Supplementary Materials

Supplementary Methods

Behavioral Tests

Behavioral tests were performed as described previously [1,2]. Experimenters were blind to mouse genotypes and in most cases software-based analysis was used to score performance. Mice were adapted to the experimental room for at least 30 min prior to experimental tasks.

Rotarod Test

Mice were placed on a rotating rod and the latency to fall was recorded automatically (Panlab Harvard, Newbury Park, USA). The speed of rotation was accelerated from 4 to 40 rounds/min over a 5-min period. Mice were subjected to three trials and were placed on the rod for a maximum of 15 min per trial, with 30-min inter-trial intervals after 2 min of habituation each time.

Open Field Test

An ENV-510 test environment equipped with infrared laser beams and an activity monitor (Med Associates, Georgia, USA) was used to evaluate motor activity in the open field test. Mice were placed in a Plexiglas box ($27 \times 27 \times 20.3$ cm^3) and allowed 30 min of spontaneous activity. Locomotor activity was recorded by each beam break as one unit of exploratory activity using the monitoring software (Med Associates, Georgia, USA).

Fear Conditioning

FreezeFrame and FreezeView software were used to record and analyze freezing behavior. On the first day, mice were given 5 foot-shocks (0.8 mA, 2 s) at 2-min intervals, during which time the mice were able to move freely. The percentage of freezing time was measured during each inter-shock interval.

On the second day, mice were placed back in the box for 11 min without receiving any foot shocks, and freezing time was measured to test fear memory.

Morris Water Maze Test

For the first 6 days, mice were trained to swim for 60 s to find the hidden platform in each trial. If successful, the mouse was allowed to remain on the platform for 15 s. If unsuccessful within the 60 s, the mouse was guided to the platform and allowed to remain on it for 15 s. The time to find the platform was recorded to compare spatial memory and learning ability between groups. On day 7, each trial (60 s) was conducted with no platform present. The time spent and entries into the "correct" quadrant were recorded as an index of reference memory.

Supplementary Figures

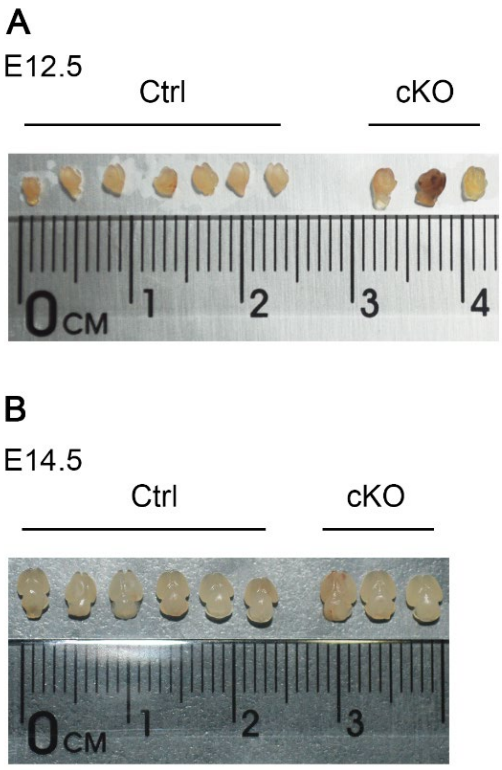

Fig. S1 Embryonic brains of cKO mice and control siblings (Ctrl), related to Fig. 2. **A**, **B** Embryonic brains at E12.5 (**A**) and E14.5 (**B**).

