Supplementary Figure Legends

Figure S1 H2A.z is expressed in progenitor cells both in vivo and in vitro, which could be effectively suppressed by H2A.z-shRNAs

(A) The temporal expression of H2A.z and H2A.v mRNAs by RT-PCR in the developing cortex.

(B) H2A.z is co-labeled with Sox2 or Pax6 in neural progenitor cells in vivo. E15 brain sections were immunostained with anti-H2A.z and anti-Sox2 or anti-Pax6 antibodies, respectively.

(C) H2A.z is expressed in neural progenitor cells and neurons cultured in vitro. The arrows indicate weakly H2A.z immunoreactivity but Tuj1-positive cells.

(D) Immunostaining images show that endogenous H2A.z is obviously reduced in H2A.z-shRNA-electroporated cells. Hatched lines are cell boundaries.

(E) Statistics indicate that the normalized intensity of H2A.z is attenuated. Each scatterplot point represents the intensity of the signal for H2A.z in an individual GFP+ cell. Quantification was conducted using the ImageJ software. n = 15-25 cells for all samples.

(F) Western blot analysis shows that exogenous Flag-H2A.z is efficiently reduced in H2A.z-shRNA-transfected N2A cells.

Figure S2 H2A.z regulates the proliferation of neural progenitor cells

(A) Mouse embryos were electroporated in utero with control, H2A.z-sh1, H2A.z-sh2 at E13 and the harvested brain sections were immunostained with mitotic marker pH3 at E16.

(B) Graph shows the percentage of pH3+GFP+ cells in VZ/SVZ. n = 6-8 for all samples.

(C) BrdU was injected intraperitoneally for 2 hours of pulse labeling and the harvested brain sections were immunostained with anti-BrdU.

(D) Percentage of GFP+BrdU+ cells among GFP+ cells. n = 7 for all samples.

(E) Immunostaining shows that the expression of H2A.z is depleted in E13 H2A.z cKO brains.

(F) The apoptosis levels between H2A.z fl/fl and H2A.z cKO brains show no significant difference. E16 brain sections of H2A.z fl/fl and H2A.z cKO mice were subjected to TUNEL assay.

(G) Graph shows the numbers of apoptotic cells per field. n = 6 for all samples.
(H) Ablation of H2A.z does not affect the proliferation of RGs. H2A.z^{fl/fl} mice and H2A.z^{cKO} mice were administered BrdU (100 mg/kg) for 2 hours of pulse labeling and were euthanized at E16. The harvested brain sections were immunostained with anti-Pax6 and anti-BrdU.

(I) Statistic of Pax6-positive cells per 100 μm² surface of VZ/SVZ. n = 6 for all samples.

(J) Percentage of Pax6⁺BrdU⁺ cells among all Pax6⁺ cells. n = 8 for all samples.

Representative images from at least three independent experiments. Error bars represent means ± s.e.m.; H2A.z^{fl/fl}, WT; H2A.z^{cKO}, cKO. Two-tailed unpaired t-test. p < 0.05 (*), p < 0.01(**), n.s., not significant. Scale bar represents 50 μm (A and C); 20 μm (E, F and H).

Figure S3 H2A.z can rescue the proliferation defect caused by the ablation of H2A.z

(A) Western blot shows that the expression of Tbr2 is increased, while the expression of Pax6 is not significantly altered when H2A.z is deleted in NPCs. E12 primary neural progenitor cells (NPCs) were isolated from H2A.z^{cKO} and H2A.z^{fl/fl} mice, and cultured in proliferated medium (suspension culture) for 3 days, and then collected the neurospheres for further analysis (A-C).

(B) Graph shows the normalized density of H2A.z, Pax6, and Tbr2. n = 7 for control samples; n = 5 for H2A.z^{cKO} samples.

(C) Relative Tbr2 mRNA levels in neurospheres derived from H2A.z^{fl/fl} and H2A.z^{cKO} mice. n = 7 for all samples.

(D) Representative images of E16 cortices electroporated with GFP/control, H2A.z into WT mice, or GFP/control, H2A.z into H2A.z^{cKO} mice at E13.

(E) Quantification of the percentage of GFP-expressing cells in different regions of the developing cortex. n = 4 for all samples.

(F) Coronal brain sections were immunostained with anti-BrdU.

(G) Percentage of GFP⁺BrdU⁺ cells among GFP⁺ cells. n = 6 for all samples.

Representative images from at least three independent experiments. Error bars represent means ± s.e.m.: Two-tailed unpaired t-test, p < 0.05(*), p < 0.01(**), p < 0.001(***). n.s., not significant. Scale bar represents 50 μm (D); 20 μm (F).

