Supplementary Methods

Plasmid constructs

ShRNAs targeted mouse Pd1 Pdl1, and Pax3 were subcloned into the pSicoR-GFP vector. The sequences were as follows: Pd1-sh1, GACATGAGGATGGACATTGTT; Pd1-sh2, GCTCGTGGTAACAGAGAGAAT; Pdl1-sh1, TCCGAAATGATACACAATTCGA; Pdl1-sh2, GCCACTTCTGAGCATGAACTA; Pax3-sh1, CCGTGGTATTAAATATGACAT; Pax3-sh2, CCGCATCCTGAGGAGTAAATT. ShRNAs targeted human Pd1 were also subcloned into the pSicoR-GFP vector. Human Pd1-sh1, CAAGGCGCAGATCAAAGAGAG; Human Pd1-sh2, GCCTAGAGAAGTTTCAGGGAA; Full-length Pd1 and Pax3 were amplified and subcloned into the pCDH-3×Flag and pCDH-3×HA respectively. The polymerase chain reaction (PCR) primer for Pd1 cDNA: forward TGGGTCCGGCAGGTACCCTGGTCATT; reverse TCAAAGAGGCCAAGAACAATGTCC; Pax3 cDNA: forward ACCACGCTGGCCGGCGCTGT; reverse CTAGAACGTCCAAGGCTTACTTTGT.

In-utero electroporation

In-utero electroporation has been widely described in our previous study. Briefly, pregnant mice purchased were anesthetized by tribromoethanol with a dose of 2.5mg per gram of body weight. Then plasmid DNA mixed with 0.02% Fast green (Sigma) and Venus-GFP (1μg/μl) were microinjected into the lateral ventricle of embryos and were electroporated into cells with a paddle electrode. Generally, 50-ms pulses at 35V with 950-ms intervals were set in the electroporator (Manual BTX, ECM830). The

electroporated embryo was put back into the abdomen of pregnant mice and brains were collected at the indicated days for further analysis.

Immunostaining

The immunostaining experiments were performed with the following antibodies: rabbit anti-Pd1 (1:1000; Proteintech; 18106-1-AP), rabbit anti-Pdl1 (1:1000; Proteintech 17952-1-AP), rat anti-BrdU (1:1000; Abcam; ab6326), rabbit anti-Ki67 (1:1000; Abcam; ab15580), rabbit anti-phospho-Histone H3 (pH3) (1:1000; Cell Signaling Technology; 3377S), rabbit anti-Pax3 (1:500, Proteintech, 21386-1-AP), mouse anti-Nestin (1: 200; Millipore; MAB353), rabbit anti-Tuj1 (1:1000; Sigma; T2200), mouse anti-Tuj1 (1:2000; Millipore; MAB1637), mouse anti-Flag (1:3000; Sigma; F1804), rabbit anti-IgG (1:1000; Bioss; bs-0295p), mouse anti-Satb2 (1:500; Abcam; ab51502), rabbit anti-Tbr2 (1:1000; Abcam; ab23345), rabbit anti-Tbr1 (1:1000; Abcam;ab31940), mouse anti-MAP2 (1:400; Millipore; MAB3418).

Confocal Imaging

All images were acquired by a Zeiss LSM780 or 880 scanning confocal microscope system. Images were further analyzed by Zen 2012 and Adobe Photoshop CS6. Generally, 2-3 brain slices per mouse were analyzed.

Western Blotting

Proteins were extracted from cells or tissue by using RIPA buffer with protease inhibitors. Then the protein samples were separated by SDS-PAGE and subsequently transferred onto NC membranes. Immunoblots were performed with primary antibodies and then visualized by IRDye 800CW or 680CW secondary antibodies. Uncropped

western blots for all relevant figures are provided in Supplemental Material.

Lentivirus preparation and infection

For lentivirus preparation, plasmids were transfected into HEK293FT cells by GenEscortI (Wisegen). The lentivirus particles in the medium are was harvested 48 hours after transfection and centrifuged at 3,000 rpm for 5 minutes to eliminate cell debris. The viral titer is then detected by infecting HEK293FT. After that, the virus infected primary NPCs with MOI (multiplicity of infection) equals 5 and then incubated in the medium for 12h with 4μg/ml polybrene. The infected NPCs were cultured continually and subjected to further analysis at the indicated time.

BrdU labeling

Generally, BrdU was used to label proliferated cells by intraperitoneal injection with a dose of 100mg/Kg. For cell proliferation analysis, BrdU injection was performed 2h before pregnant mice were sacrificed E16. For cell cycle exit analysis. BrdU administration was performed 24h before pregnant mice were sacrificed at E15.

RNA-seq analysis

Total RNA was extracted from E13 telencephalic tissue isolated from CKO mice and WT littermates and then subjected to cDNA library construction. RNA-seq was performed by the CapitalBio Technology Company. The sequencing data are accessible in NCBI's GEO (number: GSE207712).

qRT-PCR analysis

As previously described, total RNA (2μg) extracted from mouse brain tissue or NPCs was used for reverse transcription (RT) with Reverse Transcription Kit (Tiangen,

KR106). Then the reversed cDNA was subjected to quantitative PCR using SYBR qPCR master mix (Tiangen, FP205). The primers used for RT-PCR were as follows: Pd1:forward CTGAAAAACAGGCCGCCTTC; reverse ATGGCCCCACAGAGGTAGAT; Pax3: forward CAGATCCCAGTAGCACCGTC; reverse ACGTCCAAGGCTTACTTTGT; Actin: forward GGCTGTATTCCCCTCCATCG; reverse CCAGTTGGTAACAATGCCATGT.

Chromatin Immunoprecipitation assay (ChIP)

NPCs were isolated from E13 brains and then infected with control or β-catenin expressing lentivirus. Three days later, the cells were subjected to ChIP assays. Firstly, the cells were cross-linked in 1% formaldehyde solution for 15 minutes at room temperature and then terminated the reaction by incubating with 2.5M glycine for 10 minutes. After three times washing with cold PBS, the cells were collected in buffer 1 (50 mM HEPES–KOH, pH 7.5, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100, 1× protease inhibitors) and centrifuged at 4000rpm for 5minutes at 4◦C. Then, the cell pellets were resuspended in buffer 2 (10 mM Tris–HCl, pH8.0, 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 1× protease inhibitors) and incubated at 4◦C for 10 minutes. After that, the cell pellets were sonicated in lysis buffer 3 (1% SDS, 10 mM EDTA, 50 mM Tris-HCl pH 8.1, 1×Protease Inhibitor). The lysates were incubated with magnetic beads which were preincubated with the antibody of β-catenin overnight at 4◦C. The magnetic beads were washed six times with RIPA (50 mM HEPES-KOH, PH7.6, 10 mM EDTA, PH8.0, 0.7% DOC, 1% NP-40, 1×Protease Inhibitor) and washed once with TEN (10mM Tris, PH8.0,1mM EDTA, 50mM NaCl).

Then the beads were resuspended in TES (50mM Tris, PH8.0, 10mM EDTA, 1% SDS) and incubated at 65℃ to lift the crosslink. DNA extracted from the suspension was subjected to qRT-PCR on the ABI 7500 real-time PCR system (Applied Biosystems).

