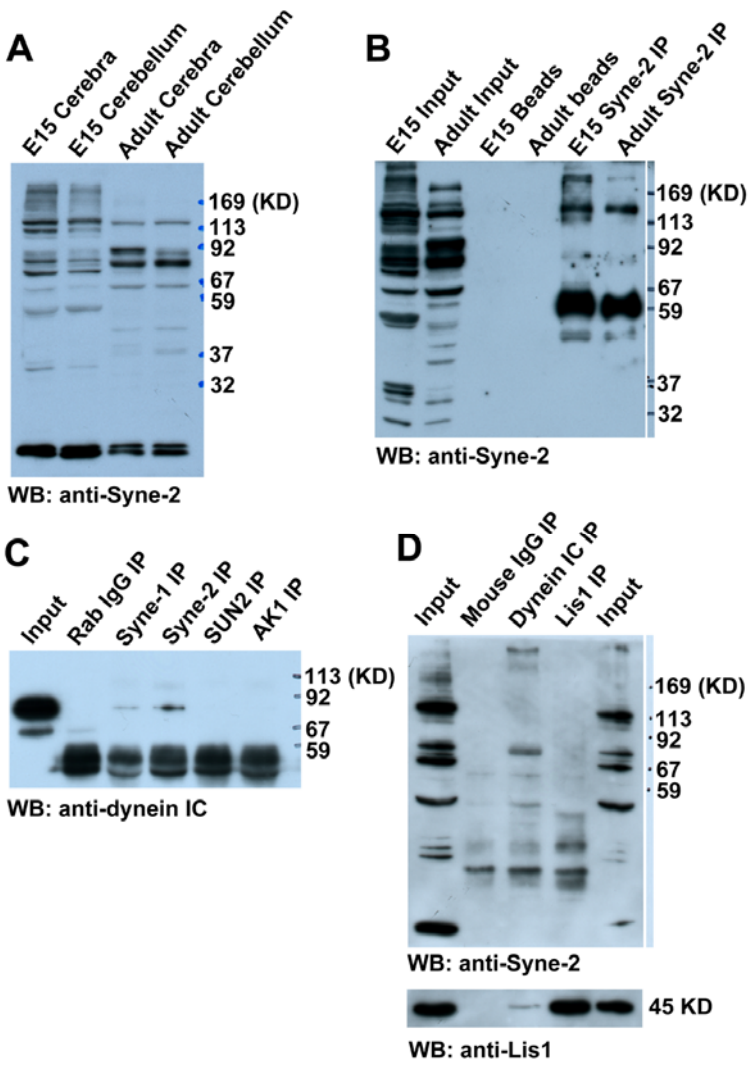

Figure S11. Syne-2 co-immunoprecipitates with the cytoplasmic dynein complex in mouse brain lysates

(A) Western blot showing the expression of Syne-2 in embryonic day E15.5 and adult brains. CA, cerebra; CB, cerebellum.

(B) Syne-2 antibody was able to immuno-precipitate itself.

(C) Antibodies against Syne-1, Syne-2, but not SUN2 or AK1 were able to pull down dynein IC from E18.5 mouse brain.

(D) The antibody against dynein intermediate chain was able to pull down Syne-2 and Lis1 from E17.5 brain lysates.

