

Figure S1. COUP-TFI is expressed in a subpopulation of neuroblasts in the adult mouse SVZ. (A) COUP-TFI/DCX double-immunostained P42 mouse brain section. (B-F) Higher-magnification images of the boxed areas in (A) showing COUP-TF+/DCX+ cells in the dorsal SVZ (B, C); COUP-TFI+ cells were not in the lateral, ventral or medial SVZ at the rostral part of the lateral ventricle. (G) COUP-TFI was expressed in about 12% CR+ cells in the GCL, but not in the periglomerular layer. Scale bars: 200 μm in A; 100 μm in G for B-G.

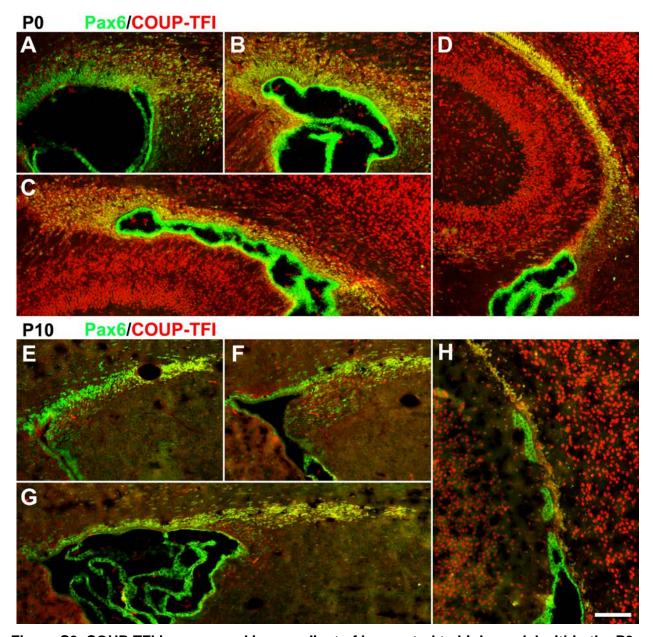


Figure S2. COUP-TFI is expressed in a gradient of low rostral to high caudal within the P0 and P10 SVZ. (A-H) Pax6/COUP-TFI double-immunostained coronal brain sections at different rostrocaudal levels of the SVZ at P0 (A-D) and P10 (E-H). Scale bar: 100 μm in H for A-H.

