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Supplemental Information

The Phosphatase PP4c Controls Spindle Orientation to Maintain Proliferative Symmetric Divisions in the Developing Neocortex

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Supplemental Figure 1

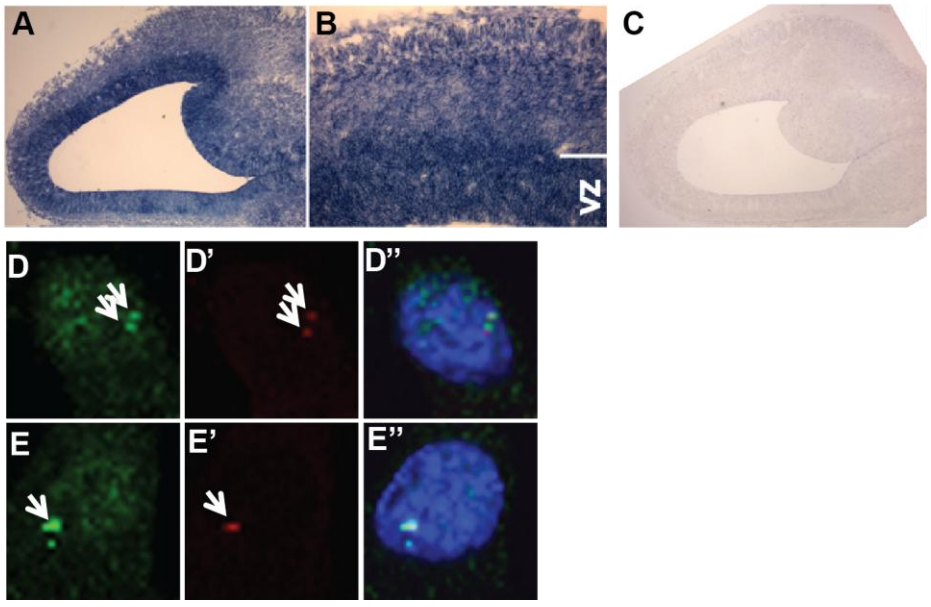

Figure S1. Expression of *pp4c* mRNA in the mouse brain and PP4c protein in NIH3T3 cells, Related to Figure 1.

(A-B) In situ hybridization of *pp4c* show its expression is ubiquitous but highly enriched in the VZ (B)

(C) Control of in situ hybridization using a sense probe from *pp4c*

(D-E) PP4c (green) localizes to centrosomes (γ-Tubulin, red) in NIH3T3 cells

Supplemental Figure 2

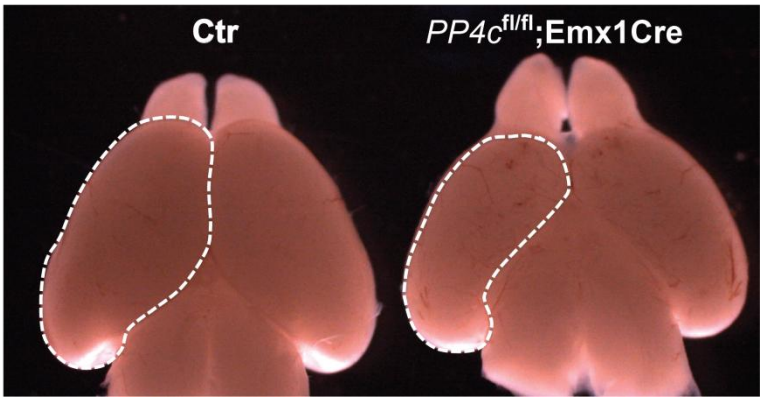

Figure S2. PP4c mutant shows reduced cortical surface area, Related to Figure 1.

Dorsal view of *PP4c*$^{fl/+}$;Emx1Cre (Ctr) and *PP4c*$^{fl/fl}$;Emx1Cre E18.5 mouse brains. The left cortical areas are marked on both brains.

