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Supplemental Information

The Ventral Hippocampus Is the Embryonic Origin for Adult Neural Stem Cells in the Dentate Gyrus

Guangnan Li, Li Fang, Gloria Fernández, and Samuel J. Pleasure

Figure S1 (related to Figure 2)

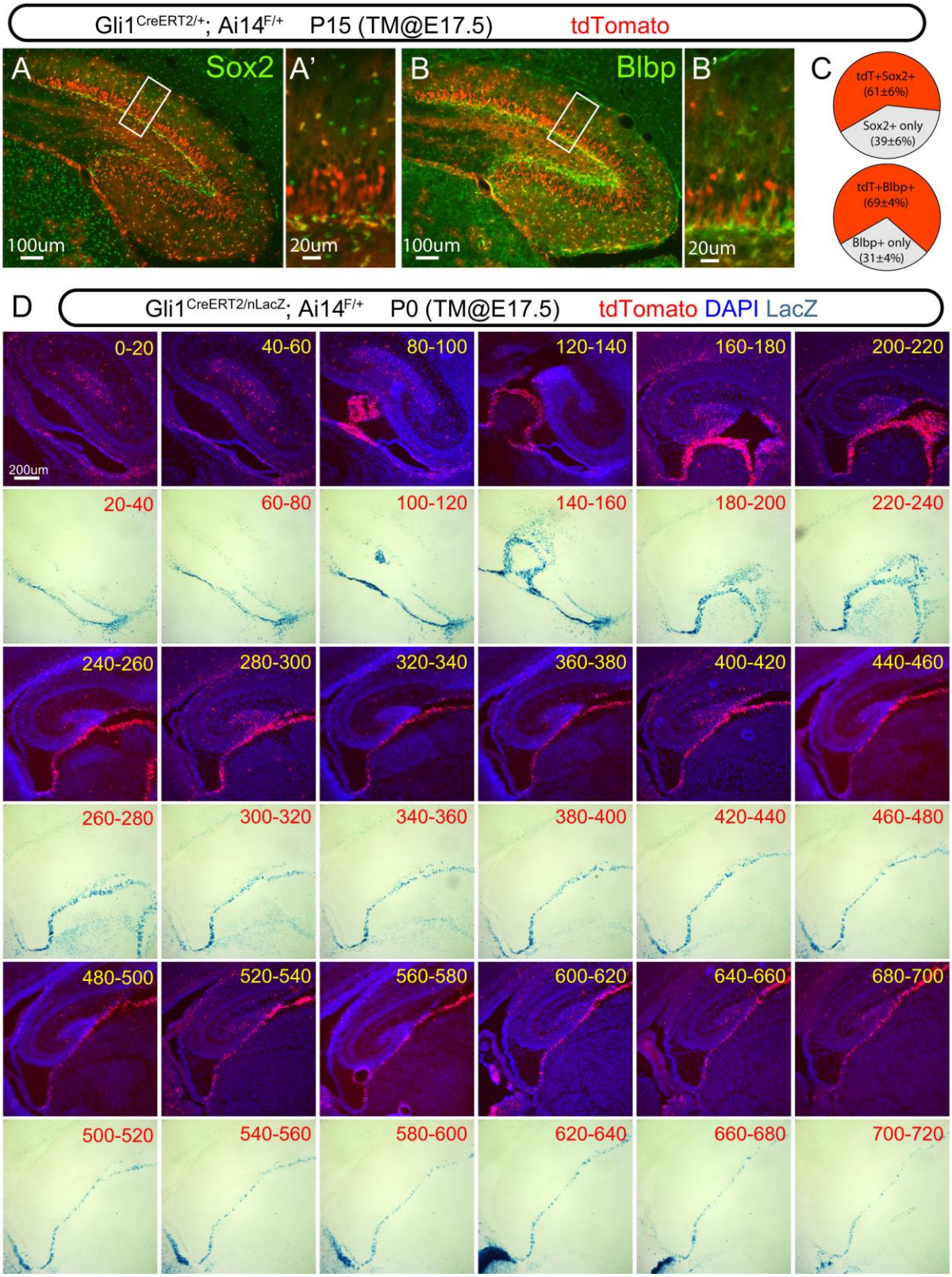

Figure S1 (related to Figure 2):

(**A**-**C**) The labeling efficiency of Gli1-CreERT2 in the SGZ was assessed by P15 with tamoxifen injection (3mg/40g animal) at E17.5 after crossing to the cre reporter Ai14. tdT+ cells in the SGZ were shown to express the radial glia markers Sox2 (**A**) and Blbp (**B**). Boxed areas in (**A**) and (**B**) were shown at the higher magnification in (**A'**) and (**B'**), respectively. 61±6% of the total Sox2+ cells in the SGZ along the septotemporal axis were tdT+ (upper panel in **C**), and 69±4% of the Blbp+ cells with radial processes in the SGZ were also tdT+ (lower panel in **C**).