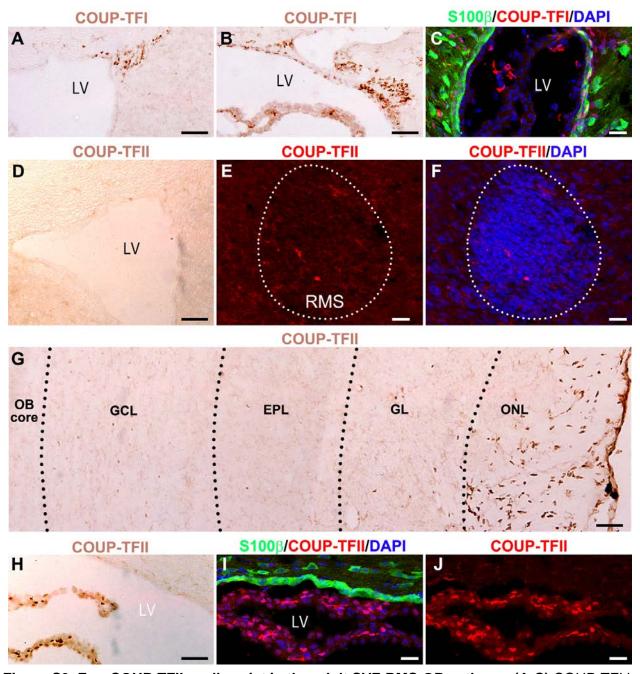


Figure S3. Few COUP-TFII+ cells exist in the adult SVZ-RMS-OB pathway. (A-C) COUP-TFI is expressed in ependymal cells in the caudal lateral wall of the lateral ventricle in adult mice ($\bf B, C$) but not in ependymal cells at the rostral level ($\bf A$). ($\bf D-G$) Very few COUP-TFII+ cells were in the adult SVZ-RMS-OB pathway. Note that COUP-TFII is strongly expressed in the ONL ($\bf G$). ($\bf H-J$) COUP-TFII is expressed in the choroid plexus. LV: lateral ventricle; GCL: granule cell layer; EPL: external plexiform layer; GL: periglomerular layer; ONL: olfactory nerve layer. Scale bars: 50 μ m in A, B, D, G, H; 20 μ m in C, E, F, I, J.

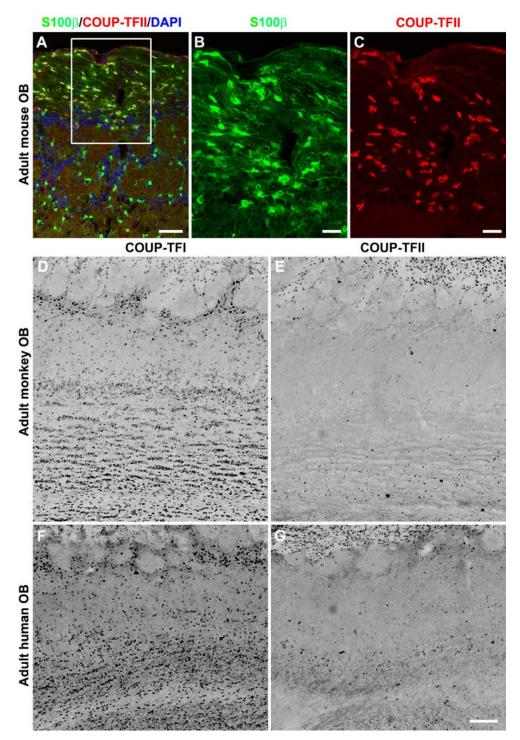


Figure S4. Expression patterns of COUP-TFI and COUP-TFII in the adult human and rhesus monkey OB are similar to those in adult mice. (A-C) COUP-TFII is strongly expressed in olfactory ensheathing cells (S100 β +) in the olfactory nerve layer of the adult mouse OB. (D-G) COUP-TFI+ cells were abundant (D, F), whereas only a small number of COUP-TFII+ cells (E, G) were in the adult human and monkey OB. Scale bars: 50 μ m in A; 20 μ m in B, C; 100 μ m in G for D-G.