Behavioral Studies

Pd1^{cKO} mice and their *Pd1*$^{fl/fl}$ littermates about 8-12 weeks were employed in behavioral experiments. The number of mice used in each experiment was more than 10 and a mixture of male and female mice was analyzed. There was no significant difference between sexes observed in all behavioral data.

Open field test

In the open field test, a square box (40cm width× 40 cm length × 40 cm height) was used to detect the locomotion and exploration of mice. Generally, the mice were placed in the center of the arena to explore freely for 5min. All the behavior was recorded by a video tracking system. The traveled distance and time spent in the center or margin areas were analyzed by EthoVision XT 14.

Elevated-plus maze test

The elevated-plus apparatus consists of two oppositely positioned closed arms(40×9.5 cm), two oppositely positioned open arms, and a central area. The maze was elevated above the floor with a height of 40cm. The test procedure was the same as previously described. The mice freely explored the maze for 5 minutes and their behavior was recorded. The preference for being in the open or closed arms was calculated by EthoVision XT 14.

Y-Maze test

The Y-maze test was used to detect spontaneous alternation. After introduction to the center of the maze, the mouse was allowed to explore three arms freely for 5 min. The number and the sequence of arm entrances were recorded to analyze the spontaneous alternation with the software EthoVision XT 14.

Forced swim test

For the forced swim test (FST), mice were placed in a cylindrical container (20cm in diameter, 35cm in height) filled with water (20cm in depth) at 25℃. The behavior of the mice placed in the water was recorded for about 6 minutes using a video tracking system. The immobility duration with the final 4 minutes was used for analysis. Immobility means the mice remained floating or only moved as necessary to keep balance in the water.

Tail suspension test

Mice were suspended by their tails with tape in a three-walled rectangular compartment (55cm height × 15cm width × 11.5cm depth). Each mouse was suspended separately so that it could not escape or hold on to nearby surfaces. The process of 6 minutes suspension was traced by a video and the escape-oriented behaviors were recorded. The immobile time during the 6 min suspension was calculated to assess the despair of mice.

Sucrose preference test

Mouse was first trained to habituate two bottles of water in individual cages for 2 days. After that, two bottles of water were replaced with two bottles of 2% sucrose, and the mouse was trained to consume 2% sucrose in the next 2 days. Then, the mouse was

deprived of water for 24h. Twenty-four hours later, one bottle of 2% sucrose and one bottle of water were supplied to the mouse. The mouse was allowed free access to two differing bottles. The position of two bottles was switched during the test to avoid side preference. Twenty-four hours later, consumption of water and 2% sucrose were measured. Sucrose preference was calculated by the percentage of sucrose consumption in the total consumption of sucrose and water.

Three-chamber social interaction test

The apparatus used for the social interaction test was a rectangular box that was divided into three equally sized chambers (25cm×25cm) by two walls. The wall retained a 5cm opening that the mice can freely move into each chamber. Two empty cylindrical cages were symmetrically placed in the left and right side chambers respectively. Firstly, the test mouse was released in the middle chamber and allowed to habituate all three chambers for 5 minutes. Then, a gender-matched unfamiliar mouse (stranger 1) was placed in the left side cage, in which the mice can have nose contact but prevent fighting. The cage on the right side keeps empty. The test mouse was released in the middle chamber and allowed to explore all three chambers for 10 minutes. The time spent in each chamber and around the cage was recorded to test the social preference for Stranger 1. After that, a gender-matched stranger mouse (stranger 2) was put into the right cylindrical cages. The test mouse was allowed to explore for another 10 minutes. The time spent in each chamber and around the two cages was analyzed using the EthoVision XT 14.

Rotarod test

Mice were placed on a long cylindrical rod that rotates along its long axis. The speed of the rod accelerates to 40rpm per minute. Latency to fall was recorded for each mouse. The test procedure was repeated tripe for one trial to yield the averaged data. Each mouse was tested in two trials with 2-3 hours intervals for a total of 3 days.

Grip strength test

The Grip strength test was performed on the GSM grip strength meter (ugo basile). Generally, the measurements are taken by keeping the torso horizontal and allowing only forepaws to attach to the grid. Then, gently pull the tail of the mouse back to ensure the mouse grips the top portion of the grid and record the maximal grip strength value of the mouse that is displayed on the screen. Repeat this procedure twice more to obtain 3 forelimb grip strength measurements.

Supplementary Figures

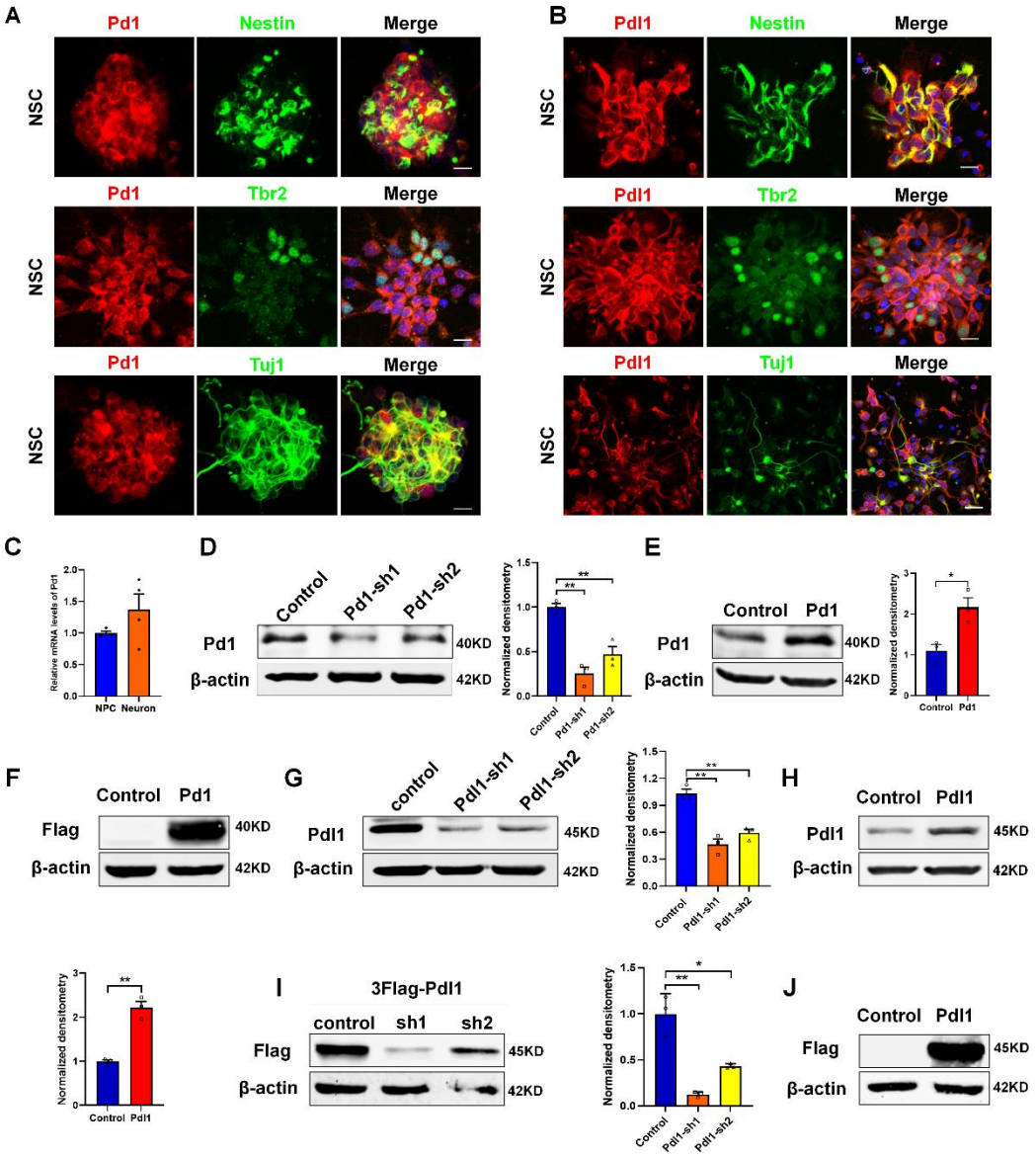

Fig. S1: Pd1 and PdL1 are expressed in NSCs cultured *in vitro* and can be effectively down-regulated by shRNA or up-regulated by overexpressed plasmids

