Supplementary Materials

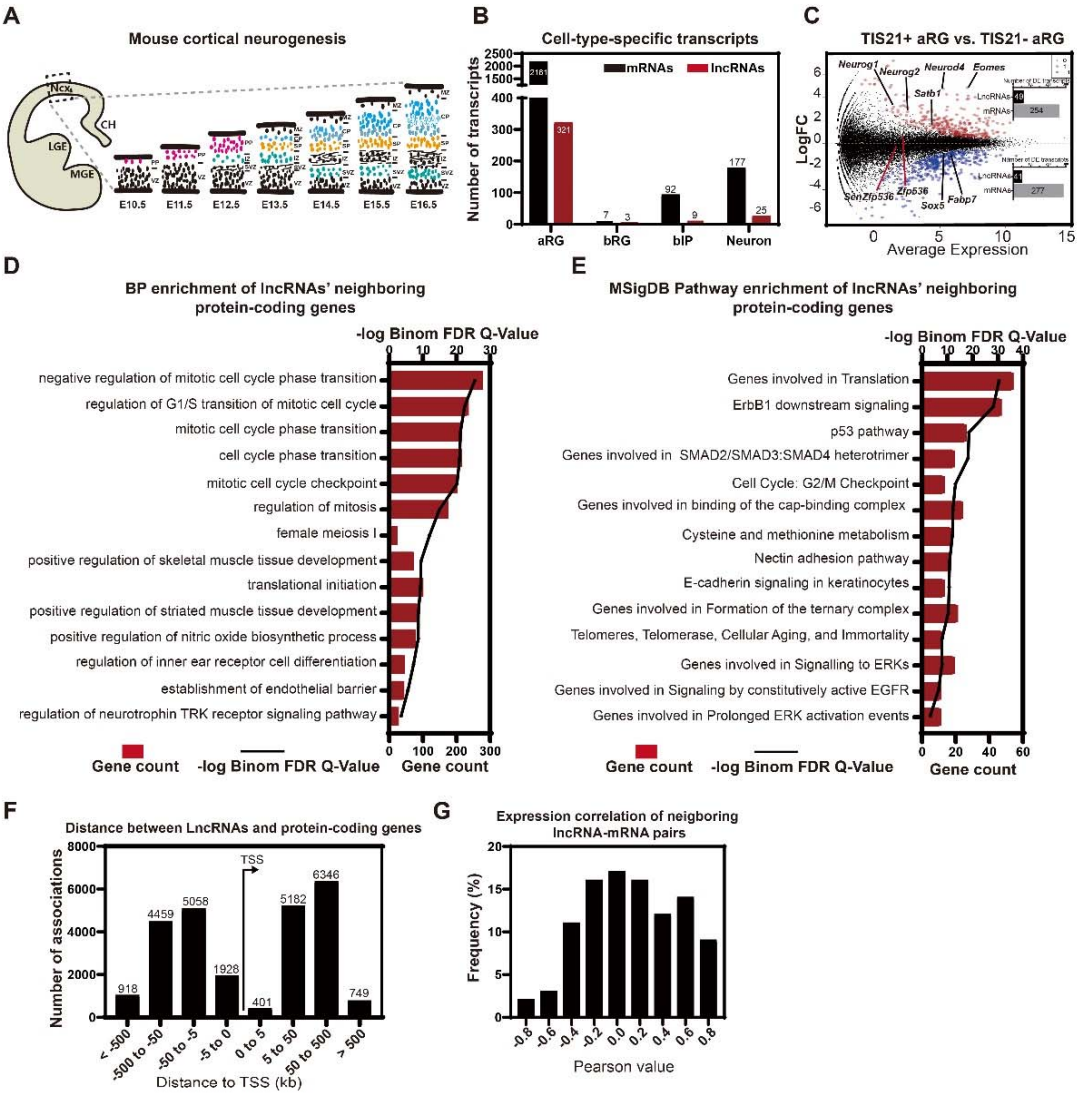

Fig. S1 A The 'inside-out' model for neurogenesis (E10.5 – E16.5) of mouse cerebral cortex. Neural progenitors reside in the VZ and subventricular zone (SVZ). The earliest born neurons form the preplate (PP), which is later split into a more superficial marginal zone (MZ), a deeply located subplate (SP), and the cortical plate (CP) that generates a multi-layered structure. Early-born neurons are located in inner layers, while later-born neurons migrate past and reside over early-born neurons. E, embryonic day; CH, cortical hem; Ncx, neocortex; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence. **B** Numbers of cell-type-specific mRNAs and lncRNAs in E14.5 aRG (apical radial glia), bRG (basal radial glia), bIP (basal intermediate progenitors), and neurons. Transcripts that are significantly enriched in all comparisons with the other three cell types are designated as cell-type-specific transcripts. **C** Scatter plots (rendered by edgeR) showing the average expression and log fold-change between proliferative (Tis21-GFP-) and differentiating (Tis21-GFP+) aRGs of E14.5 cortex. Numbers of differentially-expressed transcripts are shown on the right. Several markers, including *SenZfp536* and *Zfp536*, are highlighted. **D** The top 14 biological process (BP) GO terms of protein-coding genes adjacent to antisense, intergenic, tandem, divergent, and convergent lncRNA genes as in **F** and **G**. Bars display the gene count in each GO term and the black line chart shows –log Binom FDR Q values. **E** The top 14 MSigDB pathway terms of PCGs adjacent to antisense, intergenic, tandem, divergent, and convergent lncRNA genes as in **F** and **G**. Bars display the gene count in each term and the line shows –log Binom FDR Q values. **F** Numbers of lncRNAs categorized according to their distance from the transcription start site (TSS) of PCGs. **G** Frequency distribution of Pearson correlation coefficients of expression levels of neighboring lncRNA-mRNA pairs across RNA-seq transcriptome data derived from the dorsal forebrain (neocortex) at E10.5, E11.5, E12.5, E13.5, E14.5, E15.5, E16.5, and P0, along with VZ, SVZ/IZ, and CP tissues at E14.5.

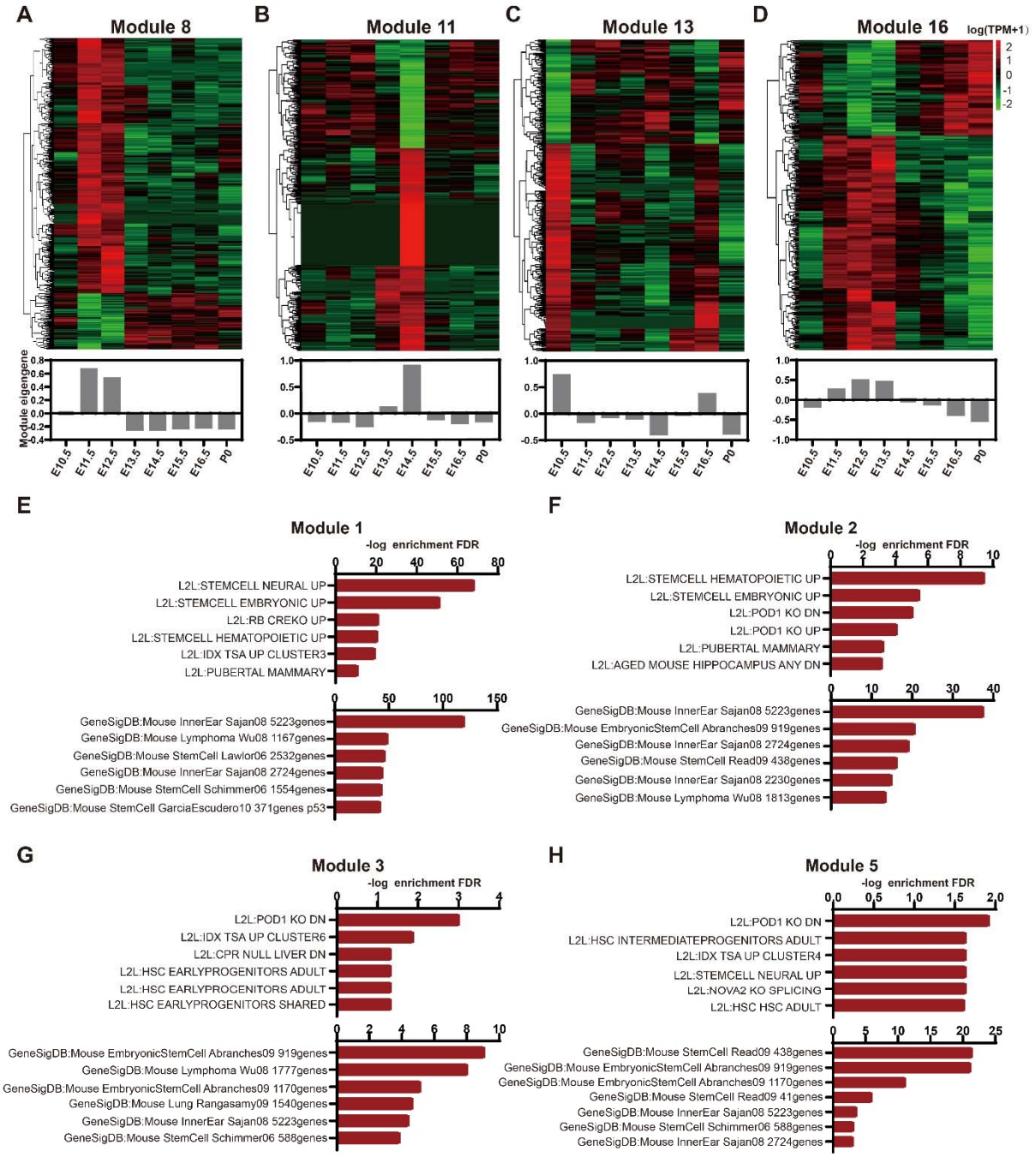
A
Module 8
B
Module 11
C
Module 13
D
Module 16
log(TPM+1)
2
1
0
-1
-2
Module eigengene
E10.5
E11.5
E12.5
E13.5
E14.5
E15.5
E16.5
P0
E
Module 1
-log enrichment FDR
L2L:STEMCELL NEURAL UP
L2L:STEMCELL EMBRYONIC UP
L2L:RB CREKO UP
L2L:STEMCELL HEMATOPOIETIC UP
L2L:IDX TSA UP CLUSTER3
L2L:PUBERTAL MAMMARY
GeneSigDB:Mouse InnerEar Sajan08 5223genes
GeneSigDB:Mouse Lymphoma Wu08 1167genes