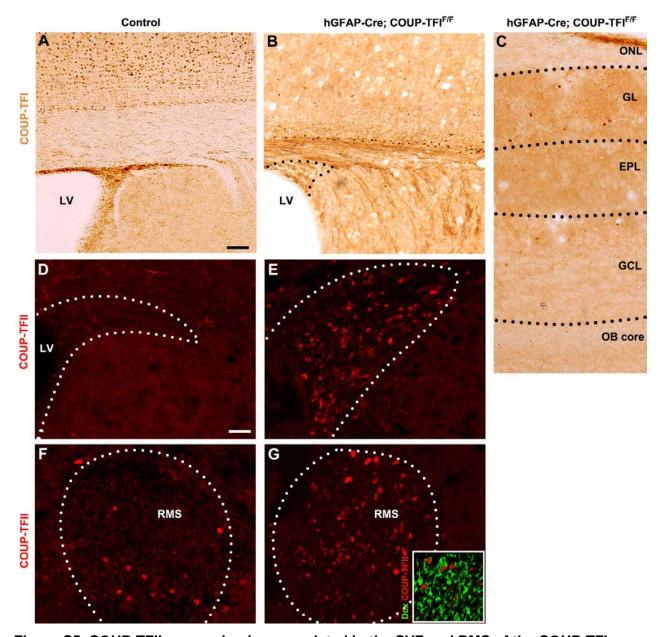


Figure S5. COUP-TFII expression is upregulated in the SVZ and RMS of the COUP-TFI conditional mutant mice. (A-C) COUP-TFI expression was eliminated in the SVZ and OB of adult hGFAP-Cre; COUP-TFI mice. Note that many projection neurons in neocortical layer VI still expressed COUP-TFI in hGFAP-Cre; COUP-TFI mice, as the hGFAP-Cre exhibits excision of floxed alleles in mouse cortical radial glia around E13.5. (D-G) In control mice, very few COUP-TFII+ cells were identified in the SVZ and RMS (D, F), but COUP-TFII expression in the SVZ and RMS was significantly upregulated in hGFAP-Cre; COUP-TFI mice (E, G). Insert in (G) showed that a subpopulation of DCX+ neuroblasts in the RMS expressed COUP-TFII. All brain sections were from P21 mice. Scale bars: 50 μm in A for A-C; 100 μm in D for D-G.

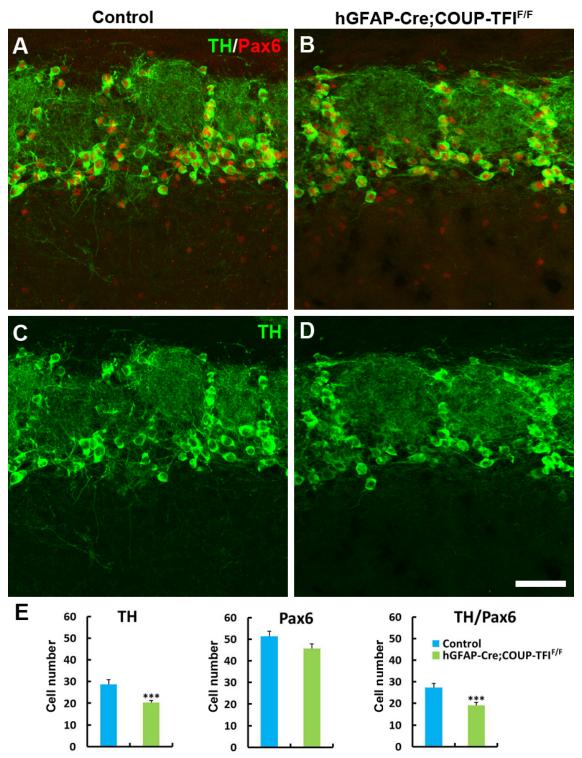


Figure S6. TH expression is reduced in the glomerular layer of the COUP-TFI conditional mutant OB. (**A-D**) OB sections double immunostained for TH and Pax6. (**E**) The number of TH+ and TH+/Pax6+, but not Pax6+ periglomerular cells was significantly reduced in the hGFAP-Cre; COUP-TFI^{flox/flox} mouse OB compared to controls at P21. *** p<0.001. Scale bar: 50 μm in D for A-D.

