Supplemental information

CIC is a Critical Regulator of Neuronal Differentiation

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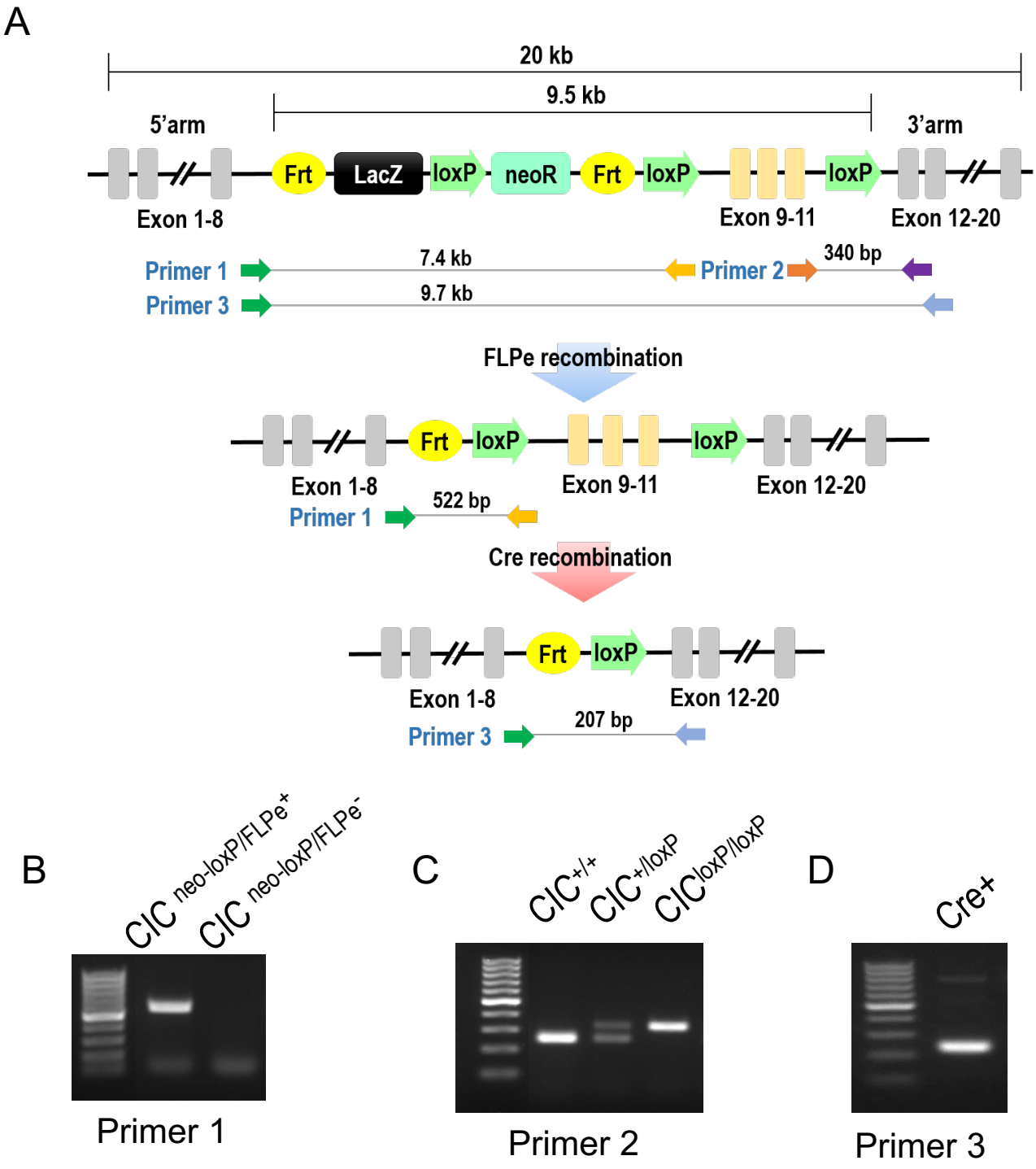