A Pd1 is expressed in neural progenitor cells and neurons cultured *in vitro*. NPCs isolated from the E13 cortex were cultured in the proliferation medium for 1 day and then used for immunostaining. n=3 individual experiments.

B Pdl1 is expressed in NPCs and neurons cultured *in vitro*. n=3 individual experiments.

C The expression of Pd1 in NPCs and neurons is further verified with RT-PCR. n=4 individual experiments.

D The expression of Pd1 can be effectively down-regulated by lentivirus-mediated shRNAs. NPCs isolated from the E13 cortex were cultured *in vitro* and infected by lentivirus expressing Pd1-shRNA or control. The cells were collected and subjected to western blotting 3 days later. The bar graph shows the normalized band intensity of Pd1-sh1 and Pd1-sh2 compared to the control. n=3 individual experiments, p=0.0008 (Control&Pd1-sh1) and 0.005 (Control&Pd1-sh2).

E Western blot analysis reveals that Pd1 is overexpressed in primary NSCs which are infected with control or 3Flag-Pd1 packaging lentivirus. The bar graph shows the normalized band intensity of Pd1 overexpression compared to the control. n=3 individual experiments and p=0.019.

F The expression of Pd1 is obviously increased by the transfection of Pd1 overexpressing plasmid in 293FT cells. n=3 individual experiments.

G Pdl1 is effectively down-regulated by lentivirus-mediated shRNAs in primary NPCs cultured *in vitro*. The bar graph shows the normalized band intensity of Pdl1-shRNA1 and Pdl1-shRNA2 compared to control. n=3 individual experiments, p=0.002 (Control&PdL1-sh1) and 0.003 (Control&PdL1-sh2).

H Pdl1 is overexpressed in NPCs cultured *in vitro*. The bar graph shows the normalized band intensity of Pd1 and control. n=3 individual experiments and

p=0.001.

I The expression of 3Flag-PdL1 can be effectively reduced by co-transfected PdL1 shRNAs in 293FT cells. n=3 individual experiments, p=0.002 (Control&PdL1-sh1) and 0.012 (Control&PdL1-sh2).

J Pdl1is significantly up-regulated in 293FT cells that transfected with Pdl1-overexpressing plasmid.

Error bars represent means ± S.E.M.; two-tailed unpaired t-test, $P < 0.05$ (*), $P < 0.01$(**), and n.s., not significant. The scale bar represents 20μm.

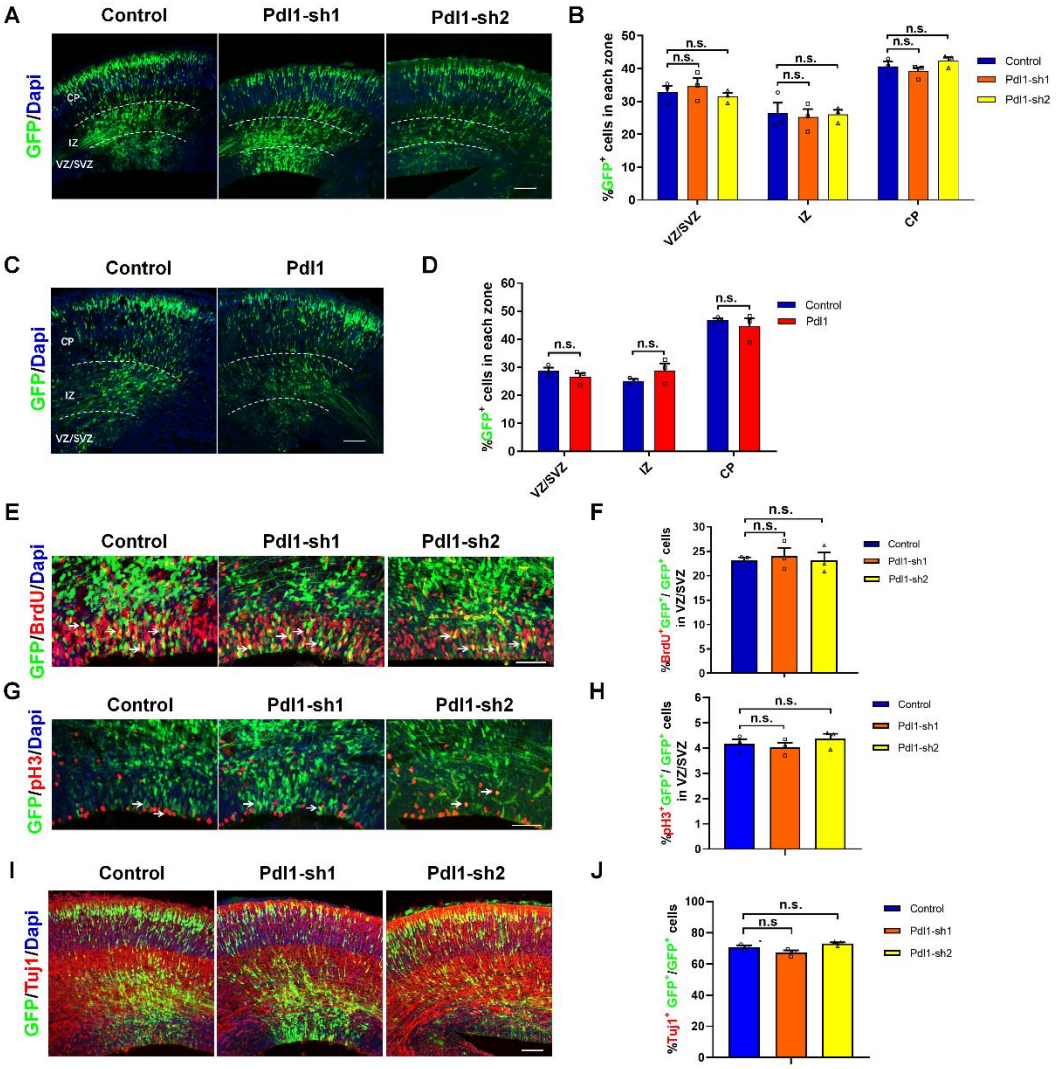

Fig. S2: Pdl1 has no obvious effect on embryonic neurogenesis

A No obvious defect in cell distribution is exhibited when the expression of Pdl1 is suppressed. Control or Pdl1 shRNA plasmid was electroporated into E13 embryonic brains, and the electroporated brains were harvested at E16. n=3 individual experiments.