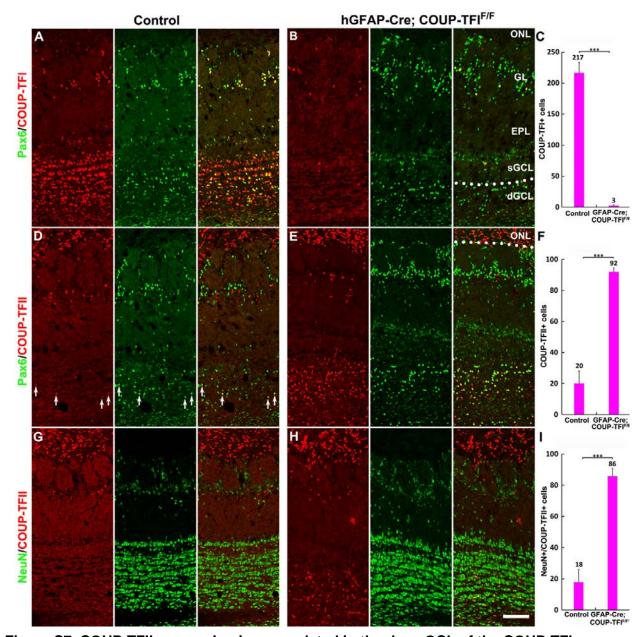


Figure S7. COUP-TFII expression is upregulated in the deep GCL of the COUP-TFI conditional mutant OB. (A-C) Only a few COUP-TFI+ cells remained in hGFAP-Cre; COUP-TFI^{flox/flox} mouse OB compared to controls. Approximately 90% Pax6+ cells in the glomerular layer and about 74.8% Pax6+ cells in the GCL expressed COUP-TFI in the control OB. (D-I) A very small number of COUP-TFII+ cells were in the GL and GCL of the control mouse OB, whereas COUP-TFII expression was significantly upregulated in the mature GCs in the deep GCL of hGFAP-Cre; COUP-TFI^{flox/flox} mice (F, I); about 78% of COUP-TFII+ cells in the GCL in the control (D, arrows) and COUP-TFI conditional mutant (E) mouse OB expressed Pax6. Note that COUP-TFII expression was not upregulated in the superficial GCL, EPL and GL (E, H). All OB sections were from P21 mice. dGCL, deep GCL; sGCL, superficial GCL; *** p<0.001. Scale bar: 100 μm in H for A-H.

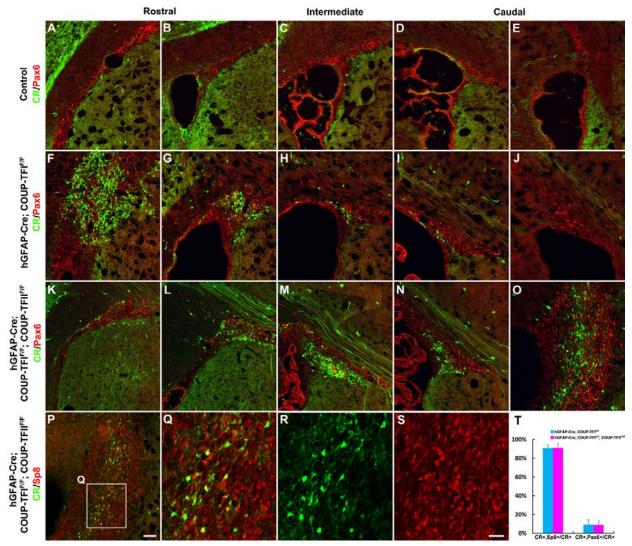


Figure S8. CR+ cells accumulate in the SVZ of COUP-TFs conditional mutant mice. (A-O) CR/Pax6 double-immunostaining showed that very few CR+ cells were in the control SVZ (A-E), whereas a subset of CR+ cells accumulate in the SVZ of hGFAP-Cre; COUP-TFI^{flox/flox} mice (F-J) and hGFAP-Cre; COUP-TFI^{flox/flox}; COUP-TFII^{flox/flox} mice (K-O). (P-S) CR/Sp8 double immunostained sections of COUP-TFs double conditional mutant SVZ. (T) Quantification data showed that about 91% of CR+ cells expressed Sp8 and about 9% of CR+ cells expressed Pax6. All brain sections were from P21 mice. Scale bars: 50 μm in P for A-P; 20 μm in S for Q-S.