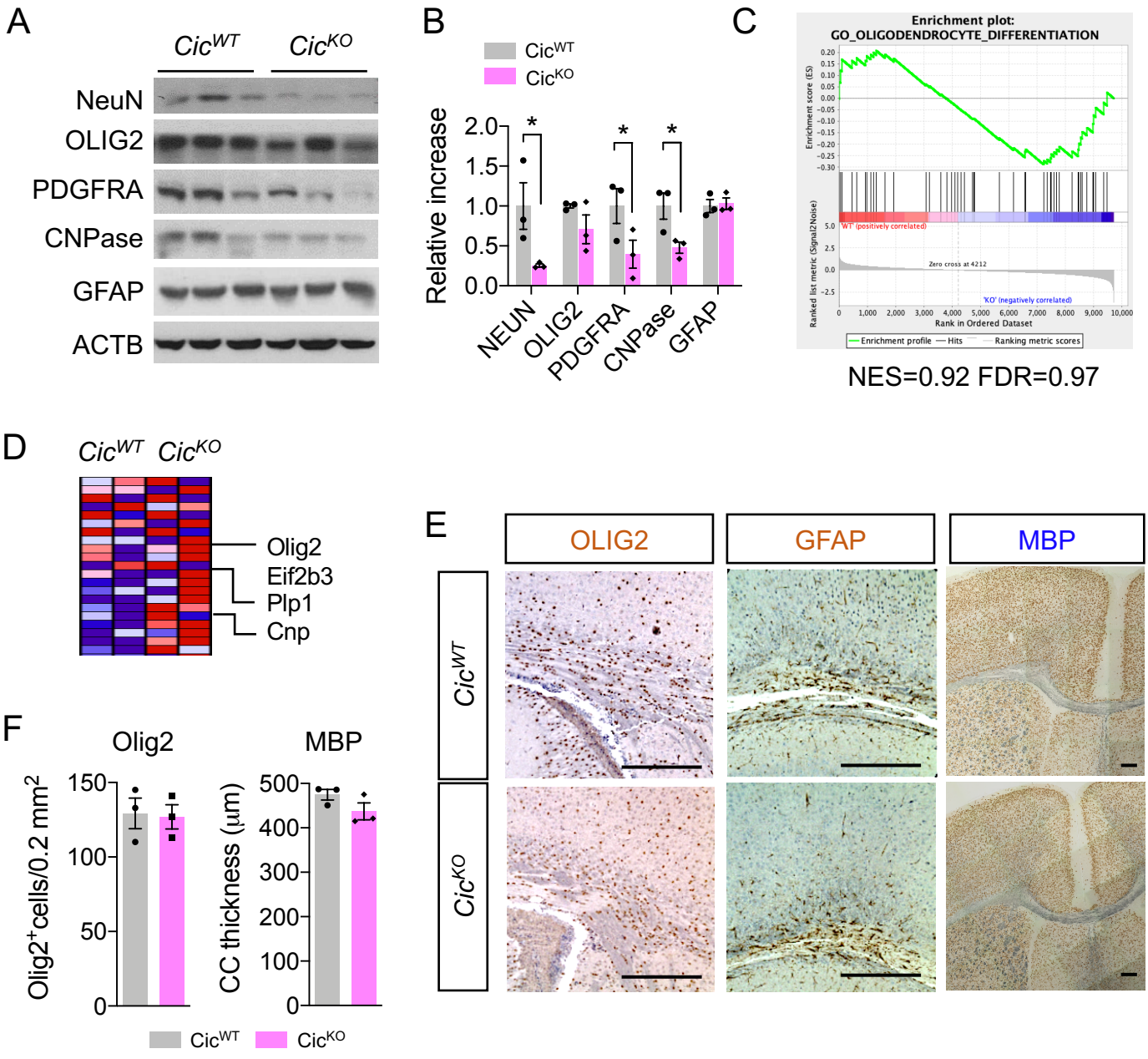
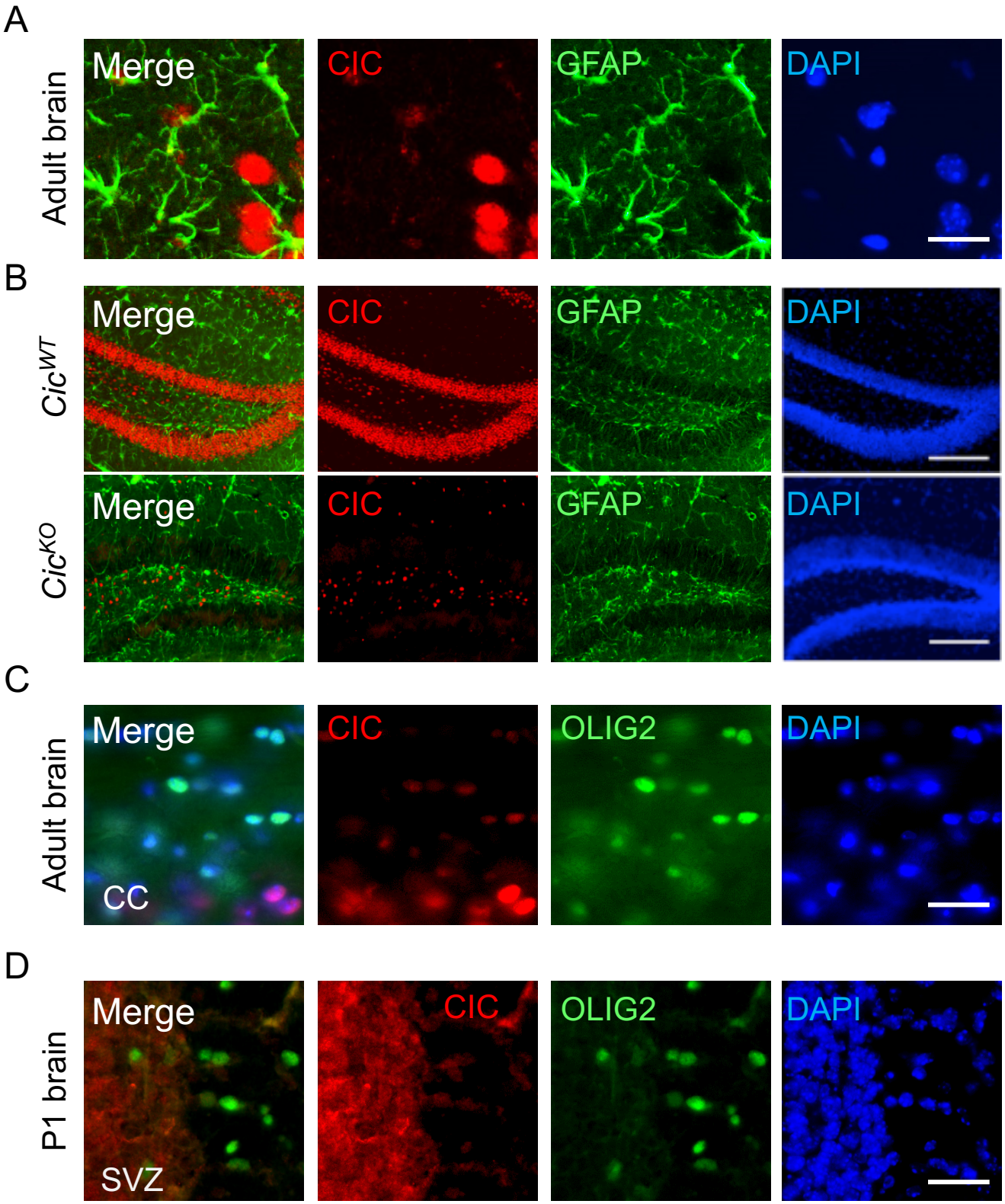