Supplement Figure 3

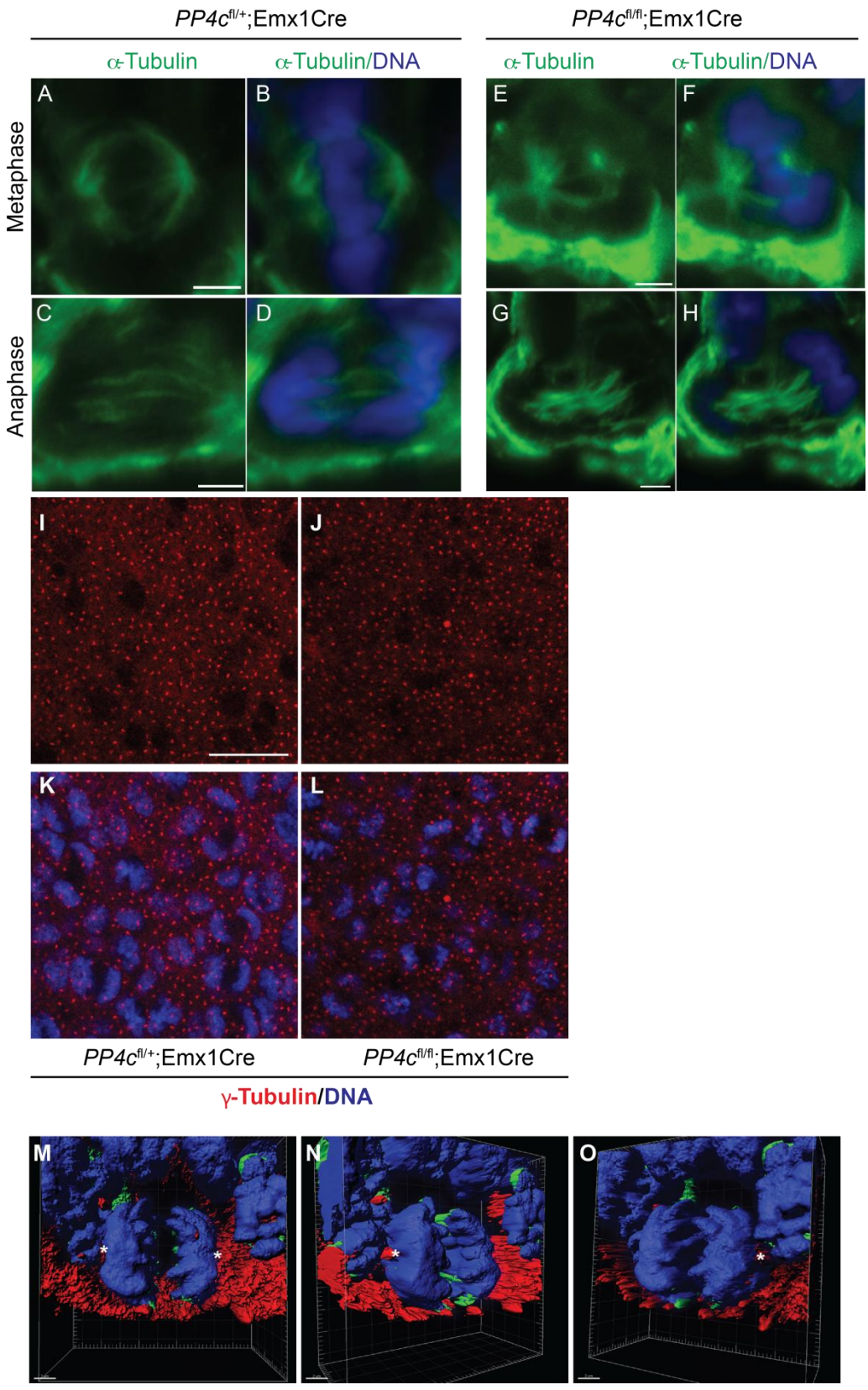

Figure S3. Spindle morphology and centrosome numbers not altered upon PP4c depletion, Related to Figure 3.

(A-H) Mitotic progenitors in *PP4c*$^{fl/+}$;Emx1Cre and *PP4c*$^{fl/fl}$;Emx1Cre brains were stained for α-Tubulin (green) and DNA (DAPI, blue) and showed normal morphology.

(I-L) Enface view of centrosomes (γ-Tubulin, red) in *PP4c*$^{fl/+}$;Emx1Cre and PP4c$^{fl/fl}$;Emx1Cre brains.

(M-O) A sample of 3D-reconstruction of the mitotic progenitor. Blue: DNA, Green: pH3, Red: centrosomes and N-cadherin which outlines the cell border. Asterisks indicate centrosomes.