Figure S4 H2A.z, but not H2A.v, regulates differentiation of neural progenitor cells

(A) Relative H2A.z and H2A.v mRNA levels in H2A.z^{fl/fl} and H2A.z^{cKO} forebrain. n = 3 for all samples.

(B) Expression analysis of H2A.v by RT-PCR in primary progenitors infected with H2A.v-sh1 or control lentivirus. n = 3 for all samples.
(C) Representative images of E16 coronal brain sections were immunostained for Tuj1. Mouse embryos were electroporated in utero with control, H2A.z-sh1, H2A.v-sh1 or H2A.z-sh1 together with H2A.v-sh1 at E13 and embryos were analyzed at E16. n = 4 for all samples.

(D) Graphs of the percentage of GFP-positive cells in the VZ/SVZ, IZ and CP.

(E) Percentage of Tuj1+GFP+ cells in all GFP+ cells.

(F) The surface of VZ/SVZ at E16 cortices labeled for Tuj1, Pax6 and DAPI. n = 4 for all samples.

(G) Quantitative analysis of fluorescent intensity of Tuj1 in VZ/SVZ.

Representative images from at least three independent experiments. Error bars represent means ± s.e.m.; Two-tailed unpaired t-test, p < 0.05(*), p < 0.01(**), p < 0.001(***). n.s., not significant. Scale bar represents 50 μm (C); 20 μm (F).

Figure S5 BrdU birth-dating shows a qualitative decreased production of upper layer neurons following loss of H2A.z cortex

(A) Representative images of cortices showing BrdU and Cux1(green; marker of layers II-III) double labeling at P0. BrdU was injected intraperitoneally to pregnant mice at E13 and incorporated by cycling progenitor cells, neurons that incorporated BrdU at E13 were analyzed at P0(A; D and G).

(B) Statistics of Cux1+ cells in upper layer.

(C) Statistics of BrdU+ and Cux1+ cells in Cux1+ cells.

(D) Neurons that incorporated BrdU at E13 were revealed by immunostaining for BrdUand Ctip2 (green; marker of layer V).

(E) Statistics of Ctip2+ cells in deep layer.

(F) Statistics of BrdU+ and Ctip2+ cells in Ctip2+ cells.

(G) Neurons that incorporated BrdU at E13 were revealed by immunostaining for BrdU and Tbr1 (green; marker of layer VI).

(H) Statistics of Tbr1+ cells in deep layer.

(I) Statistics of BrdU+ and Tbr1+ cells in Tbr1+ cells.

n = 4 for all samples. Error bars represent means ± s.e.m.; Two-tailed unpaired t-test, p < 0.05(*), p < 0.01(**), p < 0.001(***). n.s., not significant. Scale bar represents 20 μm.

Figure S6 The suppression of H2A.z results in abnormal neuronal morphology

(A) H2A.z knockdown results in abnormal neuronal morphology. Control and H2A.z-sh1 plasmid were electroporated into E13 mice to label neural progenitor cells. After 24 hours, the GFP-positive cells were isolated and cultured in differentiation medium for 4 days.

(B) Statistics of total dendrite length of neurons in control and H2A.z-sh1-electroporated cells. n = 8 cells from 5 mice.

(C) Confocal micrographs showing GFP-expressing neurons at the P5 cortex.
Neuronal morphology was analyzed at P5 after in utero electroporation at E13 (C and E).

(D) Graphs of the percentage of GFP-positive cells in different zones of the upper layer.

(E) Representative images showing GFP-expressing neurons in the upper layer at P5.

(F) Quantitative analysis of the number of dendrite branches. n = 8 cells from 3 mice.

Error bars represent means ± s.e.m.; Two-tailed unpaired t-test, p < 0.05(*), n.s., not significant. Scale bar represents 10 μm (A and E); 100 μm (C).

Figure S7 Loss of H2A.z results in abnormal cell distribution in early developmental window

(A) GFP-expressing plasmid was electroporated into E13 cerebral cortices of WT or H2A.z<sup>cko</sup> mice, and the brains were collected at P0 for analysis.

(B) Graphs of the percentage of GFP-positive cells in the VZ/SVZ, IZ, loCP and upCP. n = 4 for all samples.

(C) Images of P0 mouse cortices of WT or H2A.z<sup>cko</sup> mice electroporated at E15 with GFP.

(D) Graphs of the percentage of GFP-positive cells in the VZ/SVZ, IZ, loCP and upCP. n = 8 for all samples.