B Graphs show the percentage of GFP^+ cells in the VZ/SVZ, IZ, and CP. n=3 mice, p=0.6 (Control&PdL1-sh1) and 0.582 (Control&PdL1-sh2) for VZ/SVZ;

p=0.773 (Control&PdL1-sh1) and 0.913 (Control&PdL1-sh2) for IZ; p=0.465 (Control&PdL1-sh1) and 0.395 (Control&PdL1-sh2) for CP.

C No significant difference in cell positioning is investigated between control and Pdl1 overexpression. Control or 3Flag-Pdl1 plasmid was electroporated into E13 embryonic brains, and the electroporated brains were collected at E16. n=3 individual experiments.

D Graphs show the percentage of GFP^+ cells in the VZ/SVZ, IZ, and CP. n=3 mice, p=0.31 for VZ/SVZ; p=0.224 for IZ; p=0.522 for CP.

E BrdU incorporation is no difference between control and Pdl1 shRNA electroporated embryonic brains. The plasmids were electroporated into embryonic brains at E13 and BrdU was administrated 2 hours before the mice were sacrificed at E16. Arrowheads indicate $BrdU^+GFP^+$ cells.

F Graph shows the percentage of GFP^+ cells labeled by BrdU in the VZ/SVZ. n=3 mice, p=0.639 (Control&PdL1-sh1) and P=0.978 (Control&PdL1-sh2).

G Compared with control, no significant difference in the mitotic index of Pdl1-silenced cells is found *in utero*. The percentage of PH3 and GFP double-positive cells in the ventricular zone is shown. Arrows indicate PH3 and GFP double-positive cells.

H Graph shows the percentage of $pH3^+GFP^+$ cells in GFP^+ cells. n=3 mice, p=0.649 (Control&PdL1-sh1) and P=0.514 (Control&PdL1-sh2).

I Down-regulation of Pdl1 has no significant effect on neuronal differentiation. E16 brain sections were stained for Tuj1 after the electroporation of control or

Pdl1-shRNA plasmids into the brain at E13.

J The percentage of $Tuj1^{+}GFP^{+}$ cells relative to the total number of GFP-positive cells is displayed as a bar graph. n=3 mice, p=0.136 (Control&PdL1-sh1) and P=0.186 (Control&PdL1-sh2).

Error bars represent means ± S.E.M.; two-tailed unpaired t-test, $P < 0.05$ (*), $P < 0.01$(**), and n.s., not significant. The scale bar represents 100μm.

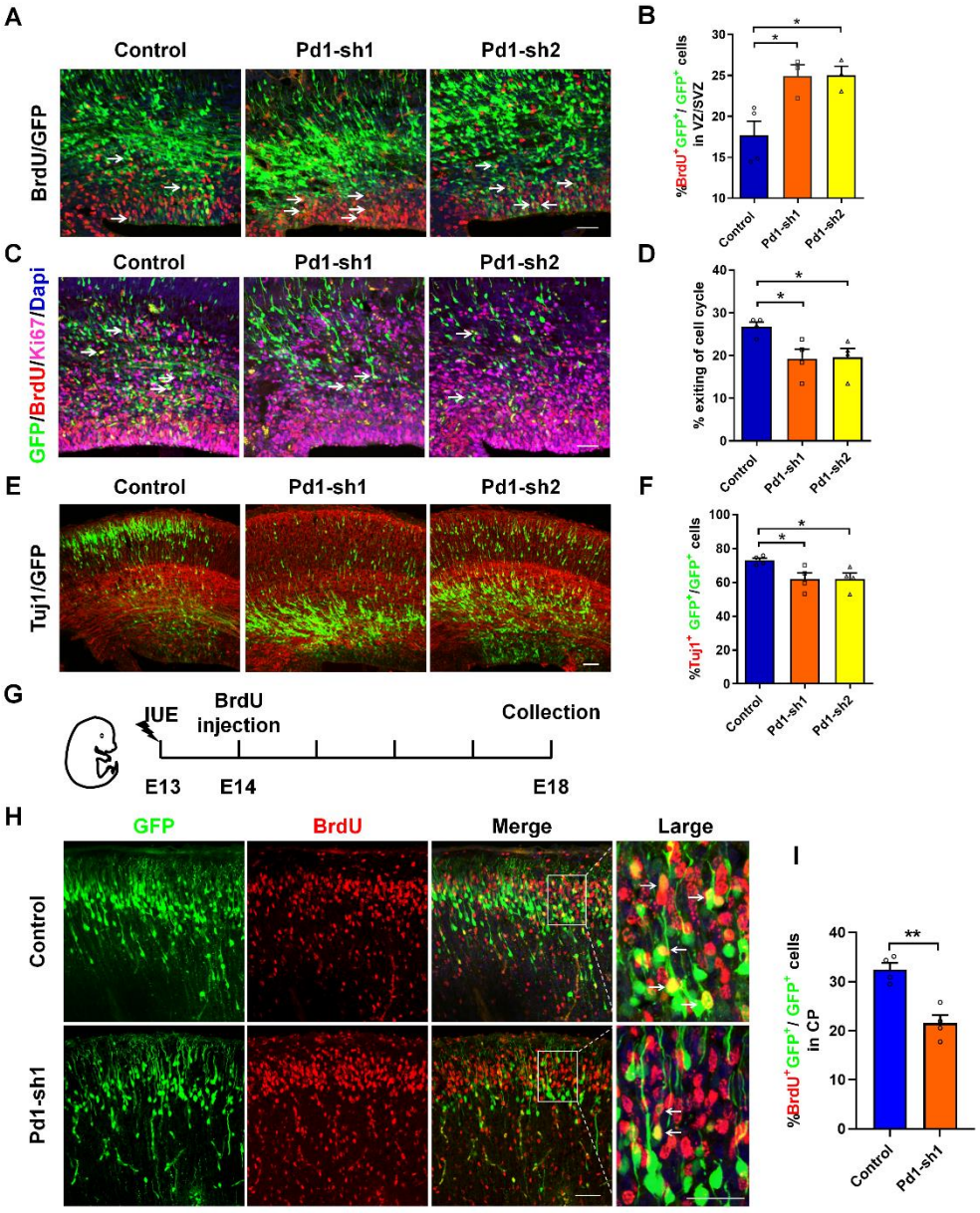

Fig. S3: Pd1 regulates the proliferation and differentiation of NSCs

A BrdU labeling is increased upon Pd1 knockdown. Pd1-shRNA or control plasmids were electroporated into the E13 brains and 100 mg/kg BrdU was injected i.p. into pregnant mice 2 h before the collection of brains at E16. The arrows indicate $BrdU^+GFP^+$ cells.

B Graph displays the percentage of $BrdU^+GFP^+$ cells in electroporated cells in

VZ/SVZ. n=3 mice, p=0.028 (Control&Pd1-sh1) and P=0.023 (Control&Pd1-sh2).