Supplemental Figure 4

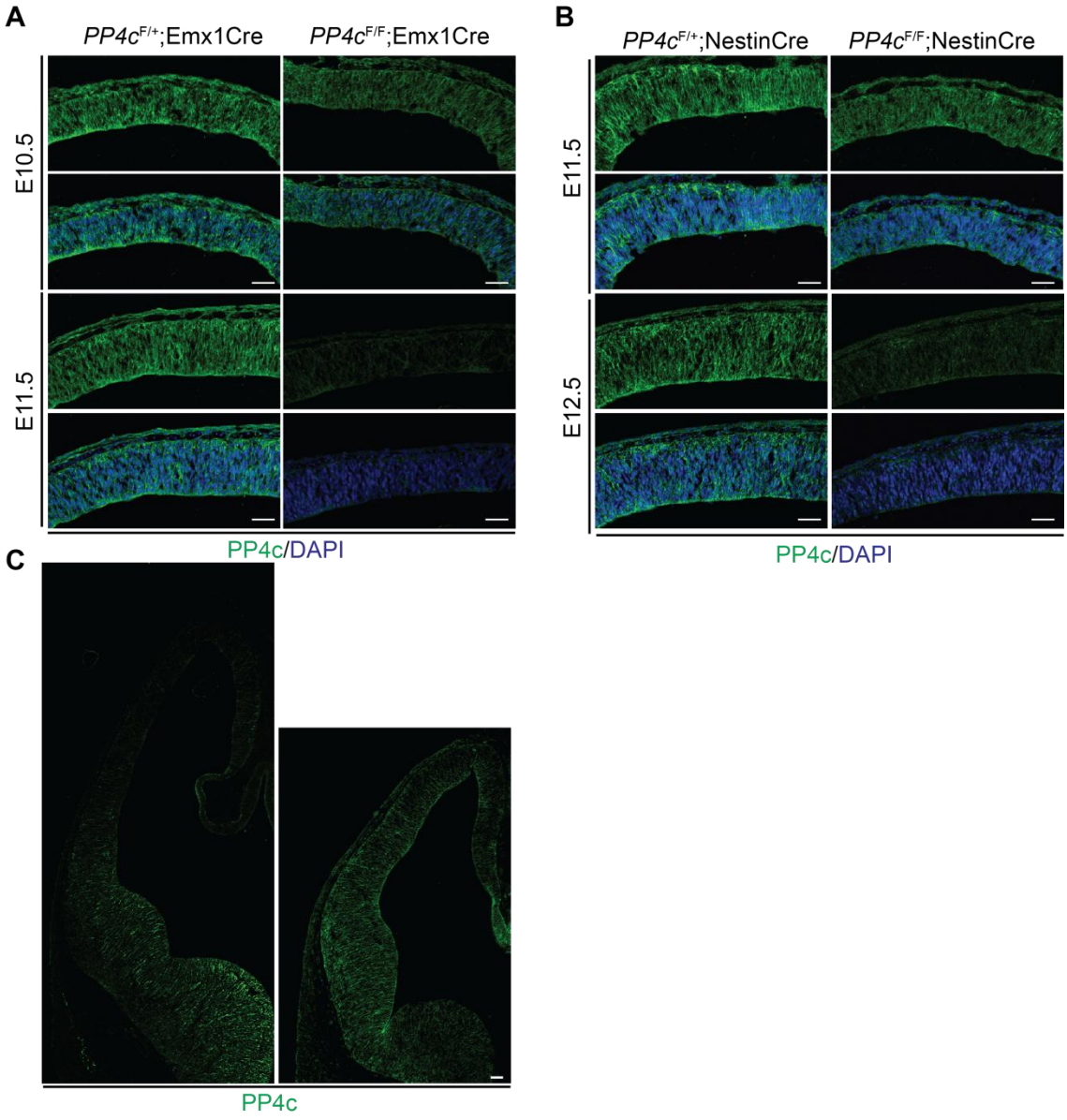

Figure S4. Emx1Cre or NestinCre mediated PP4c depletion, Related to Figure 4.

(A) Emx1Cre mediated PP4c loss at E11.5 in the dorsal cortex.

(B) NestinCre mediated PP4c loss at E12.5.

(C) The depletion of PP4c mediated by Emx1Cre at E11.5 is only occurred in the dorsal cortex where Emx1Cre activates. Left panel shows a mutant section and right panel shows a control section. Scale bars: 50μm

Supplemental Figure 5

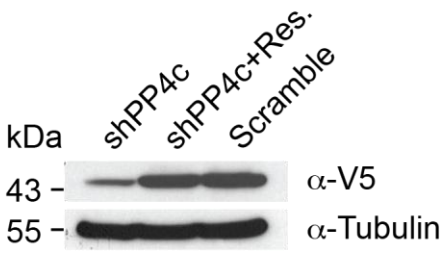

Figure S5. **Down-regulation of PP4c by shRNA in N2a cells, Related to Figure 5 and 6.**

N2a cells were transfected with a V5 tagged mouse *PP4c* construct together with either scrambled, or PP4c shRNA, or shPP4c together with RNAi resistant *PP4c*. Two days later, cells were subjected to Western blot. The membrane was blot for anti-V5 antibody and anti-α-Tubulin antibody as a loading control.