(E) Representative images showing GFP-expressing neurons in the P17 cortex. Mouse embryos were electroporated in utero with control, H2A.z-sh1, H2A.v-sh1 or H2A.z-sh1 together with H2A.v-sh1 at E13 and analyzed at postnatal day 17.

(F) Quantitative analysis of the number of dendrite branches per GFP-positive neuron. n = 5 for all samples.

upCP, upper cortical plate; loCP, lower cortical plate. Error bars represent means ± s.e.m.; Two-tailed unpaired t-test, p < 0.05(*), n.s., not significant. Scale bar represents 50 μm (A and C); 15 μm (E).

Figure S8 H2A.z<sup>cko</sup> mice weigh less than wild-type mice

(A) Survival curves of wild-type and H2A.z<sup>cko</sup> littermates, n=20-30 for all samples.

(B) H2A.z<sup>cko</sup> mice weigh less than their wild-type littermates across P0-P7. n=7 for all samples.

(C) Images of the body size of H2A.z<sup>fl/fl</sup> and H2A.z<sup>cko</sup> littermates at P0 and p10. Scale bar represents 10 mm.

(D) Representative images of the body size of H2A.z<sup>cko</sup> and H2A.z<sup>fl/fl</sup> littermates at P5 weeks. Scale bar represents 2 cm.

(E) Morphologies of the brains dissected from the P0 H2A.z<sup>cko</sup> and H2A.z<sup>fl/fl</sup> littermates are shown. Scale bar represents 1 mm.

(F) Measurement of brain weights at P0. n = 5 for all samples.
Representative whole-mount images of adult brains from $H2A.z^{cKO}$ and $H2A.z^{0/0}$ littermates. Scale bar represents 2 mm.

Measurement of adult brain weights. $n = 4$ for all samples.

Images are representative of $H2A.z^{0/0}$ and $H2A.z^{cKO}$, WT; $H2A.z^{cKO}$, cKO. Error bars represent means ± s.e.m.; Two-tailed unpaired t-test, $p < 0.05$ (*), $p < 0.01$ (**). n.s., not significant.

Figure S9 Models of three-chamber social interaction test and Y-maze

The model of the social interaction test for ‘Stranger 1-Empty’.

Representative motion trail from ‘Stranger 1-Empty’.

The model of the social novelty test for ‘Stranger 1 - Stranger 2’ trials.

Representative motion trail from ‘Stranger 1- Stranger 2’.

Representative motion trail from the Y-maze.

Figure S10 Loss of H2A.z leads to decrease the Nkx2-4 expression

Hierarchical clustering analysis of global genes with more than two-fold changes are categorized. Red represents the upregulated genes, and green represents downregulated genes. Expression levels of -4.0 to +4.0 are indicated right.

Immunostaining shows that the expression of Nkx2-4 is decreased in $H2A.z^{cKO}$ brains at E13. High-magnification images of the representative areas (broken lines) in the VZ/SVZ are shown to the down.

Quantitative analysis of fluorescent intensity of Nkx2-4 in VZ/SVZ. $n = 20$ cells for samples

Western blot analysis in E13 forebrain shows that Nkx2-4 protein is decreased upon $H2A.z$ loss.

Western blot shows that H3K36me3 levels are decreased in $H2A.z$-deleted cells. E13 embryonic cortexes were disassociated and lysed for western blot analysis. Then, the cell lysates were detected by western blotting with the antibodies indicated.

Graph shows the normalized density of H2A.z and H3K36me3, H3K27me3, H3K9me3, and H3K4me3. $n = 6$ for all samples.

Genome-wide distribution of H3K36me3 ChIP-seq in E13 forebrain.

Heatmaps of normalized density of ChIP-seq tags in E13 mouse forebrain.

Gene tracks of H3K36me3 enrichment by ChIP-seq analysis at promoter regions of Nkx2-4.

Error bars represent means ± s.e.m.; Two-tailed unpaired t-test, $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). n.s., not significant. Scale bar represents 50 μm (B, up); 20 μm (B, down).

Figure S11 The expression and localization of Nkx2-4 in the developing cortex
(A) Representative images of brain sections at E12, E15, and E18 stained for Nkx2-4 and Tuj1. Note that Nkx2-4 is expressed in a subpopulation of Tuj1+ neurons.

(B) Representative images of brain sections at E12, E15, and E18 stained for Nkx2-4 and Tbr2. Note that Nkx2-4 is expressed in Tbr2+ IP in E15.

