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Supplemental Information

**Time-Specific Effects of Spindle Positioning
on Embryonic Progenitor Pool Composition
and Adult Neural Stem Cell Seeding**

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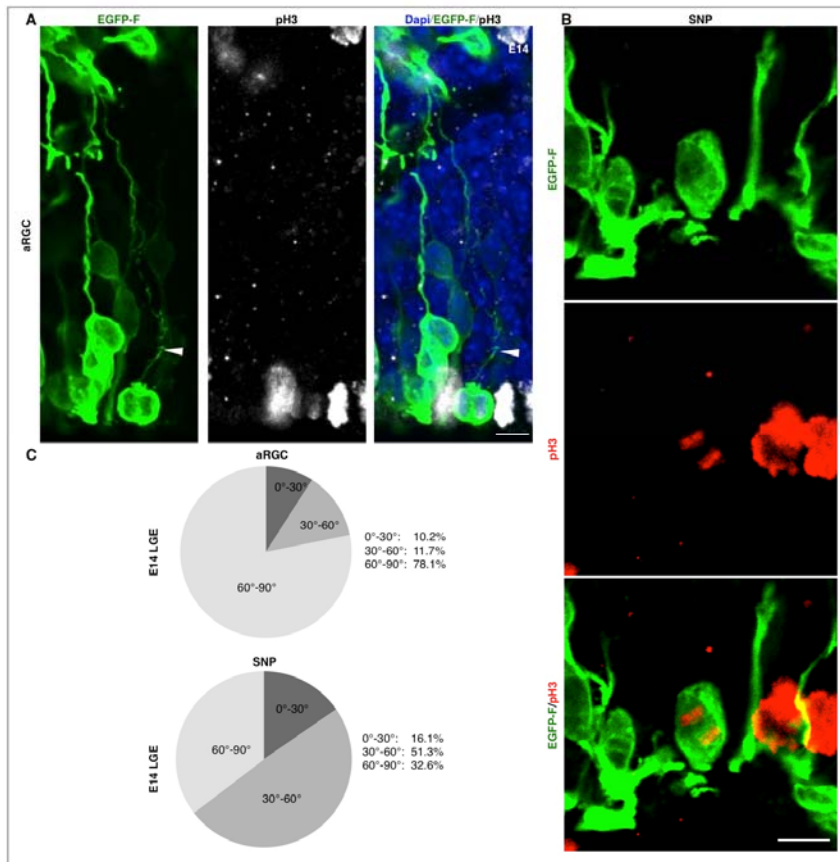


Figure S1, related to Figure 1. Quantification of cleavage angles in apical progenitor subtypes

(A) Fluorescence micrographs depicting membrane-tagged EGFP (green), the mitotic marker pH3 (white) and Dapi (blue) to determine the cleave plane in an aRGC (long basal process). (B) Fluorescence micrographs showing an SNP labeled with membrane-tagged EGFP (green), the mitotic marker pH3 (red) to determine the cleave plane. (C) Pie chart showing the distribution of vertical (60°-90°) oblique (30°-60°) and horizontal (0°-30°) cleavage angles. Note that the distribution of cleavage angles is matching the results obtained by using p-Vim as a cytoplasmic marker (Figure 1C). Scale bar: 10 μ m