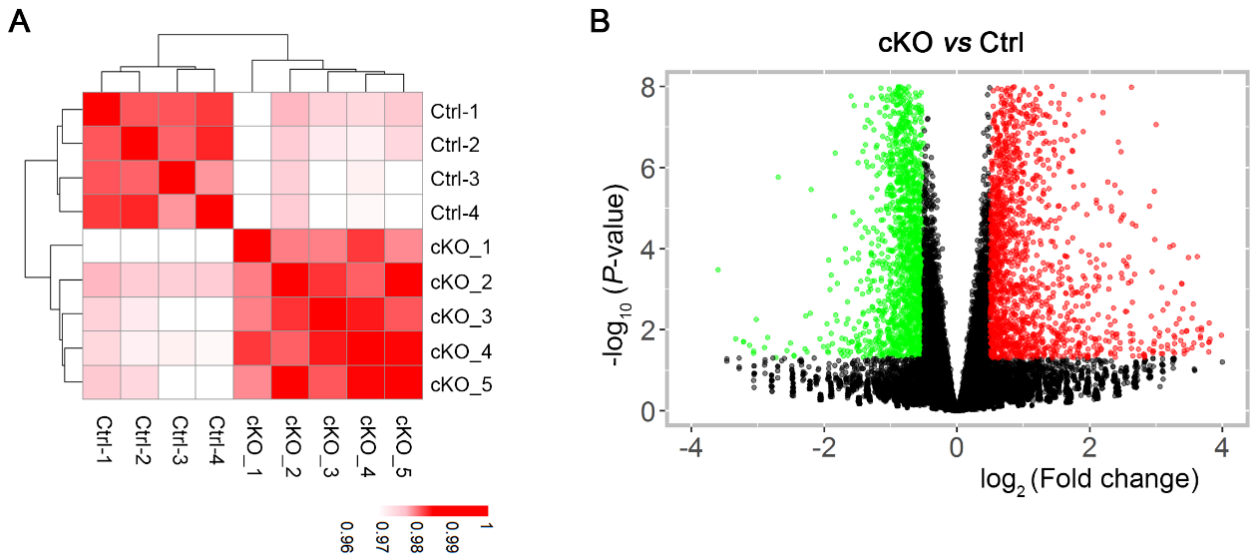

Fig. S2 Expression profiles of DEGs in ventral cells from control and cKO mice, related to Fig. 3. **A** PCA plot clearly separates the cKO samples from control samples. **B** Volcano plot distinguishes 1,366 up-regulated and 1,123 down-regulated DEGs in *Nkx2.1*-expressing progenitors from cKO embryos. DEGs with a fold-change ≥1.5 and P <0.05 are marked in red (up-regulated) and green (down-regulated).

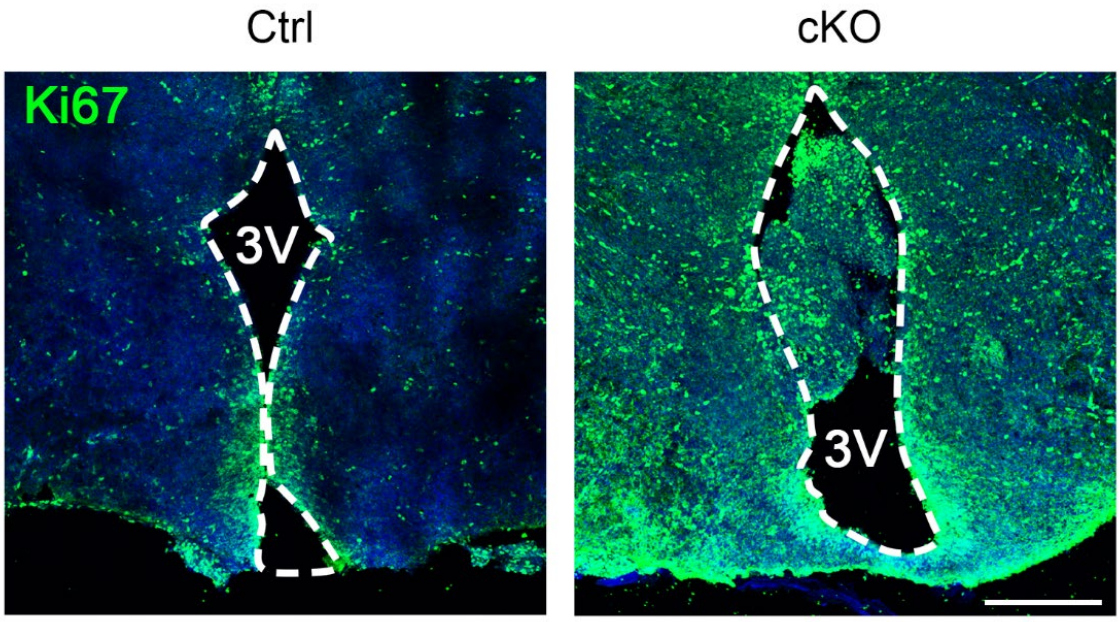

Fig. S3 Obstructed third ventricle in mutant embryo with over-proliferating hypothalamic progenitors, related to Fig. 4. Immunostaining of Ki67 in ventral third ventricle (3V) of coronal forebrain sections from E14.5 embryos. White dashed lines framed the 3V area. Scale bar, 250 μm.