(**D**) Alternate sections were used for analyzing the spatial distribution of Gli1-nLacZ+ and tdT+ cells respectively for the $Gli1^{CreERT2/nLacZ}$;$Ai14^{F/+}$ mice. The tdT+ cells at P0 represented the descendants of Hh-responding cells labeled by tamoxifen induction at E17.5. The number in each panel represents the thickness distance from the first panel. tdT+ cells clearly occupied the septal levels (480μm away from the first panel) where the Gli1-nLacZ expression was no longer detectable.

Figure S2 (related to Figure 3)

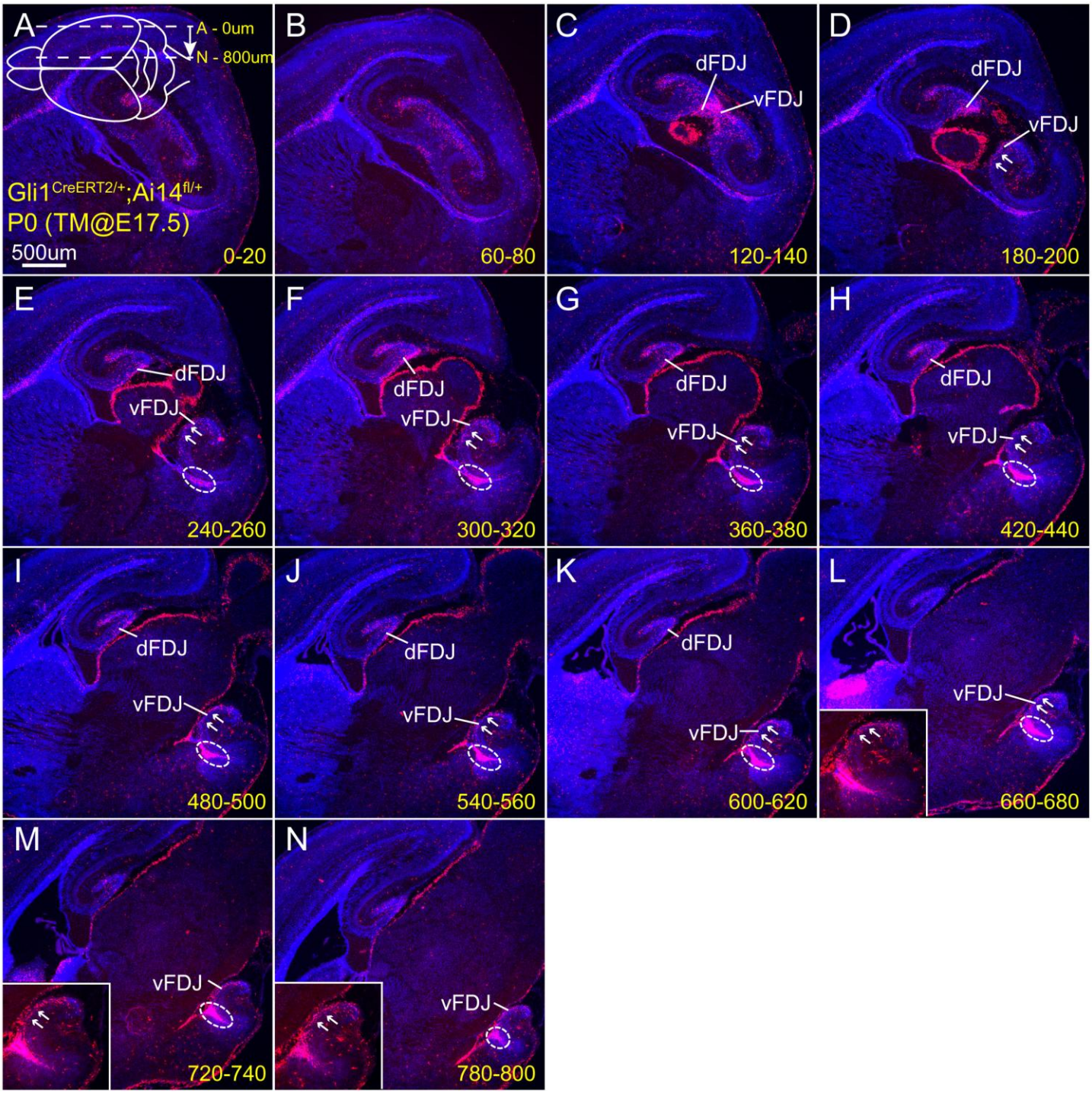

Figure S2 (related to Figure 3)

(**A**-**N**) The distribution of tdT+ cells in the Gli1$^{CreERT2/+}$;Ai14$^{F/+}$ animals were shown every 60μm by birth after tamoxifen (TM) induction at E17.5. The thickness distance as micron from the first section was shown at the bottom-right corner. Throughout the whole hippocampus (**A**-**N**), the VZ of the temporal hippocampus was the most heavily labeled by tdT (white ovals in **E-N**). The tdT+ cell stream was noticeable in the vFDJ region (arrows in **D-N**). The tdT+ cells were continuous from the vFDJ into the dFDJ at the transitional level (**C**) and tapered off in the dorsal hippocampus toward the septal pole.

dFDJ, dorsal fimbriodentate junction; vFDJ, ventral fimbriodentate junction.

Figure S3 (related to Figure 4)