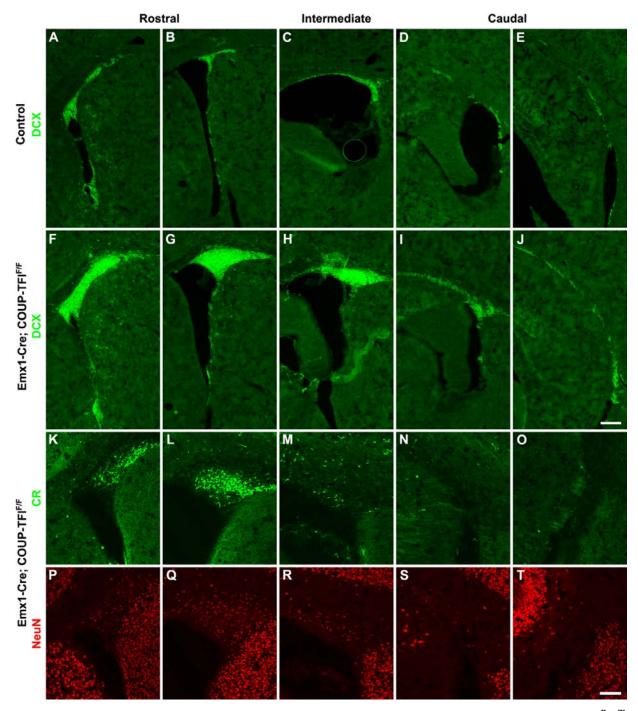


Figure S9. DCX+, CR+ and NeuN+ cells accumulate in the SVZ of Emx1-Cre; COUP-TFI^{flox/flox} conditional mutants. (A-E) DCX+ cells in the P21 control mouse SVZ. (F-J) More DCX+ cells were in the SVZ of P21 Emx1-Cre; COUP-TFI^{flox/flox} conditional mutant mice compared to controls. (K-T) A subset of CR+ (K-O) and NeuN+ (P-T) cells accumulate in the SVZ of Emx1-Cre; COUP-TFI^{flox/flox} conditional mutant mice at P21. Scale bars: 200 μm in J for A-J; 100 μm in T for K-T.

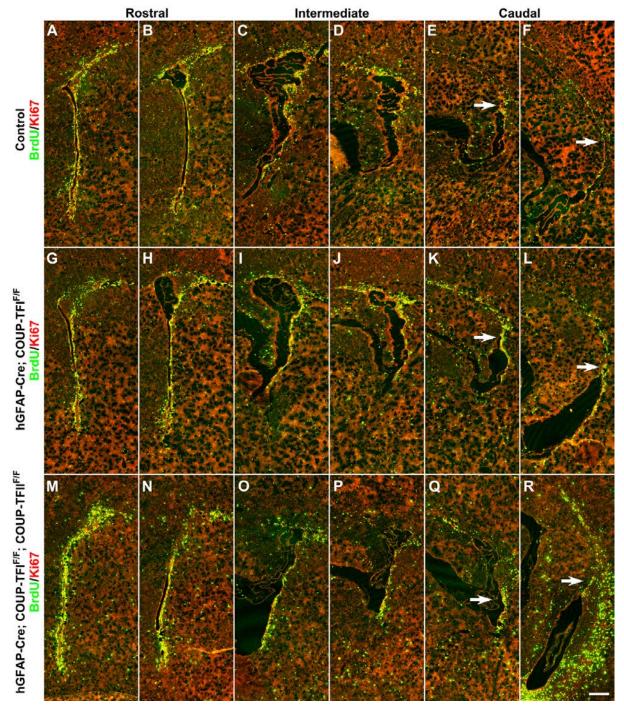


Figure S10. Cell proliferation in the SVZ of COUP-TFs conditional mutant mice. (A-R) BrdU was injected into control and COUP-TFs conditional mutant mice at P10 and mice were sacrificed 2 hours after BrdU injection. Compared to the caudal SVZ of control mice (E, F, arrows), more BrdU+/Ki67+ cells were observed in the caudal SVZ of hGFAP-Cre; COUP-TFI^{flox/flox} mice (K, L, arrows) and hGFAP-Cre; COUP-TFI^{flox/flox}; COUP-TFII^{flox/flox} mice (Q, R, arrows). Scale bar: 200 μm in R for A-R.