GeneSigDB:Mouse StemCell Lawlor06 2532genes
GeneSigDB:Mouse InnerEar Sajan08 2724genes
GeneSigDB:Mouse StemCell Schimmer06 1554genes
GeneSigDB:Mouse StemCell GarciaEscudero10 371genes p53
F
Module 2
-log enrichment FDR
L2L:STEMCELL HEMATOPOIETIC UP
L2L:STEMCELL EMBRYONIC UP
L2L:POD1 KO DN
L2L:POD1 KO UP
L2L:PUBERTAL MAMMARY
L2L:AGED MOUSE HIPPOCAMPUS ANY DN
GeneSigDB:Mouse InnerEar Sajan08 5223genes
GeneSigDB:Mouse EmbryonicStemCell Abranches09 919genes
GeneSigDB:Mouse InnerEar Sajan08 2724genes
GeneSigDB:Mouse StemCell Read09 438genes
GeneSigDB:Mouse InnerEar Sajan08 2230genes
GeneSigDB:Mouse Lymphoma Wu08 1813genes
G
Module 3
-log enrichment FDR
L2L:POD1 KO DN
L2L:IDX TSA UP CLUSTER6
L2L:CPR NULL LIVER DN
L2L:HSC EARLYPROGENITORS ADULT
L2L:HSC EARLYPROGENITORS ADULT
L2L:HSC EARLYPROGENITORS SHARED
GeneSigDB:Mouse EmbryonicStemCell Abranches09 919genes
GeneSigDB:Mouse Lymphoma Wu08 1777genes
GeneSigDB:Mouse EmbryonicStemCell Abranches09 1170genes
GeneSigDB:Mouse Lung Rangasamy09 1540genes
GeneSigDB:Mouse InnerEar Sajan08 5223genes
GeneSigDB:Mouse StemCell Schimmer06 588genes
H
Module 5
-log enrichment FDR
L2L:POD1 KO DN
L2L:HSC INTERMEDIATEPROGENITORS ADULT
L2L:IDX TSA UP CLUSTER4
L2L:STEMCELL NEURAL UP
L2L:NOVA2 KO SPLICING
L2L:HSC HSC ADULT
GeneSigDB:Mouse StemCell Read09 438genes
GeneSigDB:Mouse EmbryonicStemCell Abranches09 919genes