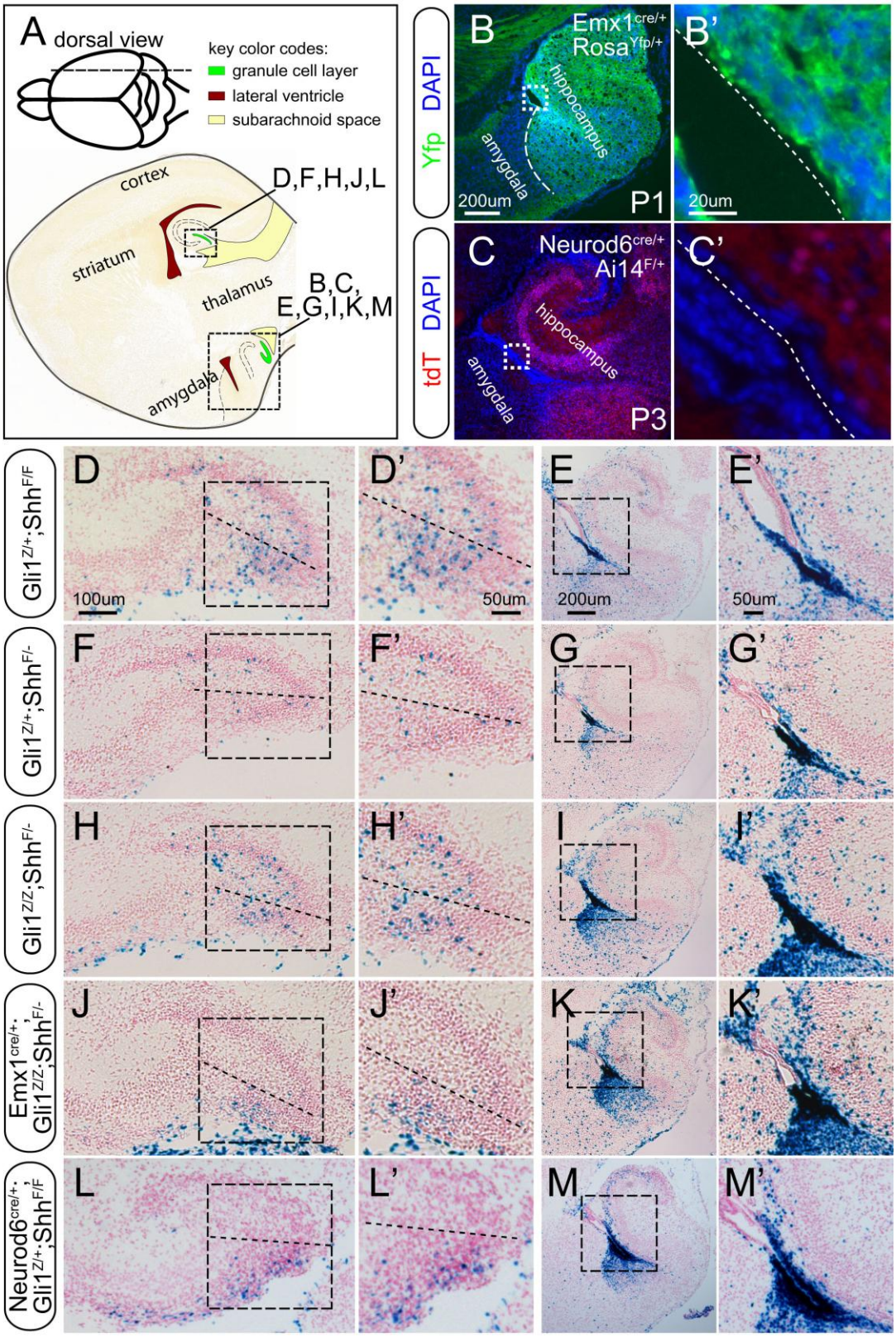

Figure S3 (related to Figure 4)

(**A**) The schemas were illustrated for the sagittal sections from the P1 animals shown in **B**-**M**.

(**B**) In the $Emx1^{Cre/+}$;$Rosa^{Yfp/+}$ animal at P1, Emx1-cre showed the recombination activity in the ventral hippocampus as the expression of Yfp (**B**), which also covered its VZ (along the dotted line in **B'**). Boxed area in **B** was shown at the higher magnification in **B'**.

(**C**) In the $Neurod6^{Cre/+}$;$Ai14^{F/+}$ animal at P3, Neurod6-cre showed the recombination activity in the ventral hippocampus as the expression of tdTomato (**C**), which didn't cover its VZ (along the dotted line in **C'**). Boxed area in **C** was shown at the higher magnification in **C'**.

(**D**-**E**) In the $Gli1^{Z/+}$;$Shh^{F/F}$ animals, Gli1-nLacZ+ cells were localized at the entrance of the hilus in the dorsal DG (**D** and **D'**). The dorsal dentate can be divided into upper and lower portions by a line connecting the tip of the CA3 field and the apex of the dentate pole. Some of Gli1-nLacZ+ cells could be detectable in the upper portion (**D'**). Gli1-nLacZ+ cells were clearly identifiable to form a stream out of the VZ of ventral hippocampus into the forming DG (**E** and **E'**). Boxed areas in **D** and **E** were shown at the higher magnification in **D'** and **E'**.