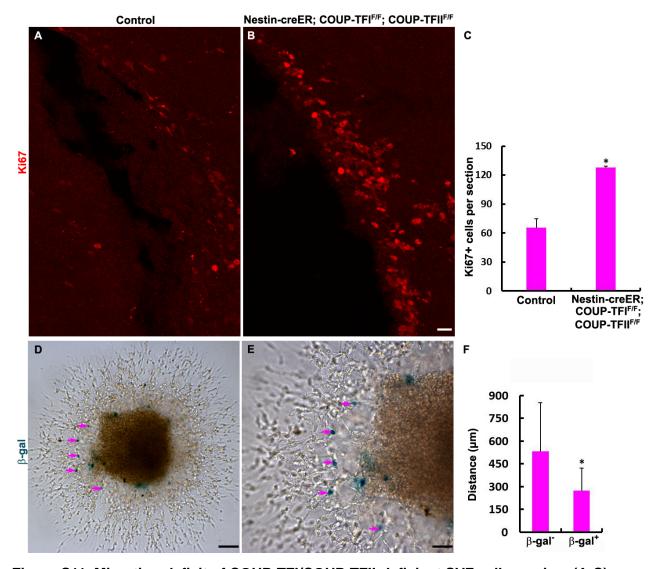


Figure S11. Migration deficit of COUP-TFI/COUP-TFII-deficient SVZ cells ex vivo. (A-C) Control and Nestin-CreER; COUP-TFI $^{flox/flox}$; COUP-TFII $^{flox/flox}$ mice were given tamoxifen at P10 by intraperitoneal injection and mice were sacrificed at P20. In the SVZ of Nestin-CreER; COUP-TFI $^{flox/flox}$; COUP-TFII $^{flox/flox}$ mice, significant increase in the number of Ki67+ cells per section were observed compared to controls. (D) SVZ explants from P1 COUP-TFI $^{flox/flox}$; COUP-TFII $^{flox/flox}$ pups were cultured with an adenovirus expressing Cre recombinase for 4 days. Most cells (β-gal-negative) showed profuse migration into the Matrigel, whereas reduced migration of COUP-TFI-/COUP-TFII-deficient cells (β-gal+, arrows) was observed. (E) Higher-magnification image of β-gal+ cells in (D). (F) Quantification of cell migration distance. * p<0.05. Scale bar: 20 μm in B for A, B; 100 μm in D; 50 μm in E.

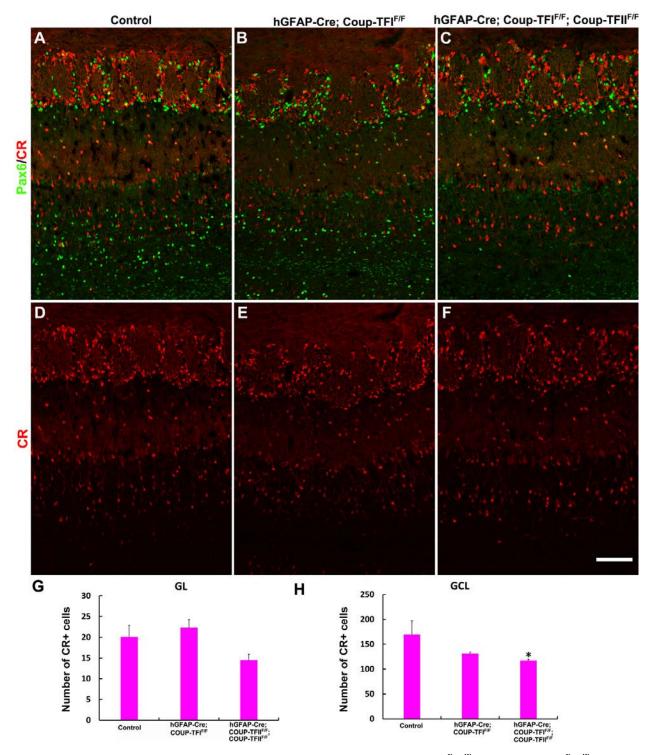


Figure S12. CR+ GCs are reduced in the hGFAP-Cre; COUP-TFI^{flox/flox}; COUP-TFII^{flox/flox} mouse OB. (A-F) Most CR+ cells in the OB GCL did not express Pax6. (G, H). The number of CR+ GCs in the hGFAP-Cre; COUP-TFI^{flox/flox}; COUP-TFII^{flox/flox} mouse OB were significantly reduced compared to controls at P21. * p<0.05. Scale bar: 100 μm in F for A-F.