GeneSigDB:Mouse EmbryonicStemCell Abranches09 1170genes
GeneSigDB:Mouse StemCell Read09 41genes
GeneSigDB:Mouse InnerEar Sajan08 5223genes
GeneSigDB:Mouse StemCell Schimmer06 588genes
GeneSigDB:Mouse InnerEar Sajan08 2724genes

Fig. S2 A–D Heatmaps and Eigengene bar plots of Modules 8, 11, 13, and 16. Upper in each panel: expression levels of lncRNAs and PCGs normalized by $\log_{10}$ (TPM+1). Lower in each panel: module Eigengene barplots representing the expression levels of all transcripts, in line with the first principal component obtained by singular value decomposition of each module. **E–H** Genes in Modules 1, 2, 3, and 5 cross-referenced with curated gene sets in L2L and GeneSigDB. Bars indicate significant enrichment of gene sets in each module.

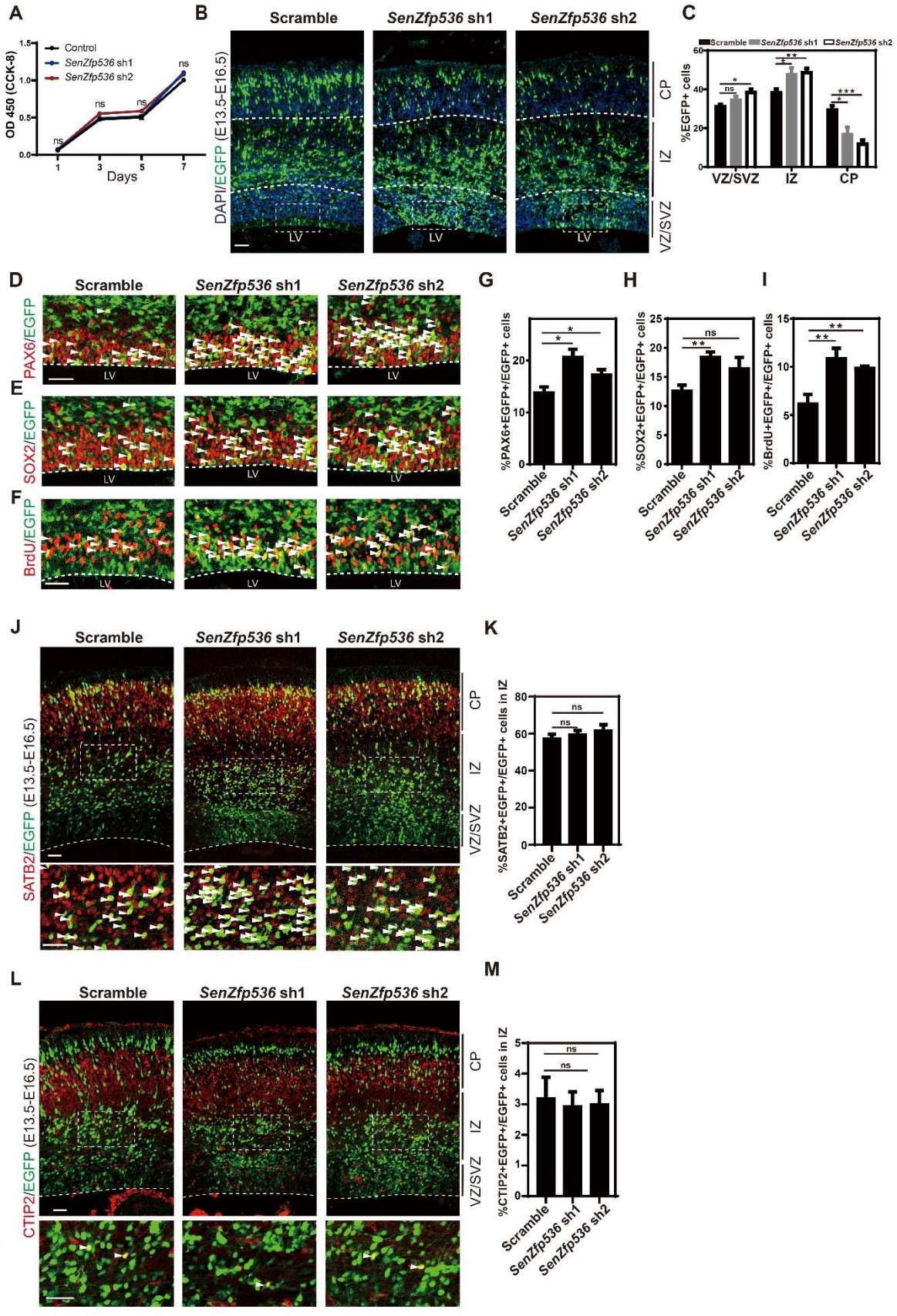
A
OD 450 (CCK-8)
Control
SenZfp536 sh1
SenZfp536 sh2
Days
ns
B
Scramble
SenZfp536 sh1
SenZfp536 sh2
DAPI/EGFP (E13.5-E16.5)
LV
CP
IZ
VZ/SVZ
C
%EGFP+ cells
VZ/SVZ
IZ
CP
D
PAX6/EGFP
E
SOX2/EGFP
F
BrdU/EGFP
G
%PAX6+EGFP+/EGFP+ cells
H
%SOX2+EGFP+/EGFP+ cells
I
%BrdU+EGFP+/EGFP+ cells
Scramble
SenZfp536 sh1
SenZfp536 sh2
J
SATB2/EGFP (E13.5-E16.5)
K
%SATB2+EGFP+/EGFP+ cells in IZ
L
CTIP2/EGFP (E13.5-E16.5)
M
%CTIP2+EGFP+/EGFP+ cells in IZ