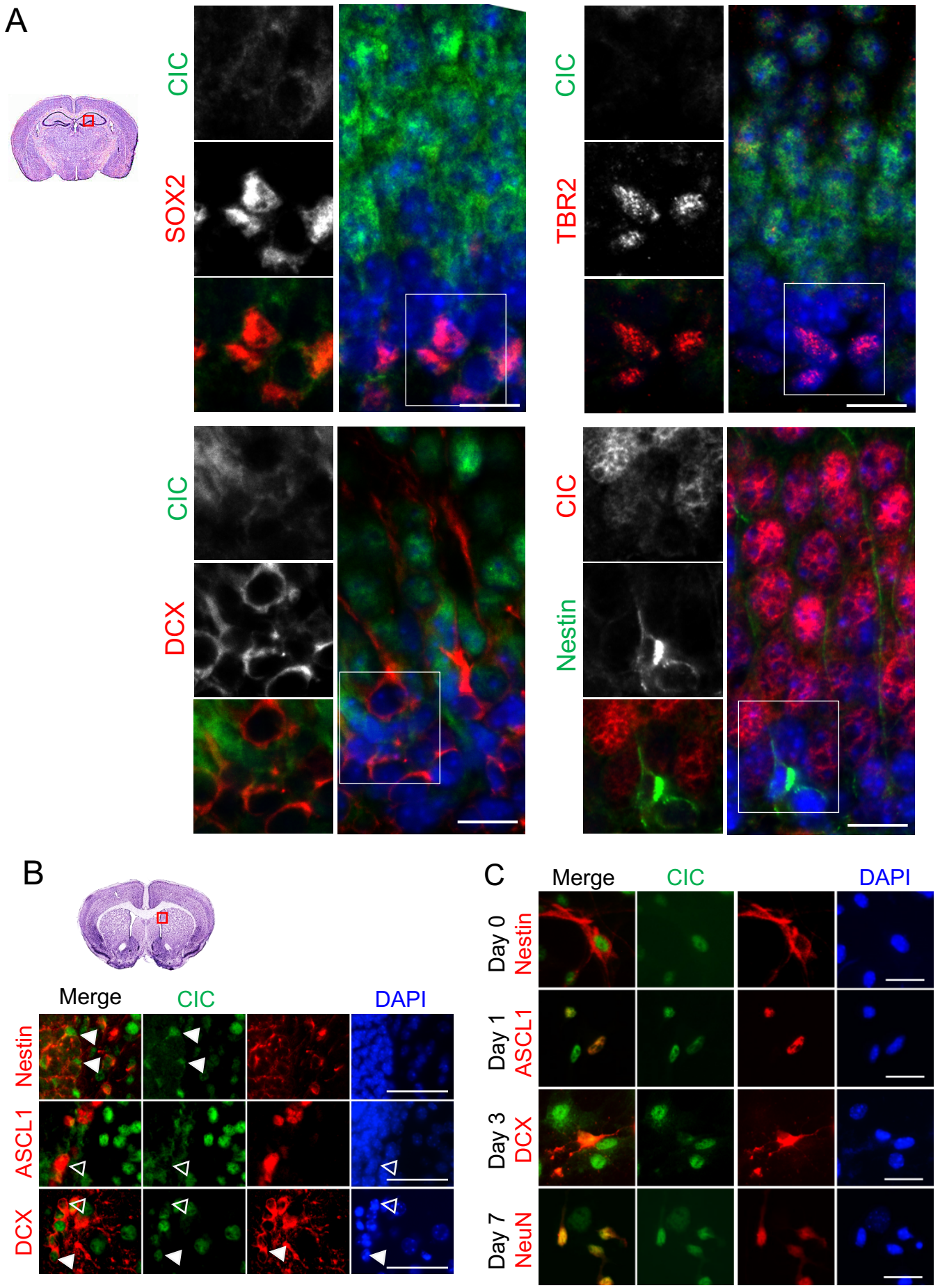
Supplemental figure 1. Generation of *Cic*^{KO} mouse. A. Diagram for *Cic*$^{L/L}$. FRT site flanked neomycin resistance cassette was removed by crossing to transgenic animals expressing hACTB:FLPe in the germ line. The LoxP site was removed by crossing to *Nes cre* transgenic mouse. B. PCR result with primer set 1 after removing FRT site. C. PCR result with primer set 2. D. PCR result of *Cic*^{KO} brain with primer set 3.