(**F**-**G**) In the $Gli1^{Z/+}$;$Shh^{F/-}$ animals, one copy of the Shh flox allele was replaced with a Shh null allele. The number of Gli1-nLacZ+ cells in the dorsal dentate was dramatically reduced in both portions (**F** and **F'**). The stream of Gli1-nLacZ+ cells leaving the VZ of ventral hippocampus was slightly diminished and there was a decrease in the expression of nLacZ in the VZ (**G** and **G'**). Boxed areas in **F** and **G** were shown at the higher magnification in **F'** and **G'**.

(**H**-**I**) In the $Gli1^{Z/Z}$;$Shh^{F/-}$ animals, an extra copy of Gli1-nLacZ was present in relation to the animals in **F**-**G.** The distribution of Gli1-nLacZ+ cells was mostly restored in both dorsal DG (**H** and **H'**) and ventral hippocampus (**I and I'**) close to the level seen in the $Gli1^{Z/+}$;$Shh^{F/F}$ animals. Boxed areas in **H** and **I** were shown at the higher magnification in **H'** and **I'**.

(**J**-**K**) In the $Emx1^{cre/+}$;$Gli1^{Z/Z}$;$Shh^{F/-}$ animals, the remaining Shh flox allele was floxed out in the Emx1 domain. Most of the Gli1-nLacZ+ cells were abolished in the upper portion of the dorsal dentate whereas residual Gli1-nLacZ+ cells were still present at the entrance of the hilus in the lower portion (**j** and **j'**), which appeared to be

continuous with the Gli1-nLacZ+ cell stream from the VZ of the ventral hippocampus (**K** and **K'**). Boxed areas in **J** and **K** were shown at the higher magnification in **J'** and **K'**.