Fig. S3 A Cell proliferation assays (CCK-8) of Neuro-2a cells transfected with *SenZfp536* and scrambled shRNAs. Results are presented as mean ± SD. Statistical significance was determined by one-way ANOVA. (**B**–**I**) Mouse cortex at E13.5 were electroporated with a mix of shRNA-expressing and EGFP-expressing vectors. Embryos were sacrificed at E16.5 for immunofluorescent analyses. Spatial distributions of transduced cells are demonstrated and quantified in **B** and **C**. VZ/SVZ immunofluorescent images and quantification for PAX6 (**D**, **G**), SOX2 (**E**, **H**), and BrdU (30 min) (**F**, **I**). White arrows indicate co-labeled cells. For VZ/SVZ in (**C**), $F = 5.867$, $P = 0.0387$; for IZ in (**C**), $F = 11.29$, $P = 0.0093$; for CP in (**C**), $F = 12.24$, $P = 0.0076$; for (**G**), $F = 8.646$, $P = 0.0171$; for (**H**), $F = 5.702$, $P = 0.0410$; for (**I**) $F = 16.61$, $P = 0.0036$ (one-way ANOVA followed by *Dunnett*'s multiple comparison test). **J**–**M** Electroporated cortices as described in **B** were immunostained with SATB2 and CTIP2. Spatial distribution of transduced cells (**J**, **L**); IZ immunofluorescent images and quantification for SATB2 (**K**) and CTIP2 (**M**). White arrows indicate co-labeled cells. Results are presented as mean ± SEM. Embryos in each experiment: scrambled, $n = 6$; *SenZfp536* sh1, $n = 4$; *SenZfp536* sh2, $n = 4$. Scale bars, 50 μm. ns, not significant (one-way ANOVA).