(C) Images of coronal sections of the developing cortex labeled for Nkx2-4 and Sox2. Note that Nkx2-4 expression is colocalized with Sox2+ NPCs.

(D) Western blot analysis shows that Nkx2-4 is efficiently reduced when Nkx2-4 is depressed. Primary progenitors from E12 brain cultured in proliferation conditions were infected with lentivirus expressing the control or Nkx2-4-shRNA for analysis. Protein lysates of infected cells were probed with the antibodies indicated.

(E) Nkx2-4-shRNA or control plasmids were electroporated into the brains of E13 embryos. At E16, the brains were harvested to investigate the cell distribution and were immunostained with the mitotic marker pH3.

(F) Graph shows the percentage of GFP+ cells in the VZ/SVZ, IZ and CP. n = 10 for control sample, 7 for Nkx2-4-shRNA sample.

(G) Percentage of pH3+GFP+ cells in the VZ/SVZ. n = 8 for all samples.

(H) Nkx2-4 knockdown inhibits the production of neurons. The brain sections with electroporated Nkx2-4-shRNA or control plasmids were immunostained with anti-Tuj1.

(I) Percentage of Tuj1+GFP+ cells in control or Nkx2-4-shRNA-electroporated brains. n = 8 for all samples.

Error bars represent means ± s.e.m.; Two-tailed unpaired t-test, p < 0.05 (*). p < 0.01(**). n.s., not significant. Scale bar represents 50 μm (A, middle and right; B, middle; E and H), 20 μm (A, left; B, left and right; C, middle and right), 15 μm (C left). All experiments were repeated at least three times.

**Figure S12 Overexpression Nkx2-4 rescues phenotypes caused by H2A.z knockdown**

(A) Representative images of E16 cortices electroporated with control, H2A.z-sh1 or H2A.z-sh1 together with shRNA resistant H2A.z or Nkx2-4 at E13. Note immunostaining for mitotic marker PH3.

(B) Graph shows the percentage of GFP+ cells in VZ/SVZ, IZ, and CP. n = 3-6 for all samples.

(C) Graph shows the percentage of pH3+GFP+ cells. n = 4 for all samples.

(D) Representative images of E16 coronal brain sections immunostained for Tbr2.

(E) Graph shows the percentage of GFP+ Tbr2+ cells. n = 4 for all samples.

(F) Representative images of E16 cortices electroporated with control, H2A.z-sh1,
Nkx2-4 or H2A.z-sh1 together with Nkx2-4 at E13. Note immunostaining for Tuj1.

(G) Graph shows the percentage of GFP+ cells in VZ/SVZ, IZ, and CP. n = 4 for all samples.

(H) Graph shows the percentage of GFP+ Tuj1+ cells. n = 4 for all samples.

(I) Representative images of P17 cortices electroporated with control, H2A.z-sh1, Nkx2-4 or H2A.z-sh1 together with Nkx2-4 at E13.

(J) Quantitative analysis of the number of dendrite branches. n = 4-5 for all samples.

Figure S13 Chromatin condensation and H2A.z binding enrichment detection in Nkx2-4 promoter

(A) The overexpression of HA-Setd2 increases the H3K36me3 levels. Primary neural progenitors were infected with control or HA-Setd2 lentivirus. Three days later, the cell lysates were detected by western blotting with the antibodies indicated.

(B) The levels of H3K36me3 are reduced when the expression of Setd2 is downregulated. Primary neural progenitors were infected with control or Setd2-shRNA lentivirus. Three days later, the cell lysates were detected by western blotting with the antibodies indicated.

(C) Interaction between H2A.z and Setd2 is verified by co-immunoprecipitation experiments. HA-Setd2- and Flag-H2A.z-expressing plasmids were cotransfected into N2A cells. Three days later, the transfected cells were subjected to immunoprecipitation using HA beads. Bound proteins were detected by western blotting for the antibodies indicated.

(D) Images of E16 mouse cortices electroporated at E13 with plasmid indicated.

(E) Graph shows the percentages of GFP-positive cells in the VZ/SVZ, IZ and CP. n = 4-5 for all samples.

(F) H2A.z binding enrichment on Nkx2-4 promoter revealed by ChIP experiments.

(G) Micrococcal nuclease (MNase) assay was used to detect the chromatin condensation status of neural progenitor cells from H2A.z^fl/fl and H2A.z^cKO.

All data were based on at least three independent experiments. H2A.z^fl/fl, WT; H2A.z^cKO, cKO. Error bars represent means ± s.e.m.; Two-tailed unpaired t-test, p < 0.01(**). n.s., not significant. Scale bar represents 50 μm (D).

