

Supplementary Materials

Supplementary Figures and Figure Legends

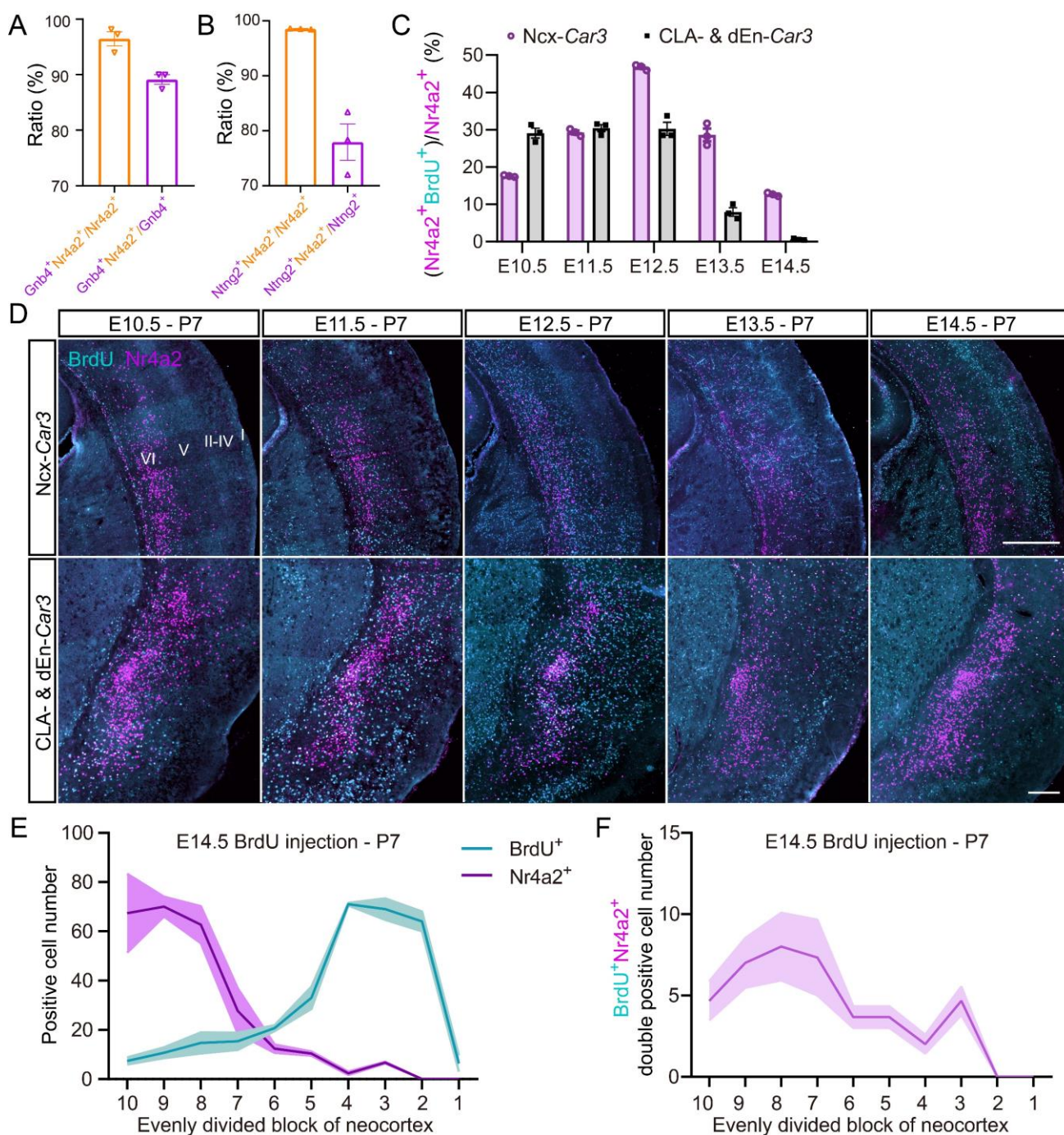


Fig. S1 The co-labeling of Nr4a2 and Gnb4 and Ntn2 in neocortex and the birth date characteristic of *Car3* populations. **A** The co-labeling ratio of $(\text{Gnb4}^+/\text{Nr4a2}^+ \text{ cell number})/(\text{Nr4a2}^+ \text{ cell number})$ and $(\text{Gnb4}^+/\text{Nr4a2}^+ \text{ cell number})/(\text{Gnb4}^+ \text{ cell number})$ is shown. $n = 3$. **B** The statistical result of the