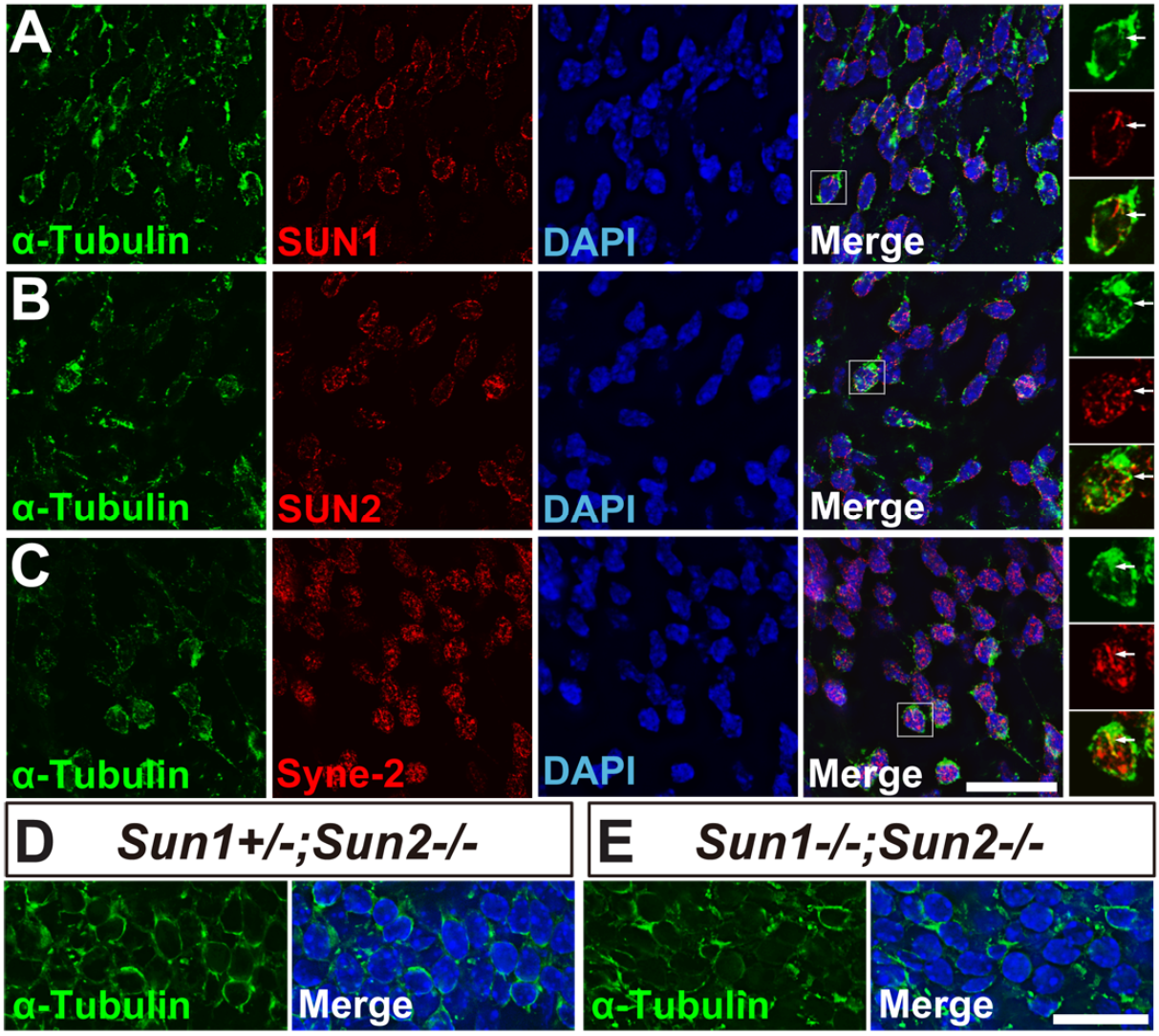

Figure S12. SUN1, SUN2 and Syne-2 partially co-localize with microtubule at the nuclear envelope and the "fork" in E15.5 mouse brain

(A-C) Coronal section of E15.5 wild-type mouse brain was stained with anti-α-Tubulin (green) and anti-SUN1 (A), anti-SUN2 (B) or anti-Syne-2 (C), and counter-stained with DAPI (blue). The α-Tubulin signals displayed round circles around the nuclei and partially co-localize with SUN1, SUN2 and Syne-2. The enlarged inserts on the right panels show that SUN1, SUN2 and Syne-2 co-localize with the "fork" structure of microtubule. Bar, 20 μm.

(D-E) Coronal section of E15.5 *Sun1/2* mutant mouse brain was stained with anti-α-Tubulin (green) and DAPI (blue). The nuclear envelope localization of microtubule was not obviously affected in *Sun1/2* DKO mouse brain compared to the control. Bar, 20 μm.