C Silencing Pd1 in progenitor cells results in reduced cell cycle exit. Control or Pd1 shRNAs plasmids were electroporated into embryonic brains at E13 and BrdU was administrated 24 hours before the mice were sacrificed at E16. The cells that exited the cell cycle are indicated $GFP^+BrdU^+Ki67^-$. Arrowheads indicate $GFP^+BrdU^+Ki67^-$ cells.

D Graph shows the percentage of $GFP^+BrdU^+Ki67^-$ cells in GFP^+BrdU^+ cells. n=4 mice, p=0.023 (Control&Pd1-sh1) and P=0.023 (Control&Pd1-sh2).

E The output of neurons is decreased when the expression of Pd1 is downregulated.

F The percentage of $Tuj1^+GFP^+$ cells in GFP^+ cells. n=4 mice, p=0.031 (Control&Pd1-sh1) and P=0.024 (Control&Pd1-sh2).

G Timeline of the BrdU birth-dating experiment. The mice were electroporated at E13, then injected with BrdU at E14, and finally harvested at E18.

H Images show the brain sections labeled by BrdU. White arrows show the $BrdU^+GFP^+$ neurons.

I The bar graph shows the percentage of $BrdU^+GFP^+$ cells in GFP^+ cells in CP. n=4 mice and p=0.002.

Error bars represent means ± S.E.M.; two-tailed unpaired t-test, $P < 0.05$ (*), $P < 0.01$(**), and n.s., not significant. The scale bar represents 100μm.

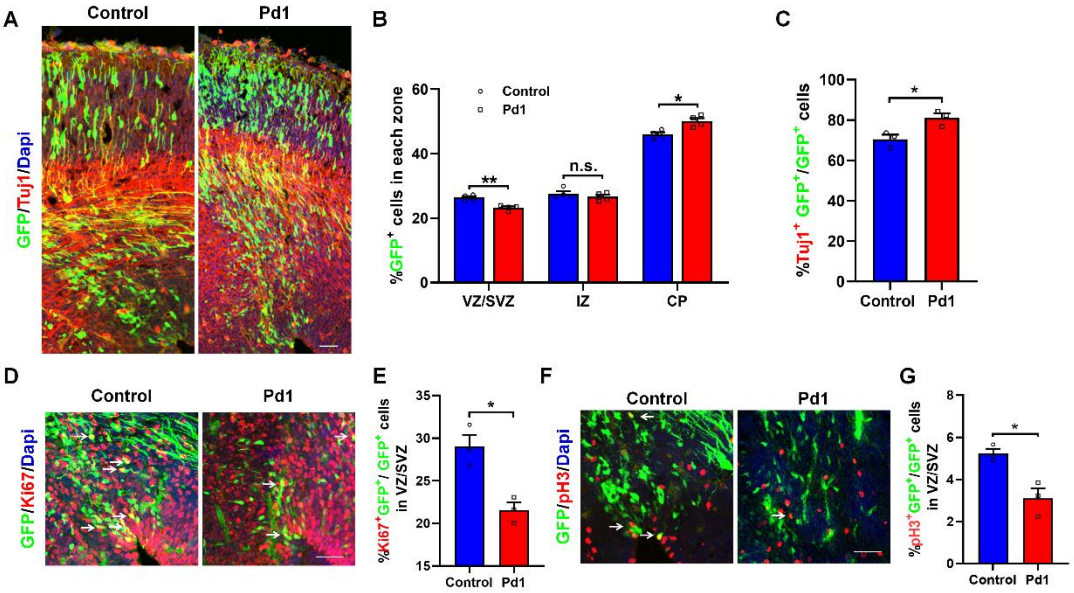

Fig. S4: Pd1 overexpression inhibits the proliferation of NPCs and promotes neuronal differentiation.

A Abnormal GFP^+ cell distribution and neuronal differentiation in Pd1 overexpressed brain. The control and Pd1 overexpressed plasmids were electroporated into E13 embryonic mouse brains, and the mice were sacrificed at E16. The harvested brain sections were subjected to Tuj1 immunostaining.

B Percentage of GFP^+ cells in each zone of the brain sections. n=4 mice, p=0.0017 for VZ/SVZ; p=0.371 for IZ; p=0.011 for CP.

C Percentage of GFP^+Tuj1^+ cell in GFP^+ cells. n=3 mice, p=0.03.

D Ki67 and GFP double-positive cells are decreased in Pd1 overexpressed brain sections. Arrowheads indicate $Ki67^+$ GFP^+ cells.

E Percentage of $Ki67^+GFP^+$ cell in GFP^+ cells in VZ/SVZ. n=3 mice, p=0.01.

F The number of $pH3^+GFP^+$ cells is reduced when Pd1 is overexpressed. Arrowheads indicate $pH3^+GFP^+$ cells.

G Percentage of $pH3^{+}GFP^{+}$ cells in GFP^{+} cells in VZ/SVZ. n=3 mice, p=0.015.

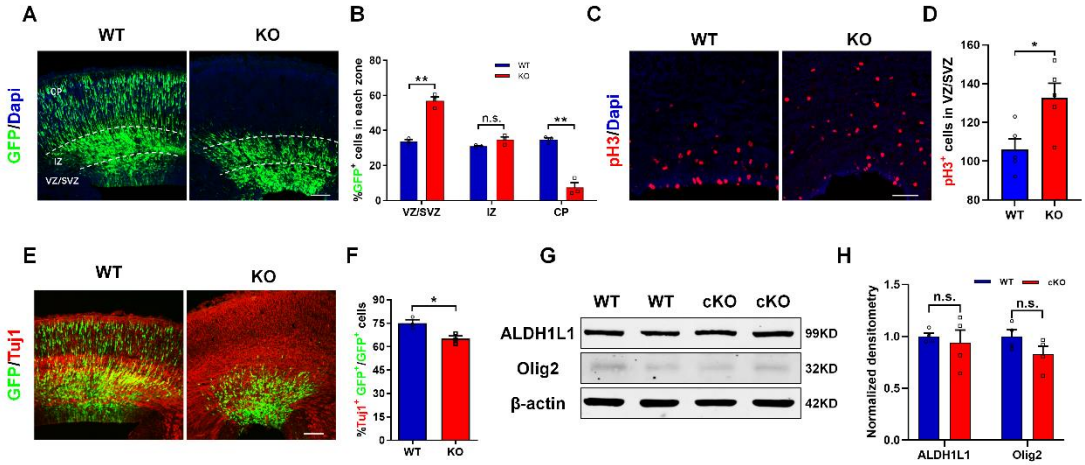

Fig. S5: *Pd1* loss results in abnormal neurogenesis

A The distribution of GFP^{+} cells is abnormal in Pd1 knockout mice.

B Percentage of GFP^{+} cells in VZ/SVZ, IZ, and CP. n=3 mice, p=0.0007 for VZ/SVZ; p=0.076 for IZ; p=0.0007 for CP.

C Images of $pH3^{+}$ cells in Pd1 WT or KO mice.

D The number of $pH3^{+}$ cells in VZ/SVZ of Pd1 WT or KO mice. n=3 mice and p=0.021.

E The number of $Tuj1^{+}$ neurons in GFP^{+} cells is reduced when Pd1 is deleted.

F Percentage of $Tuj1^{+}GFP^{+}$ cells in all GFP^{+} cells. n=5 mice and p=0.025.

G ALDH1L1 and Olig2 protein levels in WT and cKO mice brains.