co-labeling ratio of $(Ntng2^{+}/Nr4a2^{+} \text{ cell number}) / (Nr4a2^{+} \text{ cell number})$ and $(Ntng2^{+}/Nr4a2^{+} \text{ cell number}) / (Ntng2^{+} \text{ cell number})$. $n = 3$. **C** The ratios of $(Nr4a2^{+}BrdU^{+} \text{ cell number}) / (Nr4a2^{+} \text{ cell number})$ in the *Ncx-Car3* and the other *Car3* regions (CLA- and dEn-*Car3*) are calculated. $n = 3$. **D** Colocalization of BrdU (cyan) and Nr4a2 (magenta) in *Ncx*-, CLA- and dEn-*Car3* regions of P7 mice with a single pulse of BrdU injection in pregnant mice at E10.5, E11.5, E12.5, E13.5, and E14.5, respectively. I-VI, I-VI cortical layers. Scale bars, 500 μm (upper panel) and 200 μm (lower panel). **E**, **F** BrdU-single positive (**E**), Nr4a2-single positive (**E**), and BrdU⁺/Nr4a2⁺-double positive cell number (**F**) in P7 neocortex (with BrdU injection at E14.5) are counted and calculated, respectively. The neocortex is evenly divided into ten bins from deep to superficial cortex (shown as 10 to 1 in **Fig. 2A**). The wide bands in light colours around the curves are the error bands that represent SEM. $n = 3$.

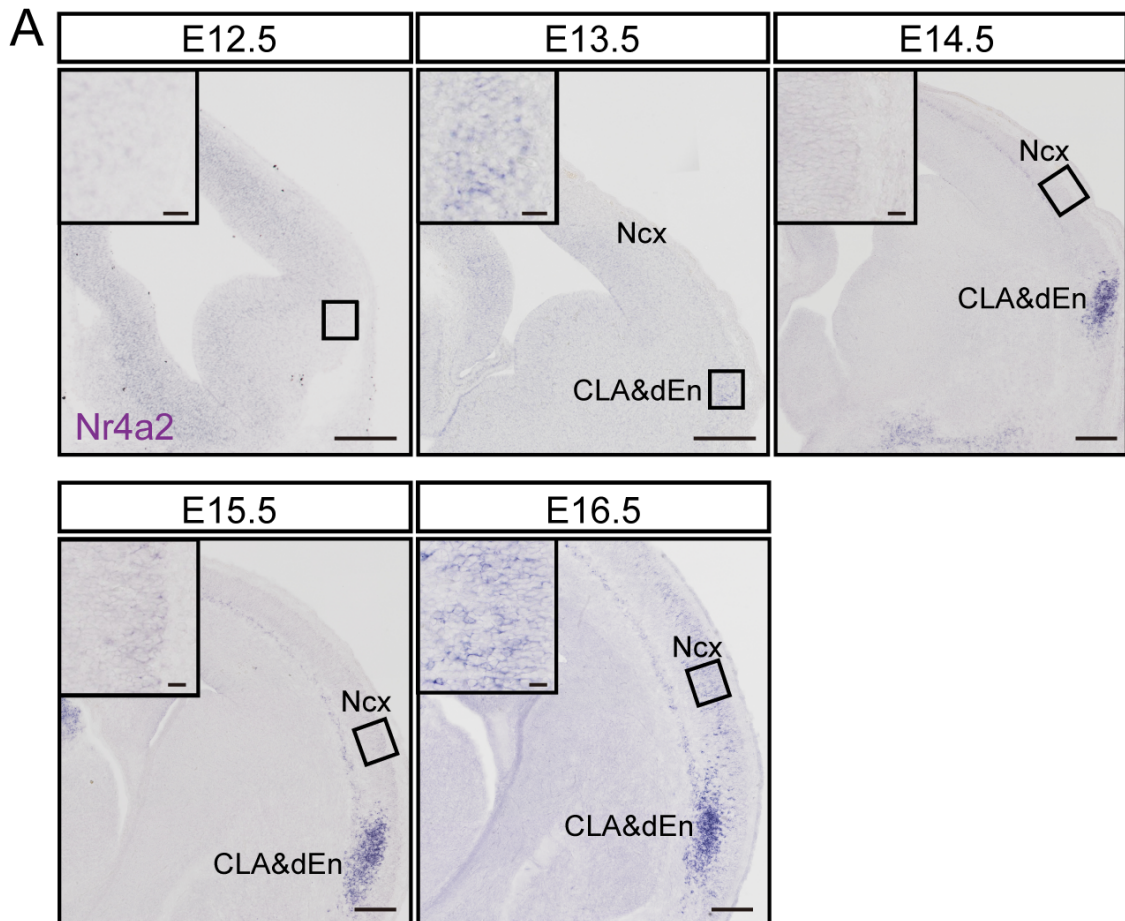


Fig. S2 The expression of *Nr4a2* in the developing cerebral cortex. **A** The expression of *Nr4a2* in the neocortex at different embryonic stages. *Nr4a2* is visualized by *in situ* hybridization. Ncx, neocortex. CLA&dEn, claustrum, and dorsal endopiriform nucleus. Scale bars, 200 μ m and 20 μ m (inserts).