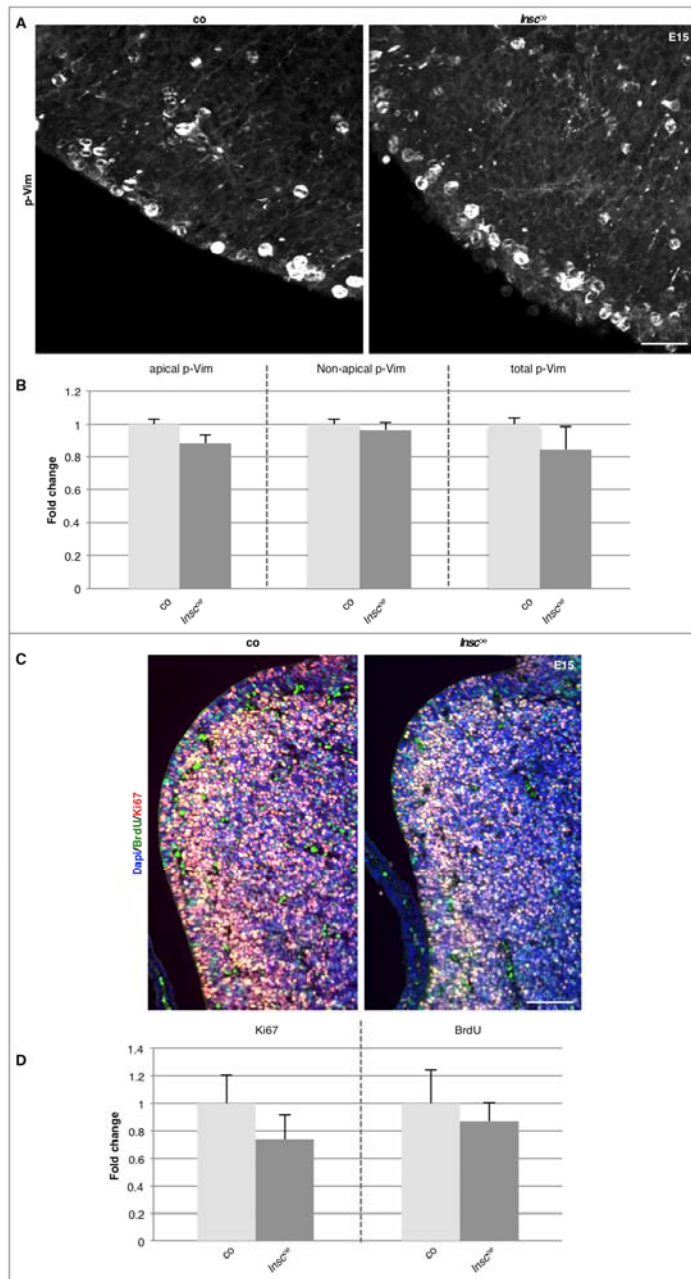


Figure S2, related to Figure 3. No altered proliferation in the LGE of *Insc^{oe}* mice

(A) Fluorescence micrographs showing staining of the mitotic marker p-Vim in control and *Insc^{oe}* LGE at E15. (B) Histograms illustrating the fold-change in apical p-Vim+ cells, non-apical p-Vim+, and total mitotic cells in a given area. Note that no significant changes are observable in *Insc^{oe}* mice. (control: 423

cells, *Insc*: 457 cells in 5 embryos each genotype; Mann-Whitney test $p > 0.05$) Scale bar = 50 μm (C) Fluorescence Micrograph showing staining for Ki67 and BrdU 20hours after BrdU injection in the LGE of control and *Insc*^{oe} animals. (D) Histograms depicting the fold change in the number of Ki67+ and BrdU+ cells per area in the LGE. Note that no significant changes are observable *Insc*^{oe} mice. (control: Ki67: 1020 cells, BrdU: 828 cells; *Insc*: Ki67: 879 cells, BrdU: 855 cells Mann-Whitney test $p > 0.05$) Scale bar = 80 μm