H Statistic of the normalized band intensity. n=4 experimental replicates. p=0.636 for ALDH1L1, and p=0.131 for Olig2.

Error bars represent means ± S.E.M.; two-tailed unpaired t-test, P < 0.05 (*), P < 0.01(**), and n.s., not significant. The scale bar represents 100μm.

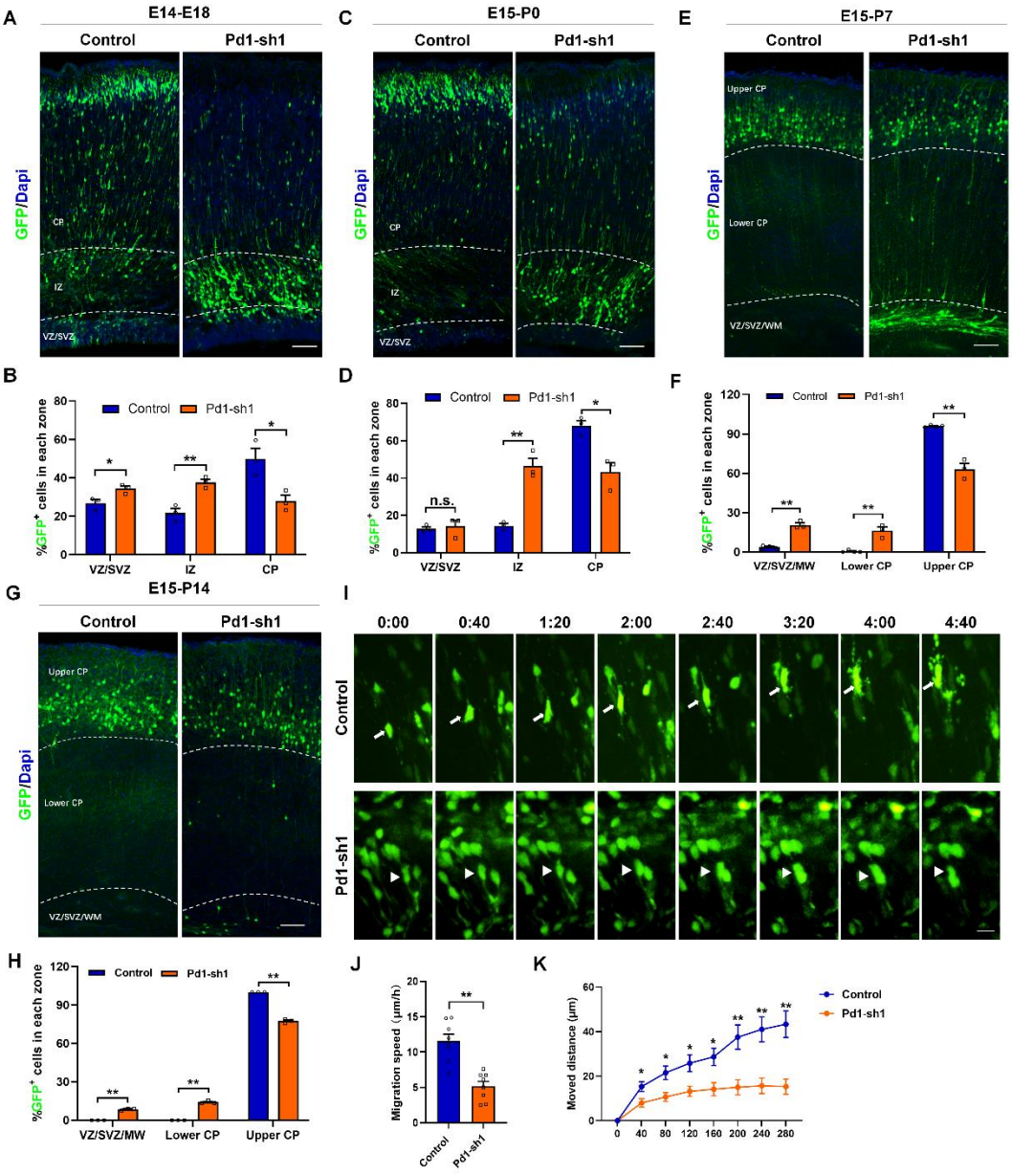

Fig. S6: Downregulation of Pd1 impairs the migration of differentiated neurons *in vivo*.

A Coronal sections of mice brains were electroporated at E14 with control or Pd1-

shRNA plasmids and harvested at E18. The scale bar represents 100μm.

B Analysis of the distribution of electroporated cells at E18. n=3 mice, p=0.033 for VZ/SVZ; p=0.006 for IZ; p=0.023 for CP.

C Representative images of P0 mice brains electroporated at E15 with control and Pd1-shRNA plasmids respectively. The scale bar represents 100μm.

D Percentage of GFP^+ cells in VZ/SVZ, IZ, and CP. n=3 mice, p=0.724 for VZ/SVZ; p=0.002 for IZ; p=0.012 for CP.

E Representative images of P7 mice brains electroporated at E15 with control and Pd1-shRNA plasmids respectively. The scale bar represents 100μm.

F Analysis of the distribution of GFP^+ cells in electroporated brains at P7. n=3 mice, p=0.0001 for VZ/SVZ/MW; p=0.001 for Lower CP; p=0.0003 for Upper CP.

G Representative graphs of P14 mice brains electroporated at E15 with control and Pd1-shRNA plasmids respectively. The scale bar represents 100μm.

H Analysis of the distribution of GFP^+ cells in electroporated brains at P14. n=3 mice, p=0.00004 for VZ/SVZ/MW; p=0.00003 for Lower CP; p=0.00002 for Upper CP.

I Time-lapse images of living cortical slices. The mice brains were electroporated with control or Pd1-sh1 plasmid respectively at E13. One day later, the electroplated brains were sliced and cultured in proliferated medium *in vitro*. The migratory behaviors of GFP^+ neurons were collected for 6 hours. White arrows and arrowheads show the migrating neurons. The experiment was repeated for at

least three times. The scale bar represents 20μm.

J Statistic of the migration speed of GFP^+ cells. n=8 neurons and p=0.0001.

K Migrated distance of GFP^+ neurons. P=0.03 for 40min; p=0.014 for 80min; p=0.016 for 120min; p=0.014 for 160min; p=0.006 for 200min; p=0.003 for 240min; p=0.002 for 280min.

Error bars represent means ± S.E.M.; two-tailed unpaired t-test, $P < 0.05$ (*), $P < 0.01$(**), and n.s., not significant.

