

Fig. S1. The expression pattern of YAP in neocortex during development. (A)Western blot detected the expression of YAP in the neocortex in different developmental stages. (B) Quantitative analysis of the relative expression level of YAP as shown in (A) (normalized to P1 neocortex, n=3). Data were mean \pm sem.

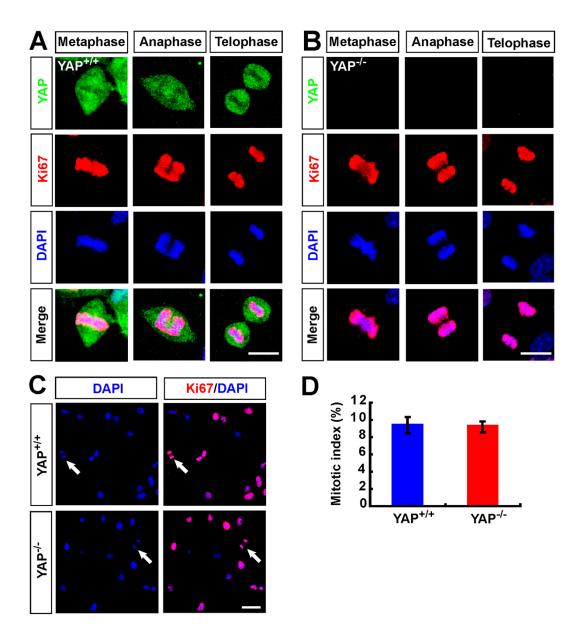


Fig. S2. Normal mitotic division in Yap-deficient NSCs. (A-B) Double immunostaining analysis of sub-cellular distributions of YAP (green) and Ki67 (red) during mitotic division stages of cultured WT NSCs (A) and YAP-deficient NSCs (B). (C) Representative images of mitotic dividing NSCs (arrowheads) from Yap^{f/f} and Yap^{nestin}-CKO mice. (D) Quantitative analysis of mitotic index (the percentage of mitotic dividing cells over total proliferating cells (Ki67 positive, red) (n=20 fields per group) of WT or YAP-deficient NSCs. DAPI (blue) was used to stain the cellular nuclei. Data were mean \pm sem. Scale bars, 20 μm.

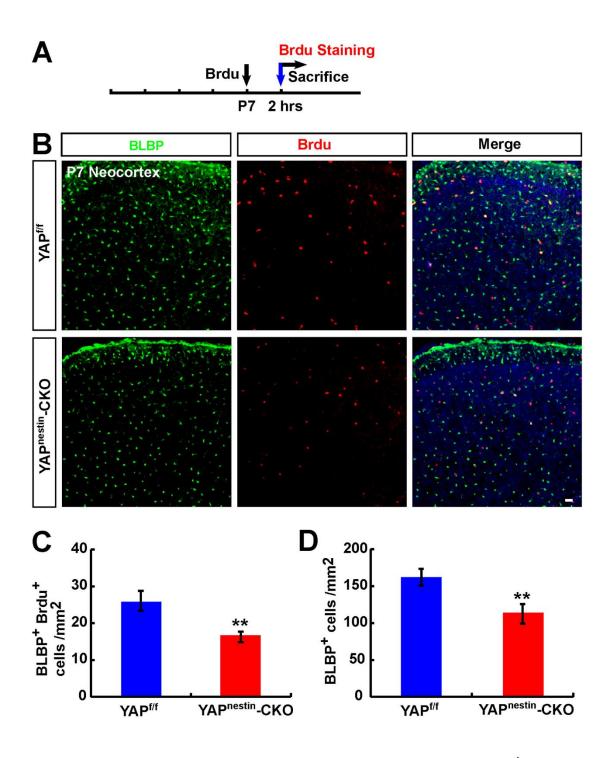


Fig. S3. Reduced local proliferation of neocortical astrocytes in P7 Yap^{nestin}-CKO mice. (A) Schematic diagram of Brdu incorporation experiments. (B) Double immunostaining analysis of BLBP (green) and Brdu (red) in neocortex of P7 Yap^{f/f} and Yap^{nestin}-CKO mic. (C-D) Quantitative analysis of the density of BLBP⁺Brdu⁺ cells (C) and BLBP⁺ cells (D) (n=10 per group) in neocortex of P7 Yap^{f/f} and Yap^{nestin}-CKO mice as shown in (B). DAPI (blue) was used to stain cellular nuclei. Scale bars, 20 μ m. Data were mean \pm sem. **P < 0.01, compared with control group, Student's t test.