(**L**-**M**) In the Neurod6$^{cre/+}$;Gli1$^{Z/+}$;Shh$^{F/F}$ animals, two copies of the Shh flox alleles were removed from the pallial neuronal lineage. The distribution of Gli1-nLacZ+ cells (**L**, **L'**, **M** and **M'**) was quite similar to the Emx1$^{cre/+}$;Gli1$^{Z/Z}$;Shh$^{F/-}$ animals. Boxed areas in **L** and **M** were shown at the higher magnification in **L'** and **M'**.

Figure S4 (related to Figure 6)

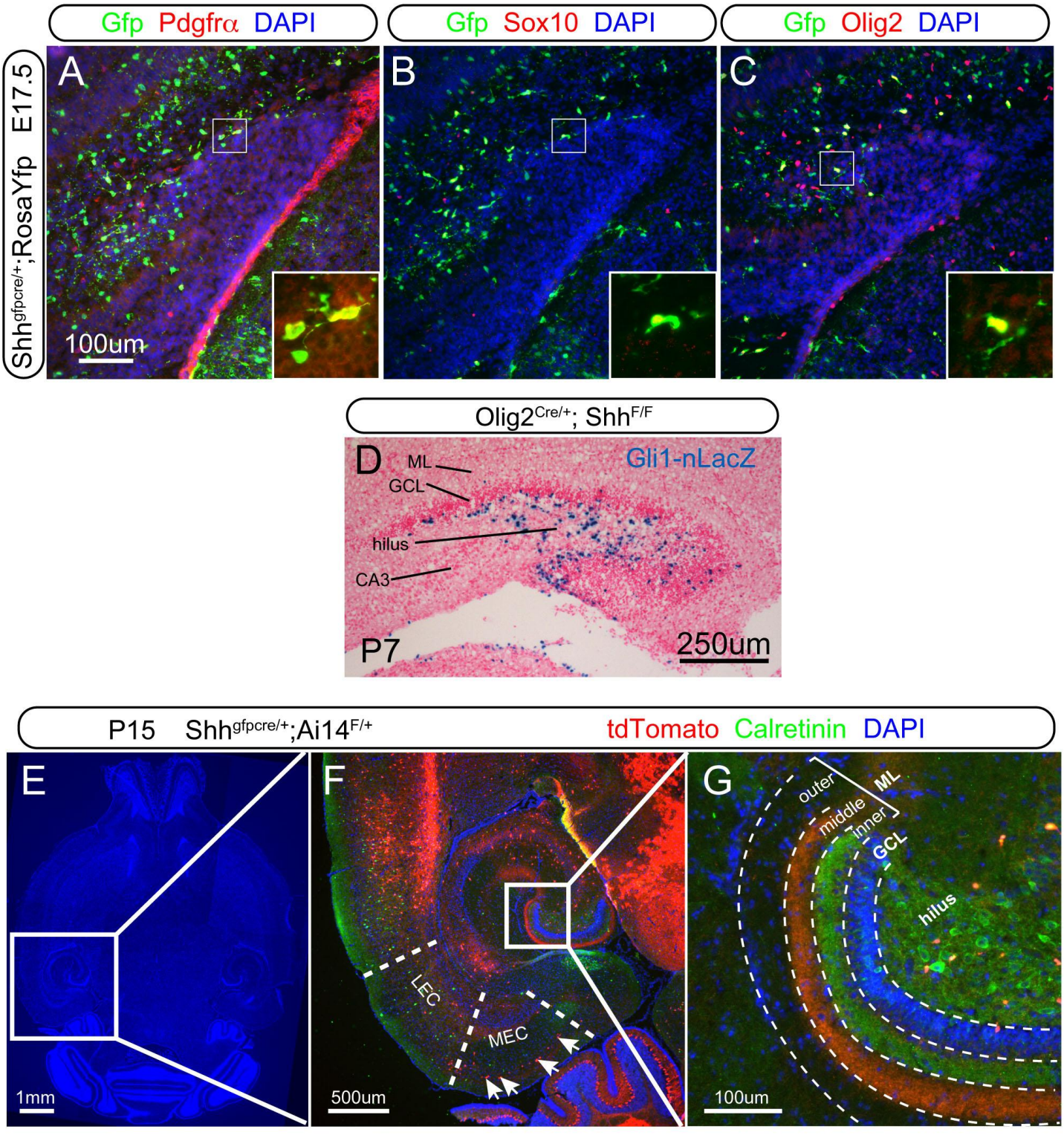

Figure S4 (related to Figure 6)