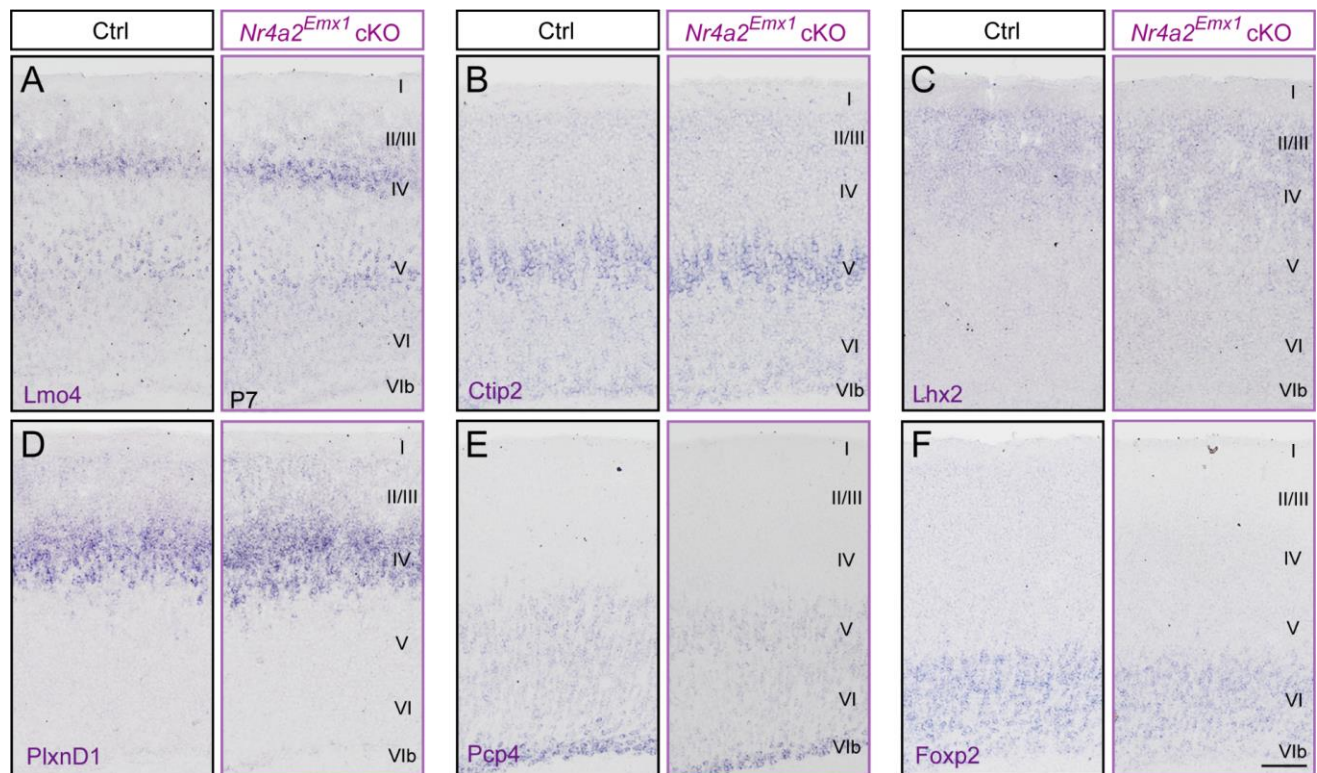


Fig. S3 The expression of layer-specific marker genes in the adult cerebral cortex of *Nr4a2^{Emx1} cKO* and control mice. **A–F** The expression of *Lmo4* (A), *Ctip2* (B), *Lhx2* (C), *PlxnD1* (D), *Pcp4* (E), and *Foxp2* (F) in the neocortex of adult *Nr4a2^{Emx1} cKO* and control mice, which is visualized by *in situ* hybridization. I-VI and VIb, I-VI and VIb cortical layers. Scale bars, 100 μ m.