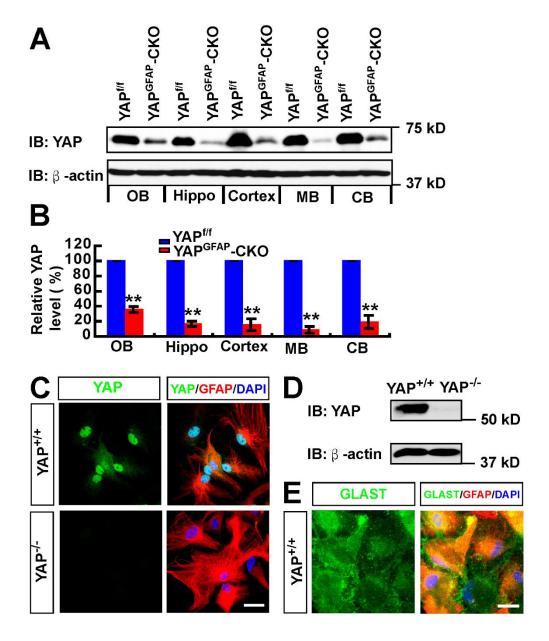


Fig. S4. Generation of Yap^{GFAP}-CKO mice. (A) Western blot analysis of YAP expression in different brain regions of P14 Yap^{f/f} and Yap^{GFAP}-CKO mice. OB, olfactory bulb; Hippo, Hippocampus; MB, midbrain, CB, cerebellum. (B) Quantification of YAP expression level shown in (A) (n=3 per group, normalized to WT group). (C) Double immunostaining analysis of YAP (green) and GFAP (red) in primary cultured astrocytes from P1 Yap^{f/f} and Yap^{GFAP}-CKO mice. (D) Western blot detected the expression of YAP in primary cultured astrocytes form P1 Yap^{f/f} and Yap^{GFAP}-CKO mice. (E) Double immunostaining analysis of GLAST (green) and GFAP (red) in primary cultured WT astrocytes. DAPI (blue) was used to stain cellular nuclei. Scale bars, 20 μm. Data were mean ± sem. **P < 0.01, compared with control group, Student's t test.

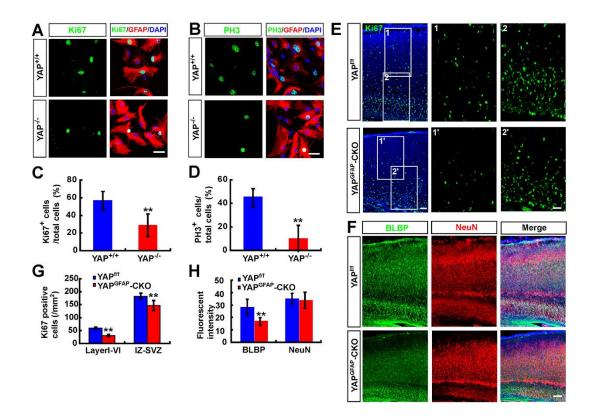


Fig. S5. Reduced neocortical astrocytic proliferation and number in Yap^{GFAP}-CKO mice.

(A-B) Double immunostaining analysis of Ki67 (green) and GFAP (red) (A), PH3 (green) and GFAP (red) (B) in primary cultured astrocytes from P1 Yap^{f/f} and Yap^{GFAP}-CKO mice. (C-D) Histograms showing the percentages of Ki67 (C) and PH3 (D) positive astrocytes over total astrocytes from P1 Yap^{f/f} and Yap^{GFAP}-CKO mice. (E-F) Immunostaining analysis of Ki67 (green) (E), double immunostaining analysis of BLBP (green) and NeuN (red) (F) in neocortex of P1 Yap^{f/f} and Yap^{GFAP}-CKO mice. (G-H) Quantification of then density of Ki67 positive cells (n=8 sections per group) (G) and fluorescent intensity of BLBP and NeuN (H) (n=9 sections per group) in neocortex of P1 Yap^{f/f} and Yap^{GFAP}-CKO mice. The selected regions were shown at higher magnification. Scale bars, 20 μm. Data were mean ± SD. **P < 0.01, compared with control group, Student's t test.