(**A**-**C**) The Shh lineage expressed markers for oligodendrocyte progenitor cells (OPCs) in the dentate gyrus at E17.5. By crossing Shh-gfpcre with Rosa-Yfp, the Shh lineage marked as Yfp+ cells (enhanced with Gfp antibody staining) in the dentate gyrus at E17.5 was colabeled with oligodendrocyte progenitor cell (OPC) markers -- Pdgfrα (**A**), Sox10 (**B**) and Olig2 (**C**).

(**D**) In the $Olig2^{cre/+}$;$Shh^{F/F}$ animals, Shh was removed from the oligodendrocyte lineage. However, the distribution of the Gli1-nLacZ+ cells in the dorsal DG was unaffected by P7.

(**E**-**G**) By crossing Shh-Gfpcre with the cre reporter Ai14, a horizontal section at P15 (**E**) showed the Shh lineage also marked the cells in the MEC (arrows in **F**), which made projection to the middle molecular layer in the DG (**G**).

LEC, lateral entorhinal cortex; MEC, medial entorhinal cortex.

Figure S5 (related to Figure 7)

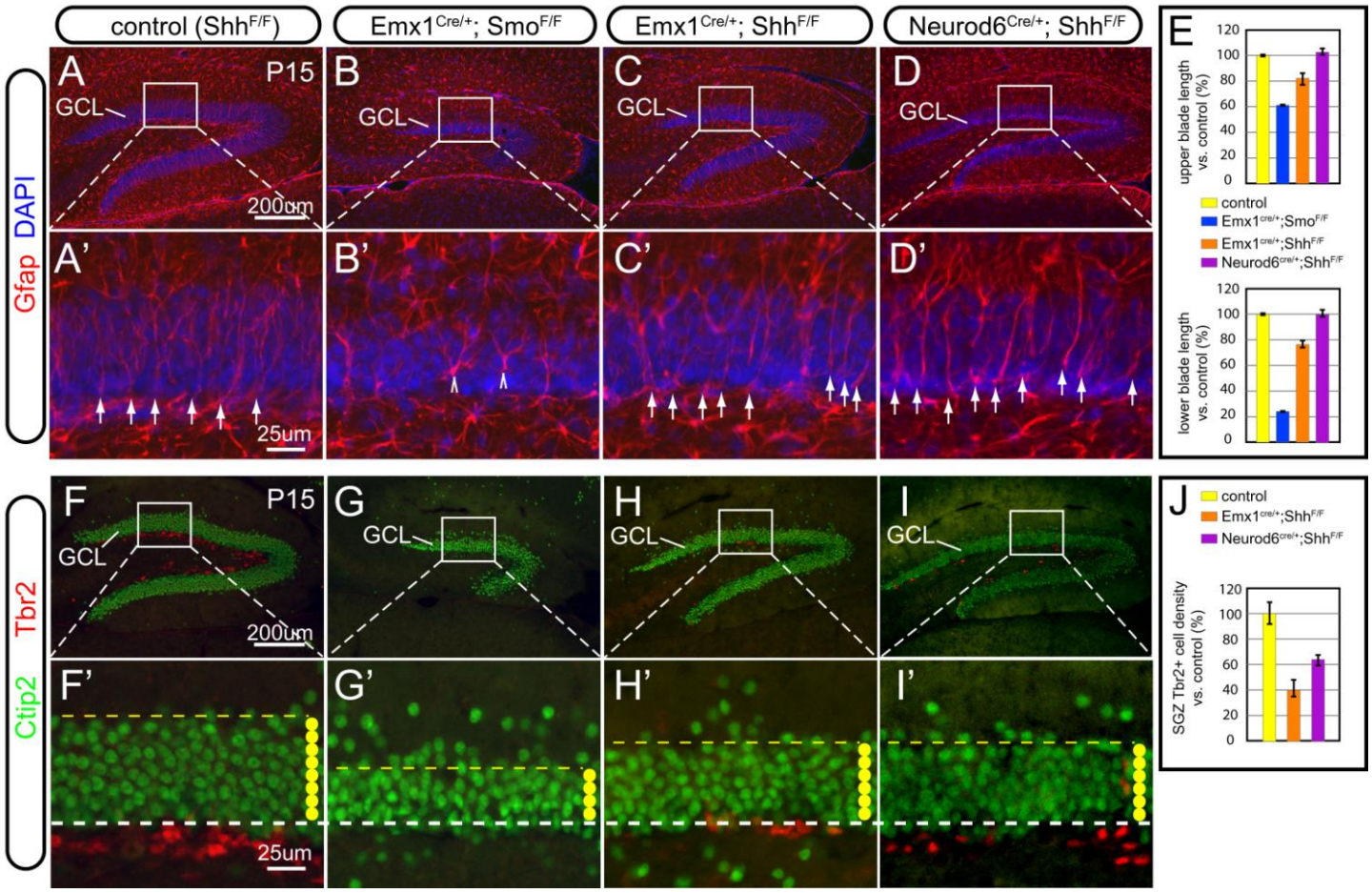

Figure S5 (related to Figure 7)

(**A**-**D**) and (**A**'-**D**') Gfap staining at P15 showed the radial glial scaffolding in the control (**A**, **A'**), Emx1-Smo cKO (**B**, **B'**), Emx1-Shh cKO (**C**, **C'**), Neurod6-Shh cKO (**D**, **D'**). Compared to the control (arrows in **A'**), the radial glia spanning the GCL poorly developed in the Emx1-Smo cKO (arrowheads in **B'**), whereas the radial glial fibers were rather intact in the Emx1-Shh cKO (arrows in **C'**) and Neurod6-Shh cKO (arrows in **D'**).

