Figures and figure supplements

GSK-3 signaling in developing cortical neurons is essential for radial migration and dendritic orientation

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Figure 1. GSK-3 signaling is essential for proper lamination of the developing cortex. (A–A’) Cux-1 staining (red) in coronal sections from control and Gsk3:Neurod6 mice at E19.5. Cux-1 neurons are strikingly mislocalized in Gsk3:Neurod6 mutants (orange arrows) including a small population of neurons that remain in the ventricular zone (yellow arrowhead). Nuclei were counterstained with DRAQ5. Scale bar = 500 μm. (n = 4). (B–B’) Cux-1 staining in parasagittal vibratome sections from control and Gsk3:Neurod6 mutants at E18.5. Cux-1 expressing neurons (arrows) are mislocalized in Gsk3:Neurod6 mutants and populate the deeper layers of the cortex along the entire rostral/caudal axis. Scale bar = 200 μm. (C) Representative Western blot confirms Figure 1. Continued on next page
strongly reduced GSK-3β protein levels in the E19.5 Gsk3:Neurod6 cortex compared to heterozygous control (n = 3 het control, n = 3 CKO). Relative Density *p<0.05, unpaired t-test.
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**Figure 1—figure supplement 1.** Migration defect apparent by E16 after Gsk3 deletion in cortical excitatory neurons.
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Figure 2. Migration defects in Gsk3-deleted mice are persistent. (A) P0;Cux 1 staining (red) in coronal sections of Gsk3$^{lox}$:Neurod6 mutants and littermate heterozygote controls. Cux1-expressing neurons are localized to layer2/3 in controls (denoted by yellow dashed lines) while Cux1-expressing neurons are localized throughout the cortical plate in the mutants. (n = 5, scale bar = 100 μm). (B) Representative Western blot of P0 cortical lysates confirms strongly reduced GSK-3α and GSK-3β protein levels in Gsk3$^{lox}$:Neurod6 mutants when compared to Gsk3a$^{lox/lox}$Gsk3b$^{lox/lox}$ controls. GAPDH was probed as a loading control (n = 3 control, n = 3 CKO). (C) P0 quantification of control and Gsk3$^{lox}$:Neurod6 Cux1 neurons using 8 bin analysis spanning white matter (WM) to the pial surface (PS), (n = 2 het control, n = 2 CKO). (D–D’) P7: Gsk3$^{lox}$:Neurod6 mutants stained with Cux1 (red) show persistent altered lamination with Cux1-expressing neurons spread throughout all layers of the cortex. Littermate controls show normal Cux1 distribution in layer 2/3. Scale bar = 200 μm. DOI: 10.7554/eLife.02663.005
Figure 2—figure supplement 1. Gsk3 overexpression enhances radial migration.
DOI: 10.7554/eLife.02663.006
Figure 3. GSK-3 signaling is dispensable for tangential migration, but required for radial hippocampal migration.

(A–A’) E19.5 coronal sections showing EYFP-expressing interneurons in heterozygous control and Gsk3:Dlx5/6 mutants crossed with the Ai3 reporter line. Gsk3-deleted interneurons (green) enter the cortex in two streams in both controls and mutants (arrowheads). Mutants showed no overt migration defect. Nuclei were counterstained with Hoechst. (n = 3). (B–B’) E19 coronal sections of control and Gsk3:Neurod6 mutants showing CTIP2 (green) expressing neurons in the hippocampus. In the Gsk3:Neurod6 mutants, the pyramidal cell layer (green) does not extend laterally into a compact CA1 region and remains dispersed (yellow arrowheads). Fimbrial axonal projections appear normal in Gsk3:Neurod6 mutants (orange arrow). Nuclei were counterstained with DRAQ5.

Figure 3. Continued on next page
Figure 3. Continued

Scale bar = 500 μm. (n = 3). (C–C’) Higher magnification of hippocampal area shown in (B). The Gsk3:Neurod6 mutants show disrupted cytoarchitecture. In the mutants, DRAQ5-labeled cells are mislocalized and diffuse (arrowheads) and fail to form clearly defined CA1/CA3 regions of the hippocampus. The Gsk3:Neurod6 mutant mice also lack a clearly defined hippocampal sulcus (green bars) and dentate gyrus (DG). (D) Representative Western blot of E18 MGE lysates confirm strongly reduced GSK-3β protein after recombination with Dlx5/6-Cre. (E) Quantification of protein knockdown in D (n = 3 WT, n = 3 CKO, unpaired t-test). DOI: 10.7554/eLife.02663.007

Figure 3—figure supplement 1. No apparent migration defect in Gsk3:Dlx5/6 mice. DOI: 10.7554/eLife.02663.008
Figure 4. GSK-3 deletion delays the multipolar to bipolar transition. (A–A') Representative E19 coronal sections after in utero electroporation at E14.5 with Neurod1-Cre and Z/EG plasmids. Electroporated cells were visualized with anti-EGFP (green), and nuclei were stained with DAPI (blue). Gsk3-deleted neurons remain in the deeper layers of the cortex but elaborate a long pial-directed process (yellow arrowheads). Gsk3-deleted neurons elaborate axons projecting towards the corpus callosum (orange arrows). Scale bar = 200 μm (n = 5, two independent litters). (B–B') Coronal sections at P10 after E14.5 electroporation, as in A. Gsk3-deleted neurons remain in the deeper layers of the cortical plate and fail to reach layer 2/3 (denoted with yellow bars). Scale bar = 200 μm (n = 3, 2 independent litters). (C) Higher magnification of Gsk3-deleted neurons in B' (box). Gsk3-deleted neurons (green) in deeper layers co-label with Cux1 (red) (orange arrows). Nuclei were stained with Dapi. (D) Quantification of control and Gsk3-deleted neurons in Figure 4. Continued on next page
Developmental biology and stem cells | Neuroscience

upper (layer 2–3) vs deeper layers of the cortex at P10. (n = 3, 4209 total neurons counted, 2234 control vs 1975 Gsk3 deleted) **p=0.003, unpaired t-test.