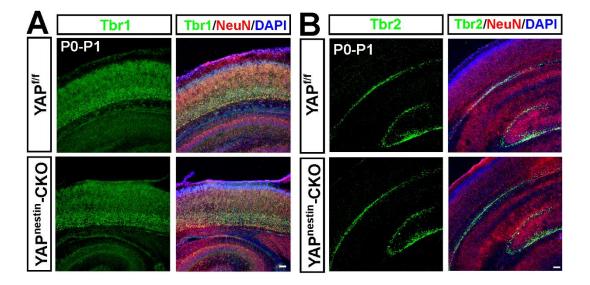


Fig. S6. Normal neurogenesis in P0-P1 Yap^{nestin}-**CKO neocortex.** (A-B) Double immunostaining analysis of Tbr1 (green) and NeuN (red) (A), Tbr2 and NeuN (B) in neocortex of P0-P1 Yap^{f/f} and Yap^{nestin}-CKO mice (n=8 per group). Scale bars, 20 μm.

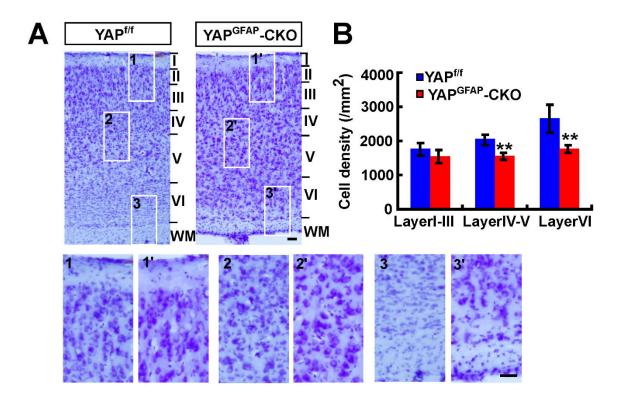


Fig. S7. Loss of neocortical neurons in Yap^{GFAP}-CKO mice. (A) Nissl staining analysis of cortical phenotypes (sagittal sections) in P27 Yap^{f/f} and Yap^{GFAP}-CKO mice. The selected regions 1, 2, 3 in Yap^{f/f}, 1', 2', 3' in Yap^{GFAP}-CKO mice were shown at higher magnification in bottom panels. (B) Quantitative analysis of cell density in indicated cortical layers (n=5 sections each group) in Yap^{f/f} and Yap^{GFAP}-CKO mice. Scale bars, 20 μm. Data are mean \pm SD. **P < 0.01, compared with control group, Student's t test.

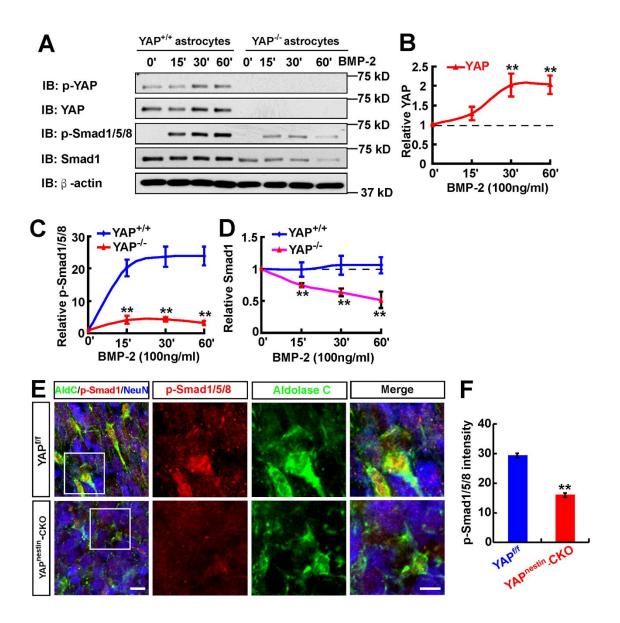


Fig. S8. YAP is required for BMP2-induced p-Smad1/5/8 signaling in astrocytes. (A) Western blot detected the downstream signaling of BMP2 in WT and YAP-deficient astrocytes before and after BMP2 treatment (100 ng/ml) at indicated time point. (B-D) Quantitative analysis of relative YAP (B), p-Smad1/5/8 (C), and Smad1 (D) as shown in (A) (n=3 per group, normalized to 0 min). (E) Double immunostaining analysis of p-Smad1/5/8 (red) and Aldolase C (green) in neocortex of P1 Yap^{f/f} and Yap^{nestin}-CKO mice. (F) Quantitative analysis of the p-Smad1/5/8 intensity as shown in (E) (n=12 per group). Scale bars, 20 μm. Data were mean \pm sem. **P < 0.01, compared with control group, Student's t test.