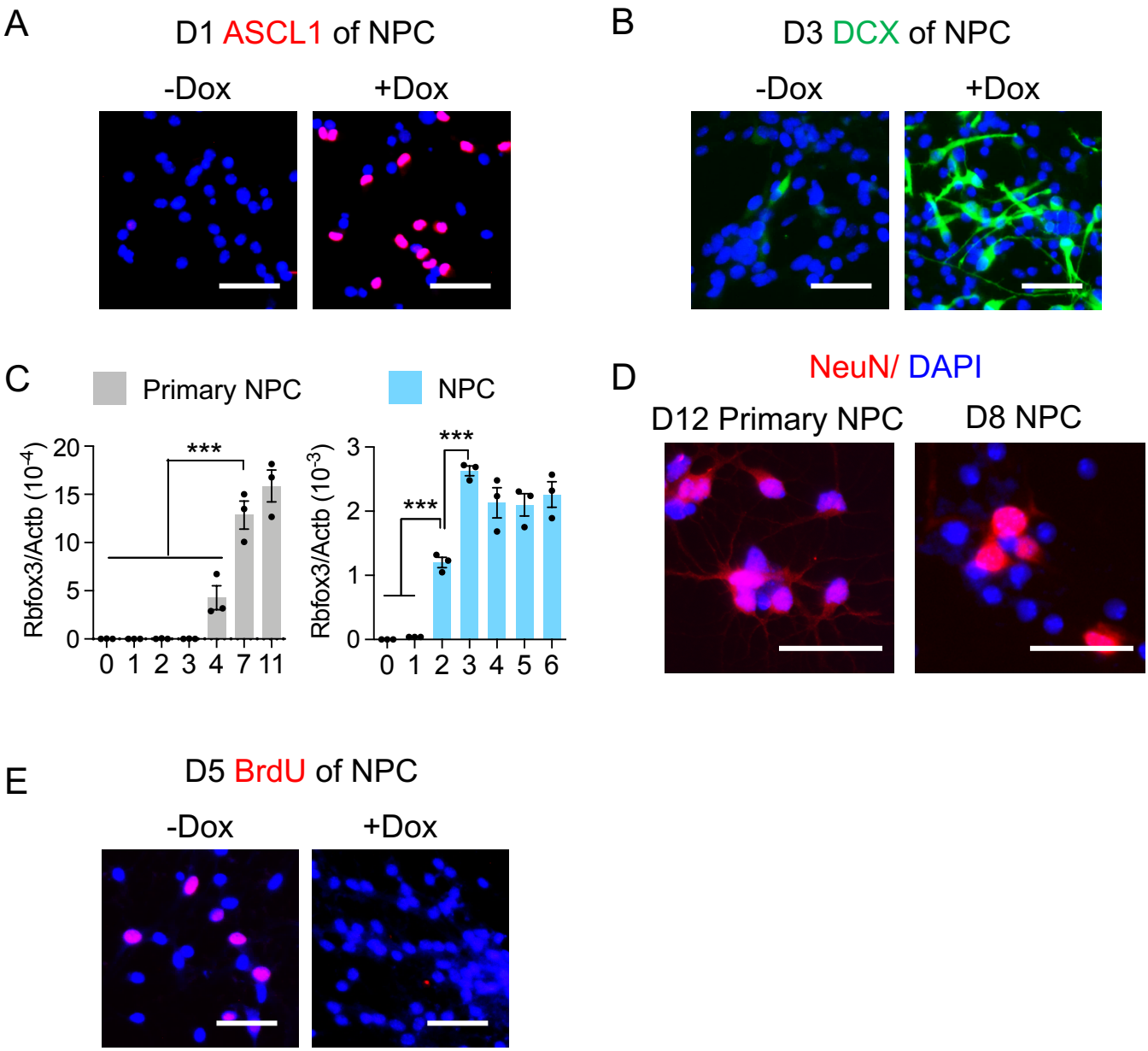
Supplemental figure 2. Gliogenesis was not altered in *Cic^{KO}* brains. A. WB analysis of P0.5 *Cic^{WT}* and *Cic^{KO}* brains. Samples were run on 4 gels and the most representative ACTB blot is shown. B. Analyze for multiple WB in (A) are plotted. Mean ± s.e.m. of 3 experimental animals. C. GSEA for oligodendrocyte differentiation. D. Heatmap for RNA-seq of P0.5 *Cic^{WT}* and *Cic^{KO}* brains. E. IHC for OLIG2 (brown), GFAP (brown), and MBP (blue) in P14 *Cic^{WT}* and *Cic^{KO}* brains. Scale bar=400 µm. F. Quantifications for (E) images are plotted. Mean ± s.e.m. of 3 images from independent animals. Statistical significance was determined by unpaired t-test for B and F. ***p<0.001. CC: Corpus callosum.