(**E**) Quantification was made for the length of the upper and lower blades in the control, Emx1-Smo, Emx1-Shh and Neurod6-Shh cKOs. In relation to the control, the length of the upper and lower blades were 61±1% (n=5, p<0.001) and 24±1% (n=5, p<0.001) in the Emx1-Smo cKO, whereas they were 81±5% (n=5, p<0.01) and 76±3% (n=5, p<0.01) in the Emx1-Shh cKO, and 102±4% (n=5, p=0.25) and 98±3% (n=5, p=0.31) in the Neurod6-Shh cKO.

(**F**-**I**) and (**F'**-**I'**) Ctip2+ cells in the GCL and Tbr2+ neurogenic precursors in the SGZ were shown for the control (**F, F'**), Emx1-Smo cKO (**G, G'**), Emx1-Shh cKO (**H, H'**) and Neurod6-Shh cKO (**I, I'**). Emx1-Smo cKO displayed complete deficiency for the Tbr2+ cells in the SGZ and the thinnest GCL (~4 cells thick, yellow dots in **G'**). Emx1-Shh cKO also had diminished number of Tbr2+ cells in the SGZ and thinner GCL (~6 cells thick, yellow dots in **H'** and **I'**), compared to the control (~8 cells thick, yellow dots in **F'**).

(**J**) Quantification of Tbr2+ cell densities in the SGZ was made for the control, Emx1-Shh cKO and Neurod6-Shh cKO. As compared to the control (100±9%), it was 40±7% (n=5, p<0.001) in the Emx1-Shh cKO and 62±4% (n=5, p<0.001) in the Neurod6-Shh cKO.

Data were shown as mean±SEM and p values for the indicated sample sizes were returned by Student's *t*-test for two-tailed distribution with unequal variance.

Figure S6 (related to Figure 8)

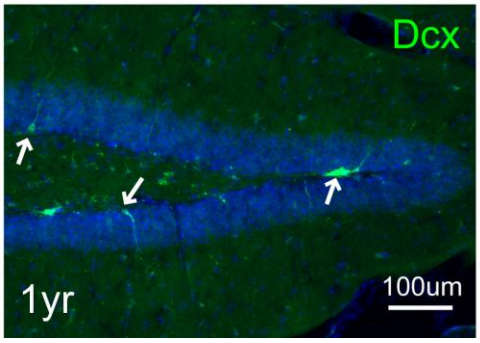

Figure S6 (related to Figure 8)

Only very few Dcx+ immature neurons (arrows) were detectable in the DG in one year old animals