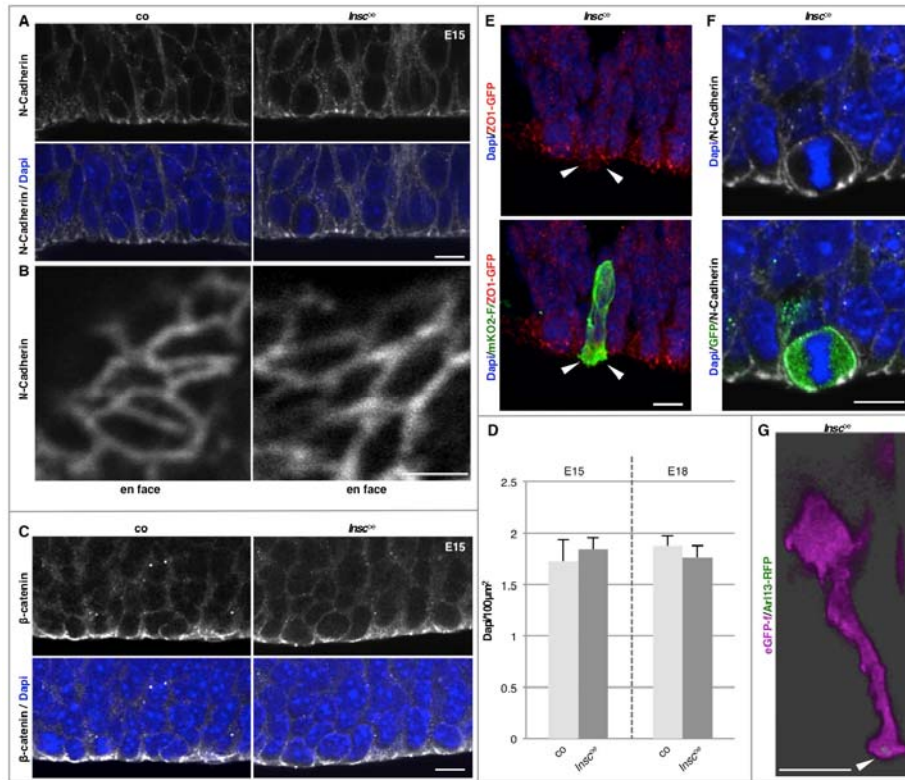


Figure S3, related to Figure 3. Normal expression of adhesion and polarity proteins in *Insc^{OE}* LGE

(A) Fluorescence micrographs of antibody staining against N-Cadherin shows that in *Insc^{OE}* LGE the expression is similar to control LGE. (B) en-face pictures showing N-Cadherin from the apical side also reveals no recognizable differences. (C) Staining for the intracellular linker protein β-catenin is unchanged in *Insc^{OE}* LGE. (D) Histograms showing the quantification of cell densities at E15 and E18. No significant changes are observable *Insc^{OE}* mice (control: E15: 696 cells, E18: 302 cells; *Insc^{OE}*: E15: 998 cells, E18: 330 cells; Mann-Whitney test $p > 0.05$) (E) Fluorescence micrograph showing a SNP (without basal process) co-electroporation of membrane-tagged mKO2-f with ZO1-GFP. Note the SNPs are integrated in the apical ZO1-belt. (F) Fluorescence micrograph of GFP electroporated,

apically dividing SNP (no basal process) double stained with N-Cadherin shows integration in the Cadherin belt during M-phase. (G) Fluorescence Micrograph of a SNP (no basal process) after co-electroporation of membrane tagged eGFP and the ciliary marker Arl13b-RFP. Strikingly the cilium is localized in the apical endfoot. Scale bars: 10 μm .

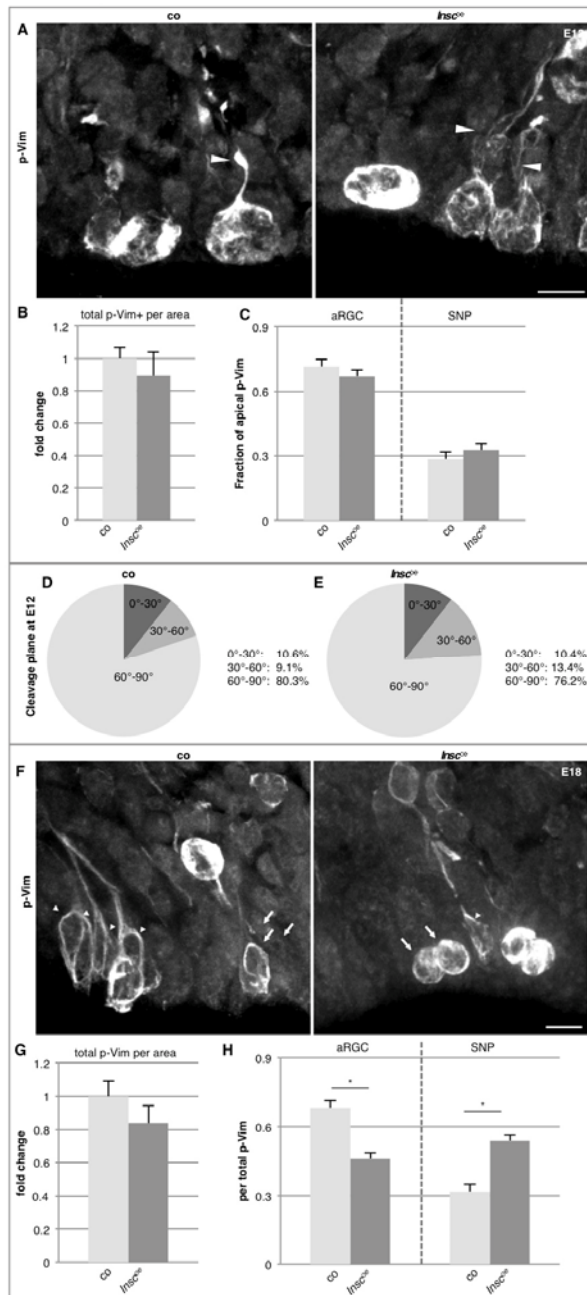


Figure S4, related to Figure 3. Differences in proliferation and progenitor pool composition are specific to a critical time window