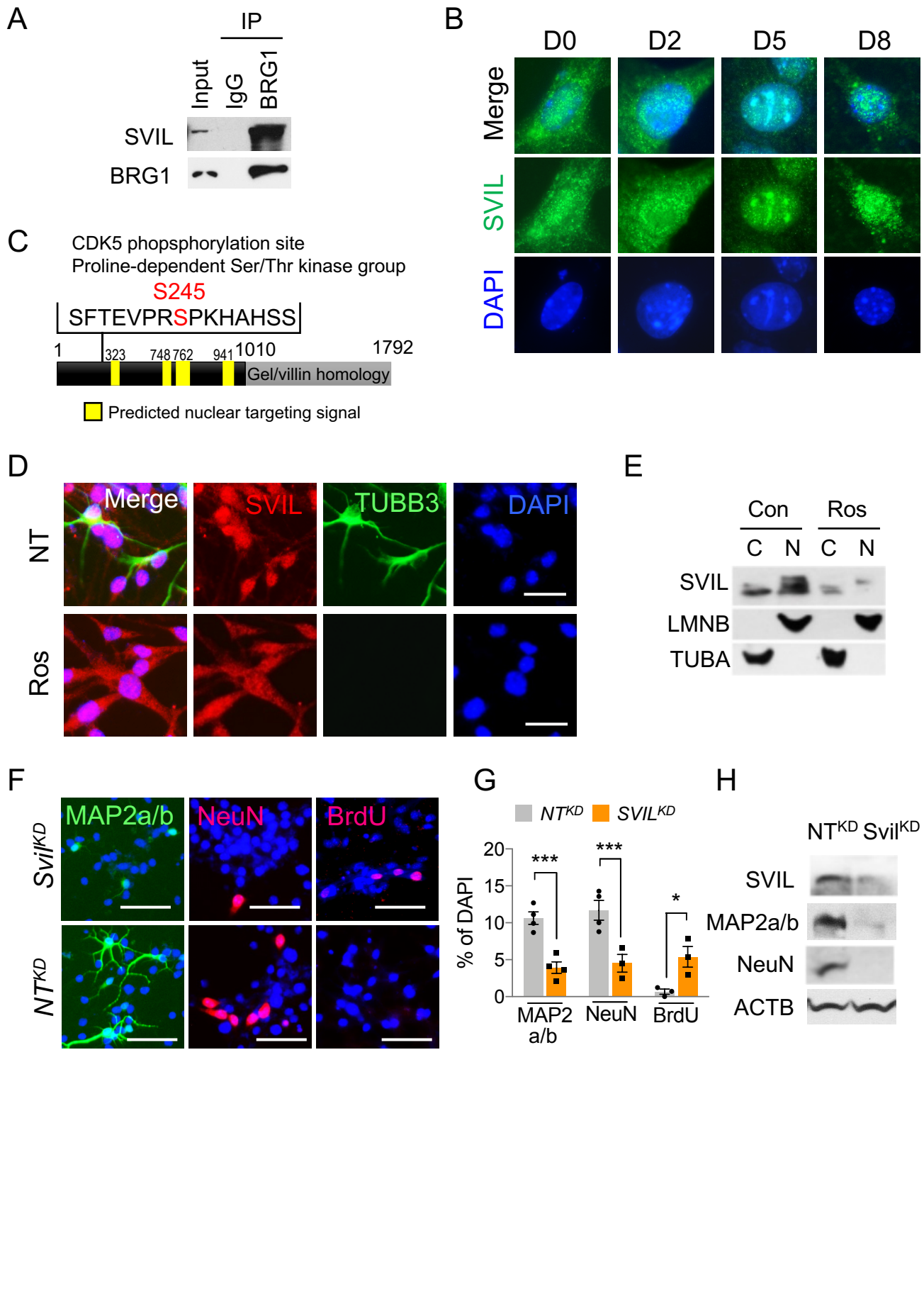
Supplemental figure 3. The expression of CIC in oligodendrocytes and astrocytes. A and B. Co-IF for GFAP and CIC in P14 brains. Scale bar=25 μm (A) and 200 μm (B). C and D. Co-IF for OLIG2 and CIC in P14 (C) and P0.5 (D) postnatal brain (D). CC: Corpus callosum, SVZ: Subventricular zone, Scale bar=25 μm. 3 experiments were conducted and the most representative experiment is shown for A-D.