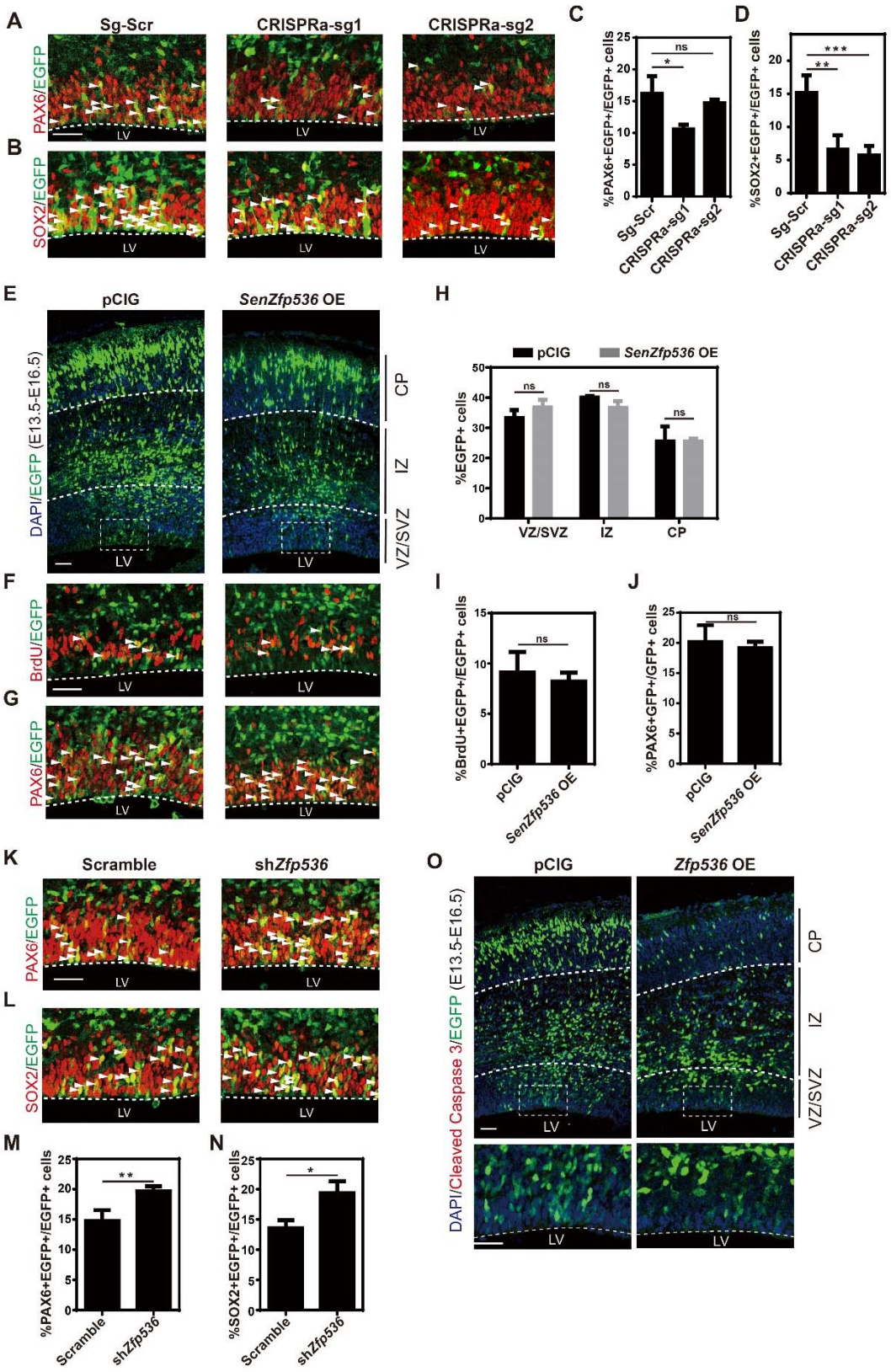
A
Sg-Scr
CRISPRa-sg1
CRISPRa-sg2
PAX6/EGFP
B
SOX2/EGFP
LV
C
%PAX6+EGFP+/EGFP+ cells
ns
*
Sg-Scr
CRISPRa-sg1
CRISPRa-sg2
D
%SOX2+EGFP+/EGFP+ cells
***
**
E
pCIG
SenZfp536 OE
DAPI/EGFP (E13.5-E16.5)
CP
IZ
VZ/SVZ
H
pCIG
SenZfp536 OE
%EGFP+ cells
VZ/SVZ
IZ
CP
F
BrdU/EGFP
G
I
%BrdU+EGFP+/EGFP+ cells
J
%PAX6+GFP+/GFP+ cells
K
Scramble
shZfp536
L
M
%PAX6+EGFP+/EGFP+ cells
N
%SOX2+EGFP+/EGFP+ cells
O
pCIG
Zfp536 OE
DAPI/Cleaved Caspase 3/EGFP (E13.5-E16.5)

Fig. S4 A–D A mix of vectors expressing sgRNAs, dCAS9-VP64-EGFP and MS2-P65-HSF1-GFP were electroporated into mouse cortex at E13.5 and transduced cells were labeled with EGFP. Embryos were sacrificed at E16.5 for immunofluorescent analyses. VZ/SVZ immunofluorescent images and quantifications for PAX6 (**A**, **C**) and SOX2 (**B**, **D**). White arrows indicate co-labeled cells. In (**C**), $F = 12.34$, $P = 0.0078$; in (**D**), $F = 20.56$, $P = 0.0024$ (one-way ANOVA followed by *Dunnett*'s multiple comparison test). Embryos in each experiment: Sg-Scr, $n = 5$; CRISPRa-sg1, $n = 4$; CRISPRa-sg2, $n = 3$. **E–J** Mouse cortices at E13.5 were electroporated with *SenZfp536*-expressing or control