(A) Fluorescence micrographs of p-Vimentin (p-Vim) staining in the LGE of E12 embryos reveals the different classes of progenitor cells present at the apical side. Arrowheads indicate the basal process if present. Histograms showing the quantification of the total number of dividing cells (B) and the fraction of

aRGCs and SNPs per total apical p-Vim (C) reveals no significant differences at E12 (co = 627 cells, *Insc^{oe}* = 696 cells; 6 embryos each genotype; Mann-Whitney test: $p > 0.05$). (D and E) Pie charts illustrating the cleavage plane of apical progenitors at E12 show that no reorientation of the cleavage plane in *Insc^{oe}* LGE occurs at early developmental stages. (F) Fluorescence micrographs of p-Vim staining in the GE of E18 embryos showing the different classes of progenitor cells present at the apical side. Arrowheads indicate aRGCs and arrows SNPs. (G) Histograms illustrating the fold change in the total number of mitotic cells show no difference in the mitotic cells at E18. (H) However, when dissecting the different progenitors at the apical side it becomes evident that also at E18 aRGC are reduced and SNPs increased in numbers (co: 373 cells, *Insc^{oe}* = 422 cells; 5 embryos each genotype; Mann-Whitney test: * $p < 0.05$). Scale bars: 10 μm .

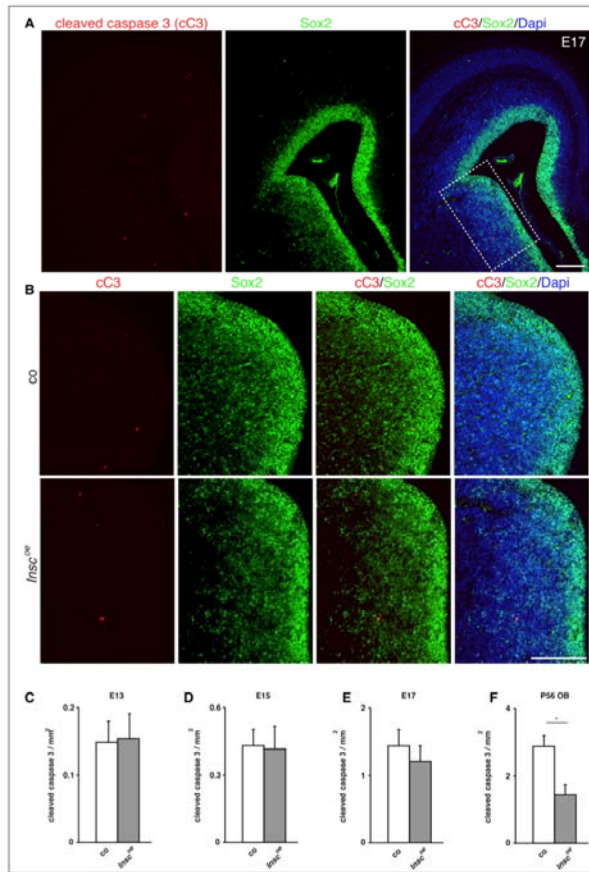


Figure S5, related to Figure 3. No difference in the amount of apoptotic cells in the LGE of *Insc^{oe}* mice

(A) Fluorescence micrographs of E17 brain illustrating the amount of apoptosis detectable in the LGE. Histograms show that after quantification no difference in the amount of apoptotic cells was detectable in the LGE at E13 (C), E15 (D) and E17 (E). However a marked reduction in the number of apoptotic cells was observed in the OB of young adult mice (P56). Scale bars: 200 μ m.