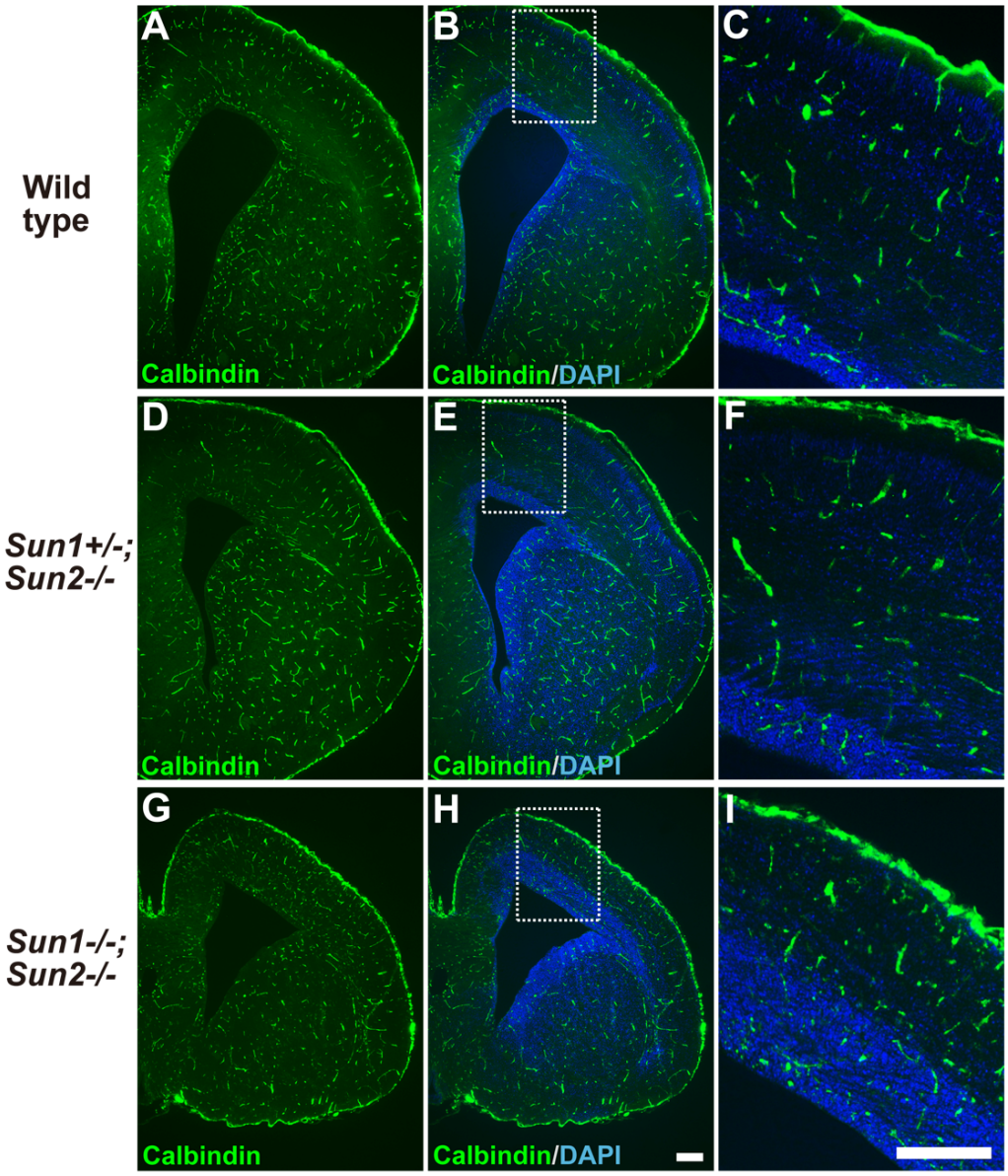

Figure S13. Calbindin positive interneuron is able to migrate to the cortical region in *Sun1/2* DKO mouse brain

Coronal sections of E18.5 mouse brain of indicated genotypes were stained with anti-Calbindin (green) and DAPI (blue). Calbindin positive cells were easily identified in the ventricular zone, intermediate zone and cortical region of both *Sun1*$^{+/-}$*; Sun2*$^{-/-}$ and *Sun1/2* DKO mouse brain. (C), (F) and (I) are enlarged views of the inserts in (B), (E) and (H), respectively. Bar, 200 μm.