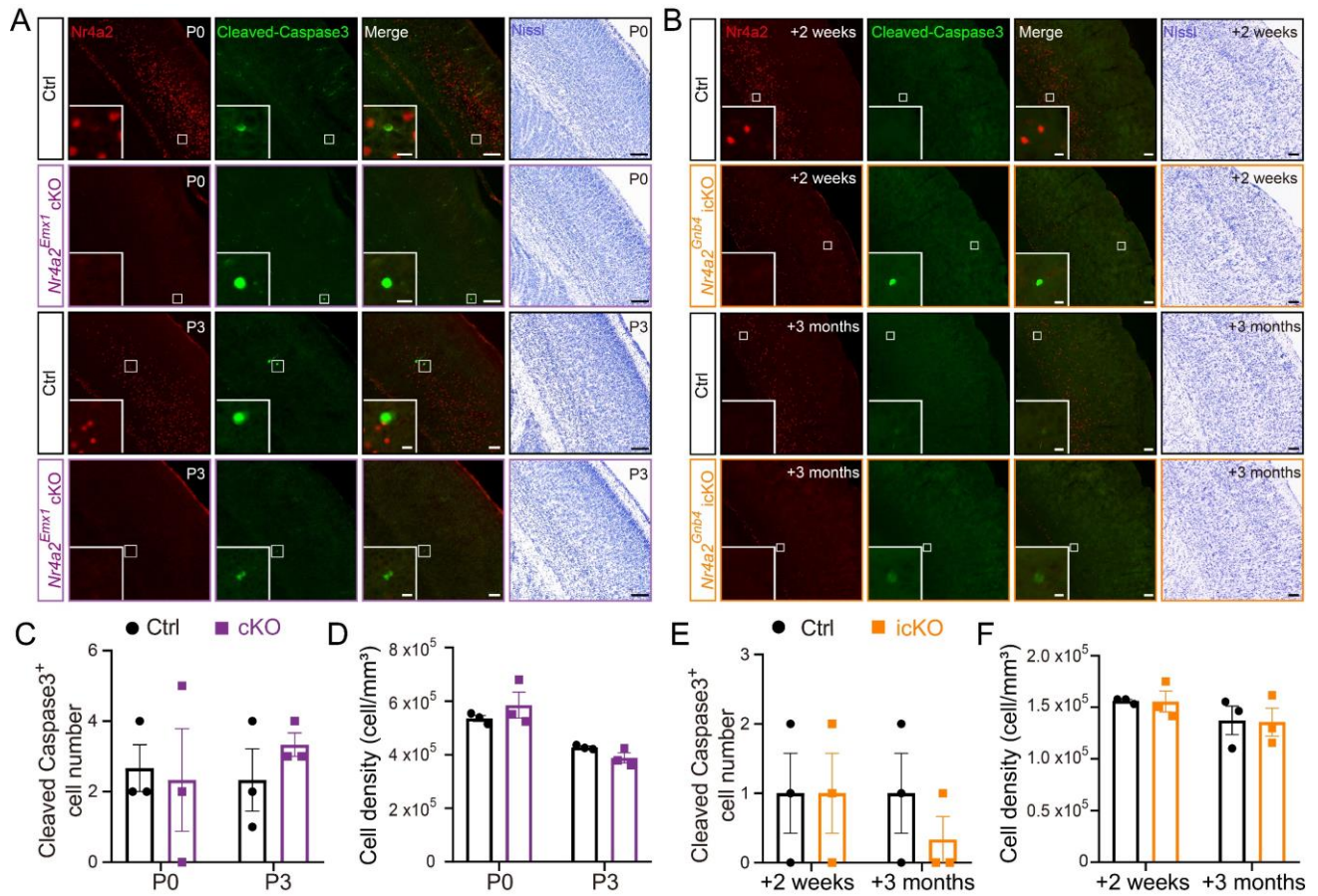


Fig. S4 No abnormal cell apoptosis occurred in the cerebral cortex after *Nr4a2* deletion at the embryonic stage and in adulthood. **A** Nissl staining (right panel) and immunostaining of *Nr4a2* (red) and cleaved-caspase3 (green) in the neocortex of P0- and P3- *Nr4a2^{Emx1} cKO* and control mice (left three panels). Scale bars, 100 μ m and 10 μ m (inserts). **B** Nissl staining (right panel) and immunostaining of *Nr4a2* (red) and cleaved-caspase3 (green) in the neocortex of adult *Nr4a2^{Gnb4} icKO* and control mice (two weeks and three months after tamoxifen administration; left three panels). Scale bars, 100 μ m and 10 μ m (inserts). **C, E** Comparisons of the cell numbers of cleaved-caspase3-positive cells in the neocortex of P0- and P3 *-Nr4a2^{Emx1} cKO* and control mice (**C**), and adult *Nr4a2^{Gnb4} icKO* mice (two weeks and three months after tamoxifen