(E) Gsk3 deletion delays the multipolar to bipolar transition. Still images from time-lapse imaging of slice cultures at 3DIV. pCAG-dsRED or Neurod1-Cre;Z/EG was injected into the ventricles of Gsk3a−/−;Gsk3blop/lop embryos and electroporated at E15. Representative images were taken at time 0, 6, and 12 hr. Control dsRed neurons migrate through the cortical plate (yellow, red, and blue arrows show individual neurons at the different time points). (n = 2 controls). Gsk3-deleted neurons fail to migrate through the cortical plate and exhibit persistent multi-polar morphology (yellow arrowheads). (n = 4 mutants).

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Figure 4. Continued

Figure 4—figure supplement 1. Gsk3-deleted neurons polarize and are highly dynamic.

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Figure 5. GSK-3 signaling is required for proper dendrite orientation. (A) Gsk3 deleted neurons at P15 shown after in utero electroporation at E14.5 with Neurod1-Cre and Z/EG plasmids. Multiple neurons with obvious abnormalities in dendritic orientation were observed in the upper layers of the cortex (orange arrows). Scale bar = 200 μm. (B–B’) Control and Gsk3-deleted neurons in the upper layers at P15, immunostained with antibodies against eGFP (black) using same methods as Figure 4. Gsk3-deleted neurons have abnormally polarized arbors indicated by orange arrows. Scale bar = 50 μm. Figure 5. Continued on next page
Figure 5. Continued

(C–C’) Neurolucida reconstructions of control and Gsk3-deleted neurons in the upper layers of the cortex. The axon (red) projects towards the ventricle in control and Gsk3-deleted neurons. Both apical dendrites (orange) and basal dendrites (blue) are more branched (orange arrows) and basal dendrites (blue) are mispolarized (blue arrowheads) in Gsk3-deleted neurons. Scale bar = 100 μm. (D) Basal dendrite quantification. Dendrogram shows that basal dendrites more frequently project towards the pial surface in Gsk3-deleted neurons when compared to control basal dendrite orientation. (n = 3, n = 3 CKO; 15 control and 15 Gsk3-deleted neurons quantified). (E) Apical dendrite dendrogram indicates polarization and length of processes. Control apical dendrites project pially (90°). Numerous small apical branches form near the soma and project laterally (orange arrows). Gsk3-deleted neurons also project pially-directed apical dendrites. However, Apical branches have a pially-directed orientation, resulting in abnormal morphology (orange arrows, also see C’).

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Figure S5—figure supplement 1. Quantification of dendritic branching at P15 in control and Gsk3-deleted neurons.

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Figure 6. Lamination in other signaling mutants. (A) P0 representative coronal sections of control (heterozygous for floxed allele) and Ctnnb1\textsuperscript{Ex3}:Neurod6 mutants stained for Cux1 (red) and Hoechst (blue). (n = 5) Scale bar = 100 μm. (B) E18 Coronal sections of control and Dvl123:Neurod6 showing Cux1 (red) and DRAQ5 (blue) staining. Cux1 neurons reach layer 2/3 in both controls and Dvl123:Neurod6 triple mutants. (n = 3). (C) Western blot verification.
of protein deletion after recombination of floxed alleles with Neurod6-Cre. Ctnnb1<sup>Flox</sup>:NeuroD6 (n = 3 WT, n = 3 CKO), Dvl123:NeuroD6 (n = 3 WT, n = 3 CKO). GAPDH was probed as a loading control. (D–F) P0 representative coronal sections of control (heterozygous for floxed allele) and indicated mutant lines stained for Cux1 and Hoechst. Scale bars are 100 μm, at least n = 5 per line. (G) Western blot verification of protein deletion in mutant lines. GAPDH was probed as a loading control. N’s refer to numbers of mutants and paired controls. Stk11:Neurod6 (n = 2), Cdc42:Neurod6 (n = 2), Pten:Neurod6 (n = 3). (H) P0 Western Blot quantification of Ctnnb1<sup>Flox</sup>:Neurod6, Dvl123:Neurod6, Stk11:Neurod6, Pten:Neurod6 and Cdc42:Neurod6 lines.

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Figure 6—figure supplement 1  Quantification of lamination in other signaling mutants.

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**Figure 7.** Phosphorylation status of GSK-3 substrates. (A–A’) Western blots of P0 cortical lysates from Gsk3<sup>loxp</sup>:Neurod6 mutants and wild-type controls performed in triplicate. Levels of GSK-3 proteins and phospho-target proteins are shown. Strong reductions in phosphorylation of doublecortin on ser327/Thr321 and CRMP-2 on Thr514 are evident. (A’) Quantification of relative densities from A. p values shown in figure (n = 3, unpaired t-test) (B–B’) Western blots of cortical lysates at P0 showing levels of other GSK-3 targets. No changes were observed in phosphorylation of dynamin, pCREB, or pFAK. No change was observed in cleaved caspase-3. GAPDH was used as a loading control. (B’) Quantification of relative protein densities (n = 3, unpaired t-test). DOI: 10.7554/eLife.02663.017