Figure S14 The model for H2A.z-mediated neurogenesis.
H2A.z regulates neural progenitor cell differentiation through Nkx2-4. The loss of function of H2A.z leads to a reduction of Nkx2-4 expression, neural progenitor differentiation defects and abnormal behaviors.
Supplementary Table S1 a short list of top up-and downregulated genes

(A) A short list of significantly upregulated genes and P-value.
(B) A short list of significantly downregulated genes and P-value.
Supplementary Figure S1

A. Graph showing relative mRNA levels of H2A.z and H2A.v over time (E11 to P1).

B. Images showing expression of H2A.z, Sox2, Pax6, and their combinations with DAPI at E15.

C. Images of NPCs showing H2A.z, Nestin, and H2A.z/Nestin.

D. Images of GFP, H2A.z, GFP/H2A.z, and GFP/H2A.z/DAPI under different conditions.

E. IHC: H2A.z expression with normalized intensity comparisons.

F. Western blot analysis showing Flag-H2A.z and β-actin levels under different conditions.
Supplementary Figure S4

A

B

C

Control  H2A.z-sh1  H2A.v-sh1  H2A.z-sh1+H2A.v-sh1

GFP/Tuj1/DAPI

CP

IZ

VZ/SVZ

D

E

% GFP+ cells

VZ/SVZ  IZ  CP

F

WT
cKO

G

Tuj1 fluorescence (a.u.) in Pax6+ cells zone
Supplementary Figure S7

A. *In utero* gene transfer at E13, analyzed at P0

B. Bar graph showing the percentage of GFP-expressing cells in different regions (VZ/SVZ, IZ, IoCP, upCP) for WT and KO mice. The graph shows a significant decrease in GFP expression in the KO group compared to the WT group.

C. *In utero* gene transfer at E15, analyzed at P0

D. Bar graph showing the percentage of GFP-expressing cells in different regions (VZ/SVZ, IZ, IoCP, upCP) for WT and KO mice. The graph shows a non-significant difference (n.s.) in GFP expression between WT and KO groups.

E. E13-P17/GFP

F. Bar graph showing the number of primary dendrites for different groups (Control, H2A.z-sh1, H2A.v-sh1, H2A.z-sh1+H2A.v-sh1). The graph shows a significant increase (n.s.) in the number of primary dendrites in the H2A.z-sh1+H2A.v-sh1 group compared to the Control group.
### Supplementary Table S1

#### A

| Gene   | Fold change | P-Val       |
|--------|-------------|-------------|
| *Nkx-2.4* | 49.7       | 2.0629E-07  |
| *Vgl2*   | 33.8       | 2.13217E-05 |
| *Prrt2*  | 21.9       | 0.000835129 |
| *Sox14*  | 15.9       | 3.13863E-08 |
| *Gsc*    | 11.9       | 0.021052506 |
| *Sim2*   | 10.9       | 0.002067265 |
| *Six6*   | 5.5        | 0.009275368 |
| *Shox2*  | 3.9        | 2.17106E-11 |
| *Otp*    | 3.7        | 7.04004E-27 |
| *Foxb1*  | 3.3        | 0.002867266 |
| *Dcc*    | 3.2        | 2.72455E-27 |
| *Ttr*    | 3.1        | 0.001466839 |
| *Fat4*   | 2.8        | 0.036263161 |
| *Slc15a2*| 2.5        | 4.40811E-16 |
| *Sytl3*  | 2.3        | 2.425E-16   |
| *Hipk2*  | 2.2        | 3.21793E-24 |
| *Tcf7l2* | 2.0        | 2.06681E-21 |
| *Ston2*  | 2.2        | 1.70079E-05 |

#### B

| Gene   | Fold change | P-Val       |
|--------|-------------|-------------|
| *Crh*  | 16.1        | 0.005462    |
| *Stabilin2* | 14.1   | 0.010526    |
| *Gpr35* | 12.1        | 0.020455    |
| *Slc52a3* | 8.0       | 0.013144    |
| *Mertk* | 3.5         | 0.002866    |
| *Nkx-2.3* | 2.0       | 3.9E-06     |
| *Lhx8*  | 1.9         | 2.94E-14    |
| *Shh*   | 1.8         | 0.005308    |
| *Hes5*  | 1.6         | 2.36E-08    |
| *Nkx-2.1* | 1.5       | 5.85E-24    |
| *Npy*   | 1.5         | 2.28E-05    |
| *Ccnd1* | 1.2         | 3.29E-06    |