administration) with their respective controls (**E**, $n = 3$). **D, F** Comparisons of the cell density displayed by Nissl staining between P0- and P3 *-Nr4a2^{Emx1} cKO* and control mice (**D**), and between adult *Nr4a2^{Gnb4} icKO* mice (two weeks and three months after

tamoxifen administration) and control mice (F, $n = 3$).

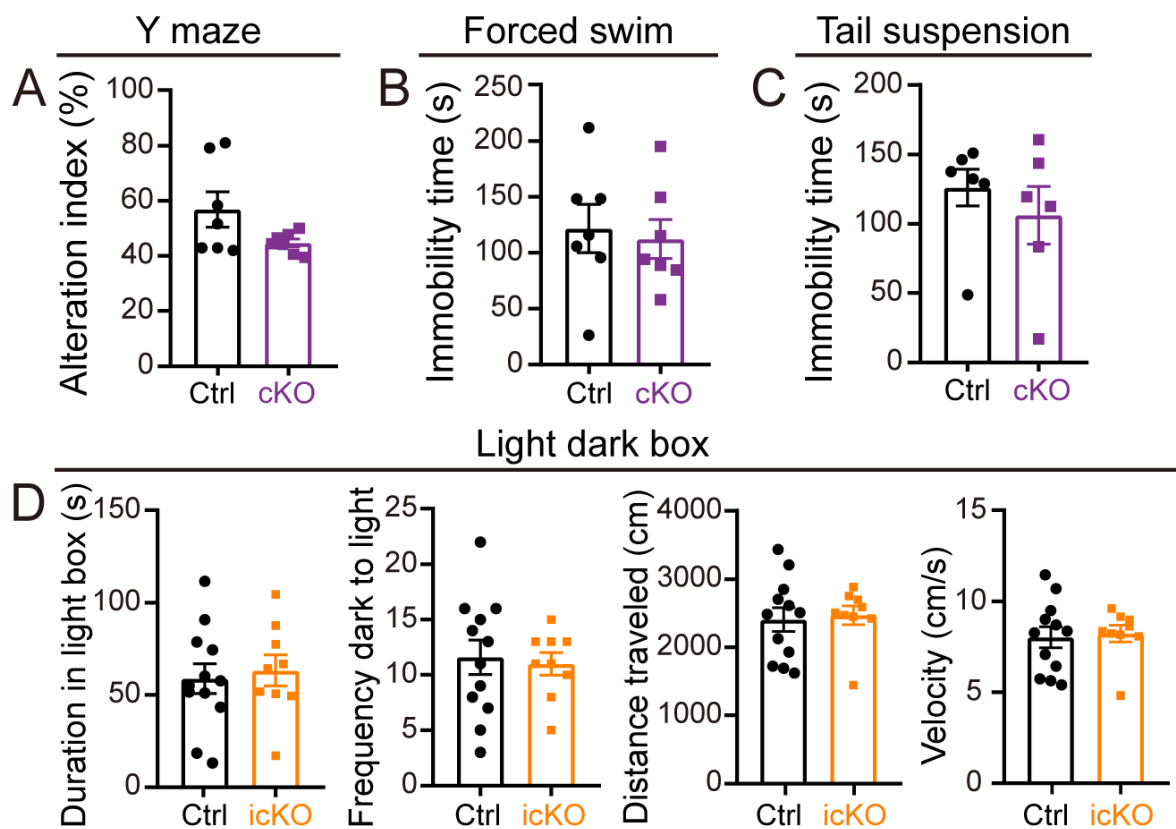


Fig. S5 *Nr4a2^{Emx1}* cKO mice show no difference in working memory and depression-like behaviors, while *Nr4a2^{Gnb4}* icKO mice show no obvious difference in the light-dark box test related to controls. **A–C** The performance of *Nr4a2^{Emx1}* cKO mice in the Y maze (**A**), forced swim (**B**), and tail suspension tests (**C**) shows no obvious difference compared with controls. $n = 7$ (**A**, **B**) and $n = 6$ (**C**). **D** The performance of *Nr4a2^{Gnb4}* icKO mice in the light-dark box test is comparable to that of controls. $n = 9$.