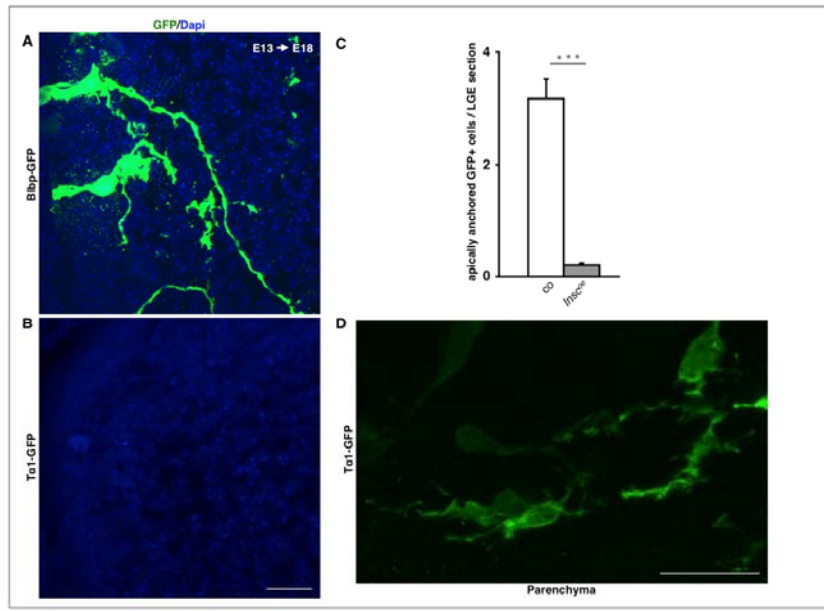


Figure S6, related to Figure 4. Tα1-GFP positive cell don't give rise to cells residing at the apical side.

Fluorescence images showing the LGE of E18 embryos electroporated at E13 with Blbp-GFP (A) and Tα1-GFP (B). Histograms illustrating the quantification of the number of reporter positive cells still present at the apical side at E18 reveals that only the Blbp-GFP signal is retained at the apical side (Blbp-GFP: 46, Tα1-GFP: 3; in 5 embryos; Mann-Whitney test: *** $p < 0.001$). (D) Fluorescence micrograph illustrating that Tα1-GFP+ cells give rise to cells in the parenchyma of the striatum. Scale bars: 20 μm .

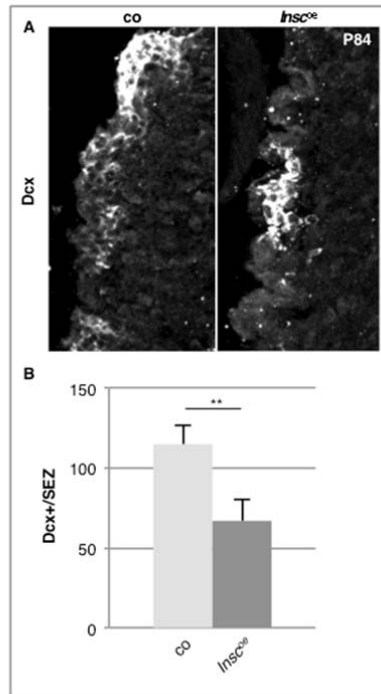


Figure S7, related to Figure 5. Reduced number of Dcx+ neuroblasts in *Insc^{oe}* animals without Tamoxifen treatment.

(A) Fluorescence micrographs of Dcx staining in control and *Insc^{oe}* animals that were not crossed to *Glast^{CreERT2}* mice and hence did not receive any Tamoxifen treatment at embryonic stages. (B). Histograms depicting the quantification of the number of Dcx+ cells in adult control and *Insc^{oe}* animals (co: 603, *Insc^{oe}*: 267; 4 animals; Mann-Whitney test: ** $p < 0.01$). Note that the reduced number of Dcx+ neuroblasts is due to *Insc* overexpression and is not affected by the Tamoxifen treatment to genetically trace the progeny labeled at E15 by *GlastCre^{ERT2}*. Scale bars: 50 μ m.

Movie S1, related to Figure 2 and 4: Time-lapse movie of an aRGC (green) producing an aRGC and a SNP and an SNP (blue) producing another SNP and 1 non-apically anchored cell (NAC)

Movie S2, related to Figure 4: Time-lapse movie of an aRGC symmetrically producing 2 SNP.

Movie S3, related to Figure 4: Time-lapse movie of a aRGC self-renewing and one daughter cell undergoing cell death shortly after its production.

Movie S4, related to Figure 4: Time-lapse movie of 2 aRGCs and their progeny. The aRGC labeled with green is asymmetrically dividing producing 1 aRGC and 1SNP. The aRGC labeled in blue is producing 1 SNP and 1 cell that is no longer apically anchored (NAC=non-apical cell).