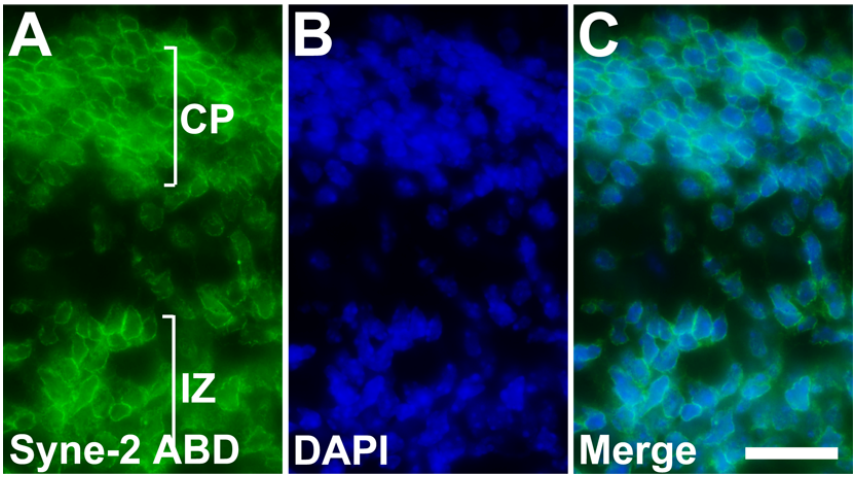

Figure S14. The ABD-containing Syne-2 is expressed and localized to the NE of E13.5 mouse brain

(A-C) Coronal section of E13.5 wild-type mouse brain was stained with anti-Syne-2 antibody (K56-386, green) and DAPI (blue). The ABD containing Syne-2 was localized around the nucleus in both cortical plate (CP) and intermediate zone (IZ). Bar, 50 μm.

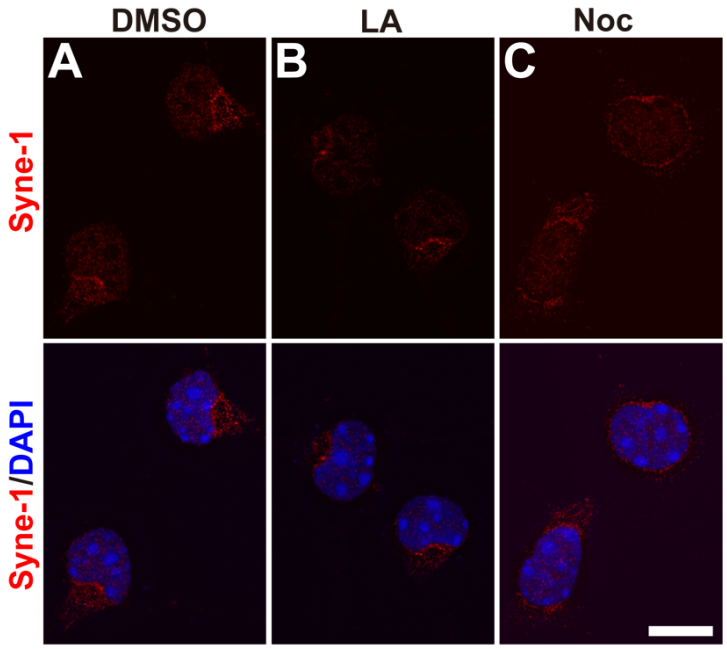

Figure S15. The asymmetric distribution of Syne-1 is dependent on microtubule, not F-actin.

Immuno-staining images showing subcellular distribution of anti-Syne-1 signals treated with either latrunculin A (LA) or Nocodazole (Noc). LA and Noc are inhibitors of actin and tubulin polymerization, respectively. Bar, 10 μm.