A
CIC
SOX2
CIC
TBR2
CIC
DCX
CIC
Nestin
B
Merge
CIC
DAPI
Nestin
ASCL1
DCX
C
Merge
CIC
DAPI
Day 0
Nestin
Day 1
ASCL1
Day 3
DCX
Day 7
NeuN

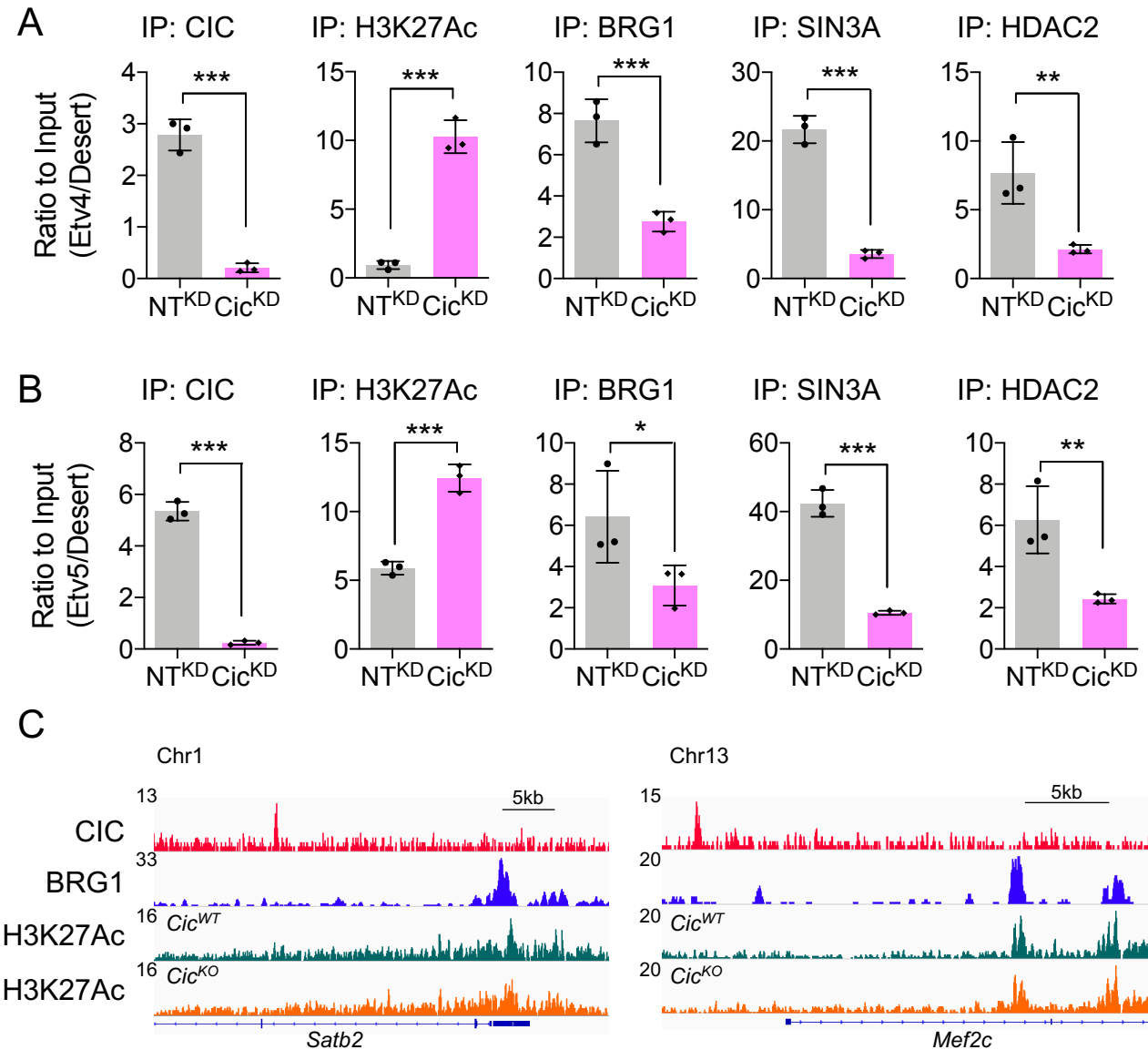
Supplemental figure 4. The expression pattern of CIC in the brain and neural progenitor cells. A. Co-IF for CIC with SOX2, TBR2, DCX, or Nestin in the hippocampus of P14 mouse. Scale bar=50 μm. B. Co-IF for CIC with Nestin, ASCL1, or DCX in the SVZ of P14 mouse. Opened arrow means CIC weak cells and closed arrow means CIC high cells. Scale bar=50 μm. C. Co-IF for CIC with Nestin, ASCL1, DCX, or NeuN in the primary NPC during differentiation. Scale bar=25 μm. 3 experiments were conducted and the most representative experiment is shown for A-C.