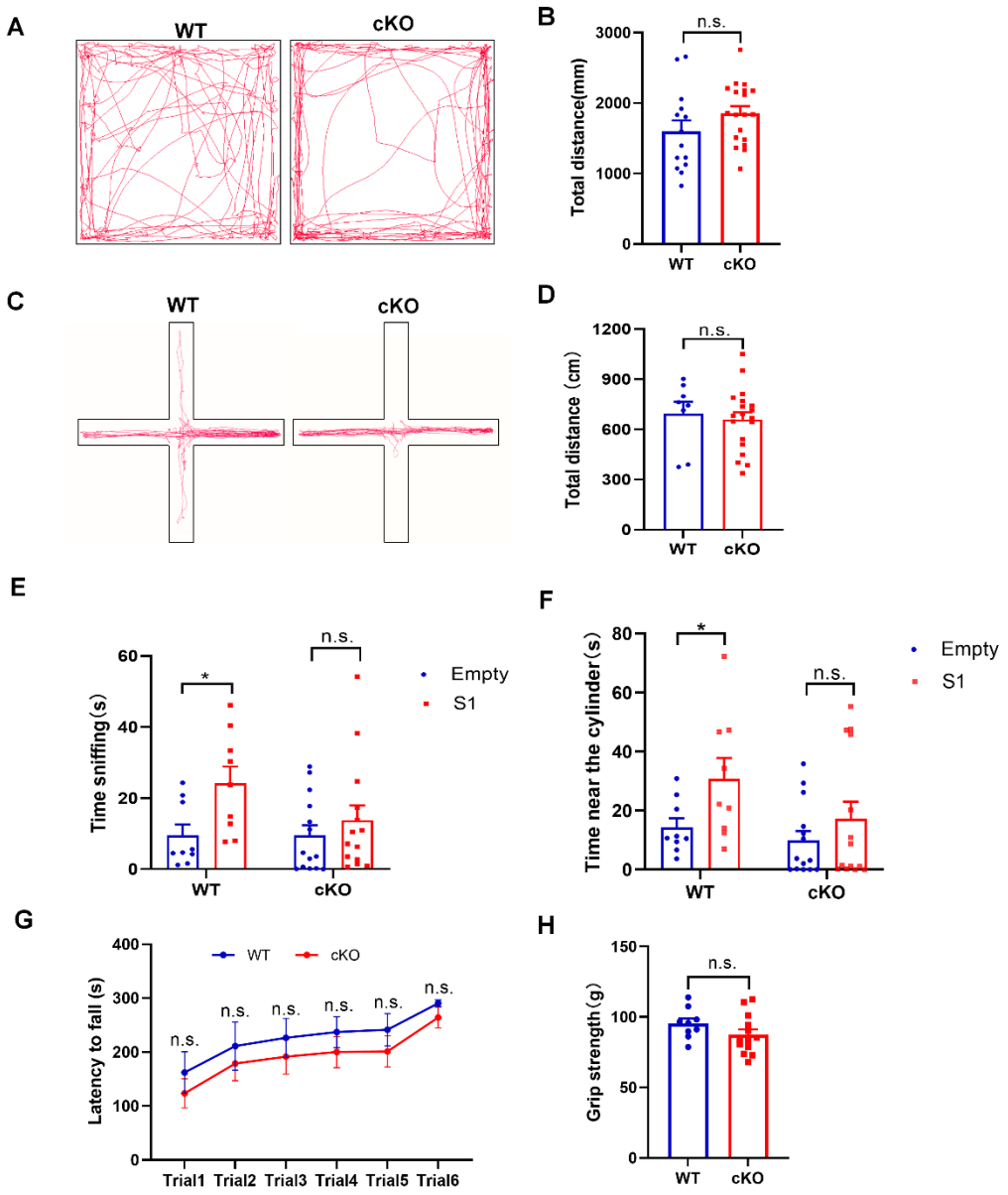

Fig. S7: Brain specific deletion of Pd1 has no obvious effect on motor function.

A Representative tracing pathway of *Pd1*$^{fl/fl}$ or *Pd1*^{cKO} mice in the open field test.

B No significant difference in total moved distance is found between *Pd1*$^{fl/fl}$ or *Pd1*^{cKO} mice in the open field test. n=14 for *Pd1*$^{fl/fl}$ mice, n=19 for *Pd1*^{cKO} mice, and p=0.150.

C Representative tracing pathway of *Pd1*$^{fl/fl}$ or *Pd1*^{cKO} mice in the test of elevated

plus maze.

D The total moved distance is no obvious difference between $Pd1^{fl/fl}$ or $Pd1^{cKO}$ mice in the elevated plus maze test. n=8 for $Pd1^{fl/fl}$ mice, n=19 for $Pd1^{cKO}$ mice, and p=0.675.

E Locomotor activity is no significant difference between $Pd1^{fl/fl}$ mice and $Pd1^{cKO}$ mice in the rotarod test. n=8 for $Pd1^{fl/fl}$ mice and n=14 for $Pd1^{cKO}$ mice. Trial 1, p= 0.292; Trial 2, p=0.306; Trial 3, p=0.331; Trial 4, p=0.251; Trial 5, p=0.219; and Trial 6, p=0.258.

F Grip strength is no significant difference between $Pd1^{fl/fl}$ mice and $Pd1^{cKO}$ mice. n=9 for $Pd1^{fl/fl}$ mice, n=13 for $Pd1^{cKO}$ mice, and p=0.164.

G The sniffing time of $Pd1^{fl/fl}$ mice is obviously increased near the cylinder of stranger 1 mice compared with the empty cylinder, while no significant difference is found in $Pd1^{cKO}$ mice. n=9 for $Pd1^{fl/fl}$ mice, and p=0.019; n=14 for $Pd1^{cKO}$ mice, and p=0.409.

H No obvious increase of $Pd1^{cKO}$ mice spend near the cylinder of stranger 1 mice is found, while $Pd1^{fl/fl}$ mice spend more time. n=9 for $Pd1^{fl/fl}$ mice, and p=0.049; n=14 for $Pd1^{cKO}$ mice, and p=0.284.

Error bars represent means ± S.E.M.; two-tailed unpaired t-test, $P < 0.05$ (*), $P < 0.01$(**), and n.s., not significant.

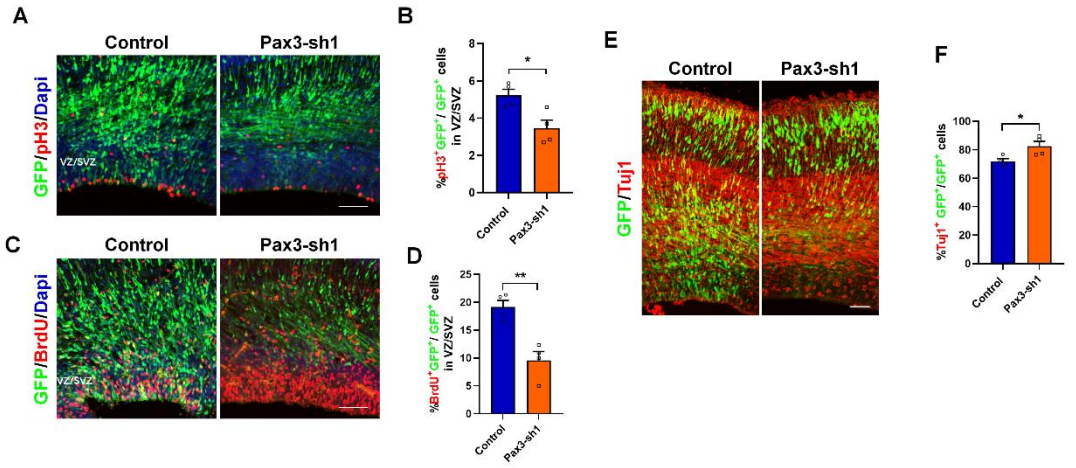

Fig. S8: Pax3 regulates NSCs proliferation and neuronal differentiation during brain development.

A The mitotic index is decreased when pax3 is down-regulated.