Supplemental Figure 6

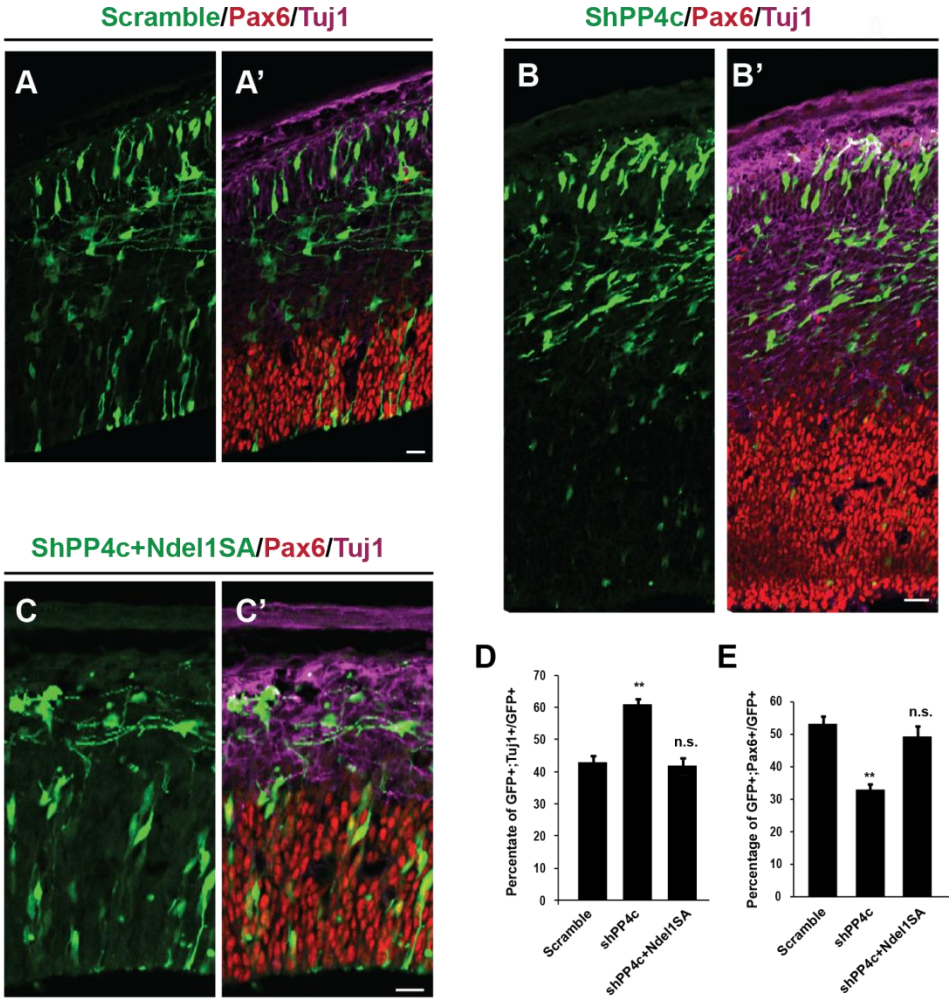

Figure S6. Ndel1SA rescues lineage defects caused by the downregulation of PP4c at the onset of neurogenesis, Related to Figure 5.

Ultrasound guided in utero electroporation was performed at E11.5 with Scramble (A-A', Green), ShPP4c (B-B', Green) or shPP4c together with Ndel1SA (C-C', Green), and brains were analyzed at E13.5. Sections were stained with Pax6 (Red) and Tuj1 (Purple) in all conditions. Note that compared to scramble electroporated brains, the number of progenitors is significantly reduced upon the downregulation of PP4c, and this phenotype can be rescued by a non-phosphorylatable form of Ndel1 (Ndel1SA) (D) The number of neurons is increased when PP4c is downregulated via shRNA, and is restored to the control level when Ndel1SA was co-electroporated with shPP4c. Note the number of neurons was quantified as GFP positive cells which are located in the Tuj1 positive region. (E) Quantification shows that the number of Pax6 positive progenitors (GFP+;Pax6+) is significantly reduced upon the downregulation of PP4c via shRNA compared to Scramble controls. When Ndel1SA was co-electroporated with shPP4c, the number of Pax6 positive progenitors is restored to the control level.