

## Supplementary Information

### Supplementary Materials and Methods

#### Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Mouse monoclonal to $\beta$ -Actin	Chemicon	MAB1501; RRID: AB_2223041
Mouse monoclonal to TUJ1	Sigma	T8660; RRID: AB_477590
Rat monoclonal to BrdU	Abcam	ab6326; RRID: AB_305426
Mouse monoclonal to BrdU	Santa Cruz	sc-32323, RRID: AB_626766
Rabbit monoclonal to Cleaved Caspase-3	Cell Signaling	9664; RRID: AB_2070042
Rat monoclonal to CTIP2	Abcam	ab18465; RRID: AB_2064130
Rabbit polyclonal to GFP	Invitrogen	A11122; RRID: AB_221569
Rabbit polyclonal to KDM2B	Millipore	17-10264; RRID: AB_11205420
Rabbit polyclonal to KDM2B	Millipore	09-864; RRID: AB_10806072

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Rabbit polyclonal to KDM2B	Labaratory of JieKai Chen	N/A	
Rabbit polyclonal to PAX6	Chemicon	AB2237; AB_1587367	RRID:
Rabbit polyclonal to TBR2	Abcam	ab23345; AB_778267	RRID:
Rabbit polyclonal to SOX2	Milipore	ab5603; AB_2286686	RRID:
Rabbit polyclonal to NeuN	Abcam	ab177487	
Rabbit polyclonal to GFAP	DAKO	Z0334	
Mouse monoclonal to FLAG	Sigma-Aldrich	F1804; RRID: AB_262044	
Mouse monoclonal to SATB2	Abcam	ab51502; AB_882455	RRID:
Goat polyclonal to UNC5D	R&D	AF1429; AB_2304199	RRID:
Sheep anti-DIG AP	Roche	11093274910; AB_514497	RRID:
Mouse monoclonal to H3K4me3	Active Motif	MABI0304; AB_514497	RRID:
Mouse monoclonal to H3K27ac	Millipore	17-683; AB_1977529	RRID:

Rabbit polyclonal to NEUROD2	Abcam	ab104430; AB_10975628	RRID:
Rabbit polyclonal to SATB1	Abclonal	A5800	
Mouse monoclonal to hnRNPAB	Santa Cruz	sc-32323	
Rabbit polyclonal to GAPDH	Cwbio	CW0101M; AB_2665434	RRID:
Rabbit polyclonal to $\beta$ -TUBULIN	Proteintech	10094-1-AP; AB_2210695	RRID:

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### Chemicals, Peptides, and Recombinant Proteins

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B27	Thermo Fisher	17504044
N2	Thermo Fisher	17502048
hEGF	Thermo Fisher	PHG0311
hFGF2	Thermo Fisher	PHG0261
Papain	Worthington	LS003118
DNase I	Sigma-Aldrich	DN-25
DMEM-F12 medium	Thermo Fisher	12634-010
Protein G agarose		
Streptavidin Agarose	Thermo Fisher	S951
5-Bromouridine 5'-triphosphate	Sigma-Aldrich	B7166
Mung Bean Nuclease	Takara Bio	2420A
Micrococcal Nuclease	NEB	M0247S

Vanadyl Ribonucleoside Complex	Sangon	B644221
	Biotech	
Protease inhibitor	Bioutil	B14001
PMSF	Sigma-Aldrich	P7626
Proteinase K	Sigma	P4032
NBT/BCIP	Roche	11681451001
DIG-NTP	Roche	11277073910
Biotin RNA labeling mix	Roche	11685597912
CDP-star	Roche	11685627001
paraformaldehyde	Sigma-Aldrich	P6148
FastGreen	Sigma-Aldrich	F7252
Lipofectamine 3000	Invitrogen	L3000-150
Trizol	Thermo Fisher	15596026

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### **Bacterial and Virus Strains**

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<i>E. coli</i> DH5 $\alpha$	TransGen	CD201-01
<i>E. coli</i> Stbl3	TransGen	CD521-01
Mus musculus BAC clone	BACPAC	RP23-21416
Lentivirus vector, pLKO.1-zsGreen		

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### **Critical Commercial Assays**

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Mouse	Neural	Stem	Cell	LONZA	VAPG-1004
Nucleofector <sup>®</sup> Kit					

HiScribe™ T7 High Yield RNA NEB E2040S

Synthesis Kit

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**Experimental Models: Cell Lines**

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Mouse Neuro-2a cell line The Cell Bank TCM29

of Chinese

Academy of

Sciences

Mouse NE-4C cell line The Cell Bank SCSP-1501

of Chinese

Academy of

Sciences

Human HEK293T cell line A gift from Dr.