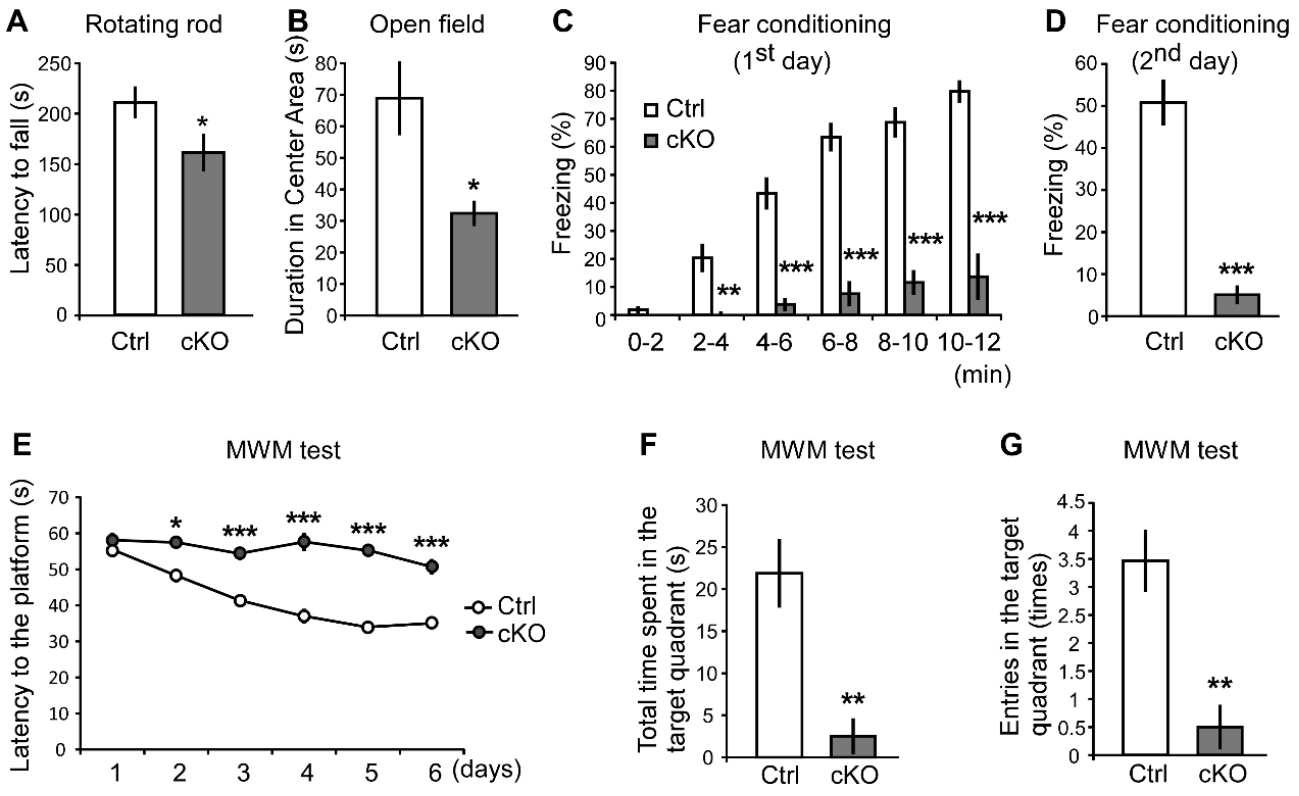

Fig. S4 Congenital hydrocephalic mice show impaired motor function, anxiety-like behavior, and learning-memory deficits. **A** Adult cKO mice show shorter latency to fall in the rotating rod test (n = 6–12 mice; *P <0.05, unpaired t-test). **B** Time spent in the center area is much shorter for cKO mice than for control mice in the open field test, revealing anxiety-like behavior in cKO mice (n = 6–12 mice; *P <0.05, unpaired t-test). **C**, **D** On day 1 of the fear conditioning test, mice are shocked six times by transient and discontinuous electrical stimuli during 12 min. Control mice show an increasing freezing time at each interval, while cKO mice display a significant reduction in freezing time (**C**). On day 2, although no stimulus is applied, control mice take half the time to freeze, but cKO mice take much less time to freeze (**D**) (n = 6–15 mice; **P <0.01, ***P <0.001, unpaired t-test). **E**–**G** In the Morris water maze (MWM) test, mice are trained to search for the hidden platform for 6 days. Latency to find the platform in cKO mice is significantly longer than that in control mice during the 6 days of training (**E**). On day 7, the cKO mice show a remarkable decrease in total time spent (**F**) and number

of entries (**G**) in the target quadrant after platform removal (n = 6–15 mice; *P <0.05, **P <0.01, ***P <0.001, unpaired t-test).

References

[1] Jia YF, Song NN, Mao RR, Li JN, Zhang Q, Huang Y, *et al*. Abnormal anxiety- and depression-like behaviors in mice lacking both central serotonergic neurons and pancreatic islet cells. Front Behav Neurosci 2014, 8: 325.

[2] Song NN, Jia YF, Zhang L, Zhang Q, Huang Y, Liu XZ, *et al*. Reducing central serotonin in adulthood promotes hippocampal neurogenesis. Sci Rep 2016, 6: 20338.