B Graph shows the percentage of $pH3^{+}GFP^{+}$cells relative to GFP^{+} cells in VZ/SVZ. n=4 mice and p=0.018.

C The proliferation cells labeled by BrdU are reduced in Pax3-shRNA electroporated cells. BrdU was labeled 2h before the brains were harvested at E16.

D Graph shows the percentage of $BrdU^{+}GFP^{+}$ cells relative to GFP^{+} cells in VZ/SVZ. n=4 mice and p=0.003.

E Images show that the output of neurons is reduced in pax3 suppressed brains.

F Graph shows the percentage of $Tuj1^{+}GFP^{+}$ cells in total GFP^{+} cells. n=4 mice and p=0.03.

Error bars represent means ± S.E.M.; two-tailed unpaired t-test, $P < 0.05$ (*), $P < 0.01$(**), and n.s., not significant. The scale bar represents 100μm.

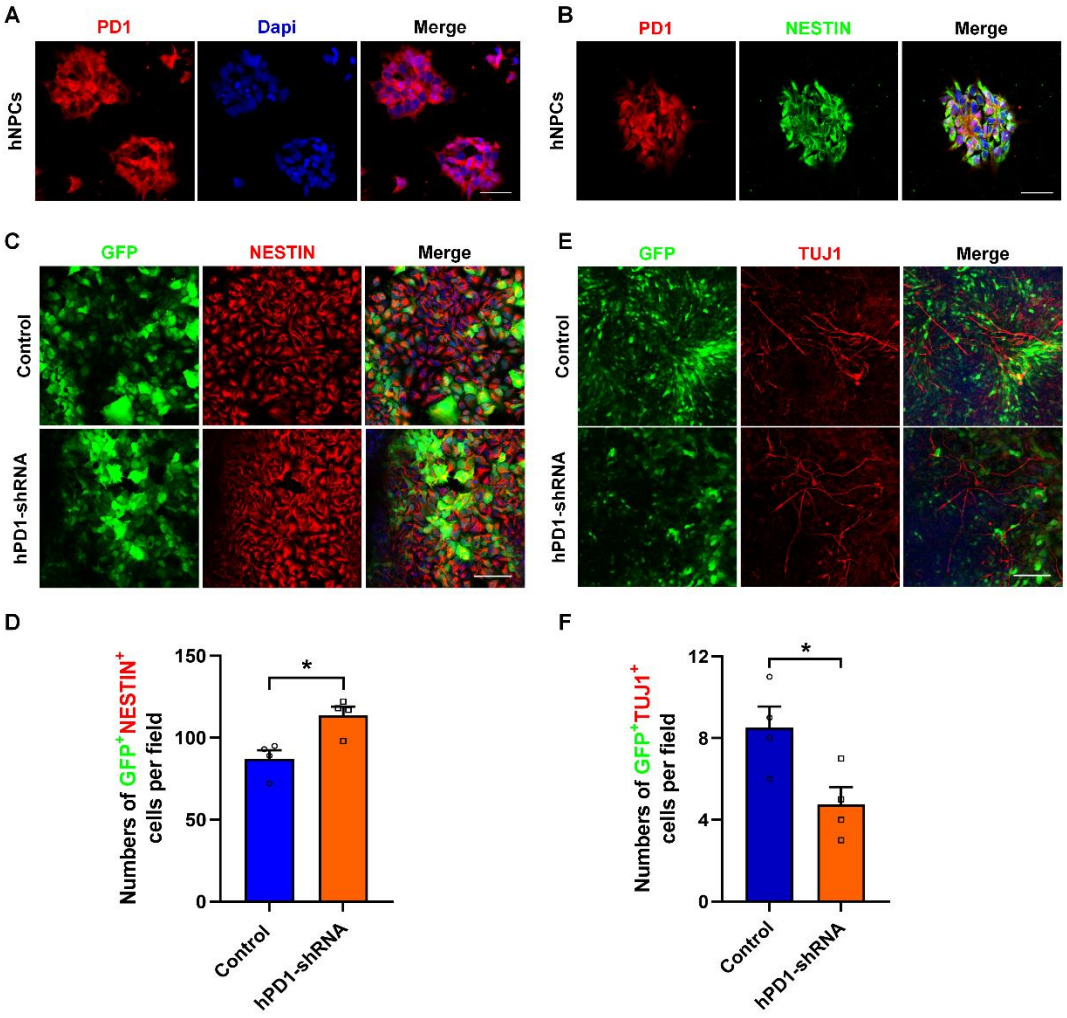

Fig. S9: PD1 controls the number of human NPCs and differentiated neurons.

A PD1 is expressed in hNPCs. Human NPCs differentiated from ES cells were used for immunostaining. n=3 individual experiments. The scale bar represents 20μm.

B PD1 is co-labeled with the hNPCs marker, NESRIN. n=3 individual experiments. The scale bar represents 20μm.

C The number of hNPCs is increased when the expression of PD1 is downregulated. The scale bar represents 100μm.

D Cells co-labeled by GFP and NESTIN per field are quantified. n=3 individual experiments and p=0.012.

E The number of neurons that differentiated from hNPCs is decreased when PD1 is downregulated. The scale bar represents 100μm.

F Statistic of neurons labeled by GFP and TUJ1 in each field. n=3 individual experiments and p=0.032.

Error bars represent means ± S.E.M.; two-tailed unpaired t-test, P < 0.05 (*), P < 0.01(**), and n.s., not significant.

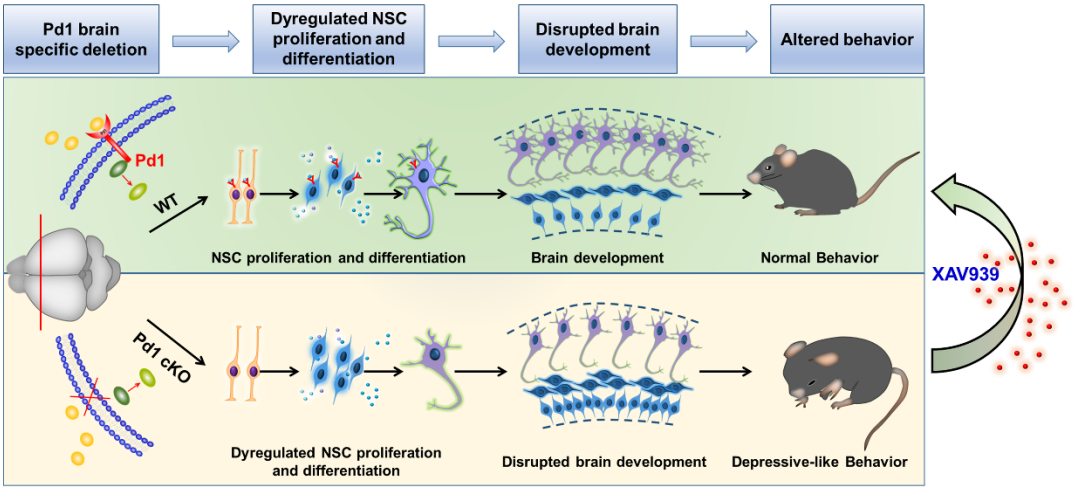

Fig. S10: Model for Pd1 function in embryonic neurogenesis and behavioral exhibition.