Supplemental figure 5. Neuronal differentiation of NPC. A. IF analysis for ASCL1 at 1 day of differentiation in NPC cultured with or without 2 μg/ml of doxycycline (Dox). B. IF analysis for DCX at 3 day of differentiation in NPC cultured with or without 2 μg/ml of Dox. C. qRT-PCR results of primary NPC (black bar) and NPC (blue bar) during differentiation. Mean ± s.e.m of 3 experiments. Statistical significance was determined by one-way ANOVA. *** $p<0.001$. D. IF analysis for NeuN in D12 primary NPC (left) and D8 NPC (right). E. IF analysis for BrdU incorporation at 5 day of differentiation in NPC cultured with or without 2 μg/mL of Dox. Scale bar=50 μm. 3 experiments were conducted and the most representative experiment is shown for A, B, D, and E.


A
IP
Input
IgG
BRG1
SVIL
BRG1
B
D0
D2
D5
D8
Merge
SVIL
DAPI
C
CDK5 phopsphorylation site
Proline-dependent Ser/Thr kinase group
S245
SFTEVPRSPKHAHSS
1
323
748
762
941
1010
1792
Gel/villin homology
Predicted nuclear targeting signal
D
NT
Ros
Merge
SVIL
TUBB3
DAPI
E
Con
Ros
C
N
C
N
SVIL
LMNB
TUBA
F
SvilKD
NTKD
MAP2a/b
NeuN
BrdU
G
NTKD
SVILKD
% of DAPI
20
15
10
5
0
***
***
*
MAP2 a/b
NeuN
BrdU
H
NTKD SvilKD
SVIL
MAP2a/b
NeuN
ACTB

Supplemental figure 6. Nuclear translocation of SVIL is required for neurogenesis. A. Co-IP analysis for endogenous proteins from P0.5 brain lysates. One experiment was conducted. B. Nuclear translocation of SVIL during differentiation. Scale bar=5 μm. C. Schematic representation of phosphosite of SVIL by CDK5. D.IF analysis for SVIL and TUBB3 in non-treated (NT) and 20 μM of Roscovitine treated cells (Ros) at 3 days of differentiation. Scale bar=25 μm E. WB analysis for nuclear fraction. Lamin B1 (LMNB) was used as a marker for nuclear fraction (N) and αTubulin (TUBA) was used as a marker for cytosolic fraction (C). F. IF analysis for MAP2a/b, NeuN, and BrdU in NT^{KD} NPC and $Svil^{KD}$ NPC at 8 days of differentiation. Scale bar=50 μm. G. Indicated proteins-positive cells of (F) images are counted. Mean ± s.e.m of 100 DAPI positive nuclei from 3 experiments. Statistical significance was determined by unpaired t-test. *$p<0.05$,*** $p<0.001$. H. WB analysis for each specific marker. 3 experiments were conducted and the most representative experiment is shown for D, E, and H.



Supplemental figure 7. mSWI/SNF-SIN3 complex is required for CIC's transcriptional regulation. A and B. ChIP-qPCR results from NT^{KD} NPC and Cic^{KD} NPC at *Etv4* (A) or *Etv5* (B) promoter region. Mean ± s.e.m. of 3 experiments. Statistical significance was determined by unpaired t-test. *p<0.05, **p<0.01, *** p<0.001. C. ChIP tracks of CIC, BRG1, and H3K27Ac in P0.5 brains of Cic^{WT} and Cic^{KO} mouse.

Supplemental Table legends

Table S1. Integrated analysis for RNA-seq and ChIP-seq, related to Figure 3.

Table S2. The list of mass spectrometry from immunoprecipitation of CIC, related to Figure 6.

Table S3. The list of oligos, related to methods.