vectors. Embryos were sacrificed at E16.5 for immunofluorescent analyses. Spatial distribution of transduced cells is illustrated and quantified (**E**, **H**). VZ/SVZ immunofluorescent images and quantifications for BrdU (30 min) (**F**, **I**) and PAX6 (**G**, **J**). White arrows indicate co-labeled cells. Samples in each experiment: pCIG, $n = 7$; *SenZfp536* OE, $n = 3$; ns, not significant (two-tailed unpaired Student's *t*-test). **K–N** Mouse cortices at E13.5 were electroporated with vectors expressing *Zfp536* shRNA. Embryos were sacrificed at E16.5 for immunofluorescent analyses. VZ/SVZ immunofluorescent images and quantification for PAX6 (**K**, **M**) and SOX2 (**L**, **N**). White arrows indicate co-labeled cells. Samples in each experiment: Scrambled, $n = 5$; *shZfp536*, $n = 4$. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant (two-tailed unpaired student's *t*-test). Results are presented as mean ± SEM. (**O**) Mouse cortices at E13.5 were electroporated with pCIG or *Zfp536*-expressing vectors. Embryos were sacrificed at E16.5 for immunofluorescent analyses. Spatial distribution of transduced cells and VZ/SVZ immunofluorescent images for Cleaved Caspase 3 are illustrated.