Hongbing Shu

*LncKdm2b* polyA Knock-in mouse This paper N/A

ES cells

*LncKdm2b* polyA Knock-in and This paper N/A

*Kdm2b* indels Mouse ES Cells

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**Experimental Models: Organisms/Strains**

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Mouse: CD-1

Hunan

SJA

Laboratory

Animal Co

Mouse: C57BL/6	Hunan	SJA
	Laboratory	
	Animal Co	
Mouse: <i>Ai14</i> reporter	(Madisen et al.,	
	2010)	
Mouse: <i>Kdm2b</i> <sup>CreERT2/+</sup>	This paper	N/A

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### Recombinant DNA

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pGEM-Teasy	Promega	A1360
pCAGGS		
pCAG-mir30		
pMD2.G	Addgene	12259
psPAX2	Addgene	12260
pGL3-basic	Promega	E1751
phRL-TK	Promega	E6921
pCALNL-DsRed	Addgene	13769

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### Software and Algorithms

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DAVID functional annotation tool	(Huang et al., 2009)	<a href="https://david.ncifcrf.gov/">https://david.ncifcrf.gov/</a>
UCSC Genome Browser	(Kent et al., 2002)	<a href="http://genome.ucsc.edu/">http://genome.ucsc.edu/</a>

CPAT	(Wang et al., 2013)	<a href="http://lilab.research.bcm.edu/cpat/index.php/">http://lilab.research.bcm.edu/cpat/index.php/</a>
PhyloCSF	(Lin et al., 2011)	<a href="http://compbio.mit.edu/PhyloCSF/">http://compbio.mit.edu/PhyloCSF/</a>
RNAfold web server		<a href="http://rna.tbi.univie.ac.at/">http://rna.tbi.univie.ac.at/</a>
Prism	GraphPad	Ver 6

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### Supplementary tables

Table S1. Divergent lncRNAs identified in this study.

Table S2. Significantly-enriched proteins in *LncKdm2b*-precipitating extracts compared to the antisense-*LncKdm2b*.

Table S3. Statistical analyses of electroporated cortices.

Table S4. Sequences for all primers used in this study.

### Supplementary figure titles and legends

**Figure S1. *LncKdm2b* and *Kdm2b* are transiently expressed in the developing mouse embryonic cortex.**

(A) Gene ontology (GO) analysis of coding genes associated with divergent lncRNAs.

The top GO terms (>1.5-fold and  $P < 1 \times 10^{-6}$ ) are shown.

(B) Protein-coding scores of *LncKdm2b* using CPAT and PhyloCSF programs.

(C) Thirteen putative ORFs of *LncKdm2b* were cloned into the pFLAG-N3 vector with

3 × Flag tag sequence fused to their 3' for HEK293T cell transfection. After 48 hours, immunoblotting was performed to detect Flag-tagged proteins. PRRX1B tagged with 3 × Flag tag served as a positive control. Data are representative of three independent experiments.

(D) Fragments per kilobase per million mapped reads (FPKM) values for *LncKdm2b*, and *Kdm2b* in mouse ESCs, mouse NPCs and mouse cortices at indicated developmental stages.

(E-L) RT-qPCR analyses of indicated markers of E10.5, E12.5, E14.5 and adult (6 weeks) mouse dorsal forebrains. The y-axis represents relative expression normalized to *Gapdh*.

(M) Representative immunoblotting of mouse dorsal forebrains using antibodies against KDM2B and  $\beta$ -TUBULIN.

(N) Northern blots of *LncKdm2b* and *Gapdh* using poly(A) RNAs extracted from mouse dorsal forebrains.

(O) Schematic illustration of *in situ* hybridization probes for mouse *LncKdm2b* and *Kdm2b*.

(P) *In situ* hybridization (ISH) of *LncKdm2b* on coronal sections of E12.5 mouse forebrain (the CD-1 strain). Scale bars, 100  $\mu$ m. The higher-magnification image of the boxed area shows immunofluorescent staining for TBR2 (green) and TUJ1 on ISH section of *LncKdm2b* (red). Scale bars, 50  $\mu$ m.

(Q) Southern blot analysis of genomic DNA from wild-type (WT) or *Kdm2b*<sup>CreERT2/+</sup>

knock-in mice.

(R-S) Immunofluorescent staining for EGFP (green), TBR2 (red), TUJ1 (blue), UNC5D (red), and DAPI (blue) on cortical sections of E14.5 *Kdm2b<sup>CreERT2/+</sup>* mice. Boxed areas are enlarged at the bottom-right corners. Scale bars, 50  $\mu$ m.

In (E-L), quantification data are shown as mean + SD (n = 3 unless otherwise indicated).

Ctx, cortex; LV, lateral ventricle; VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone.

**Figure S2. *Kdm2b*-expressing cortical cells are fated to be cortical projection neurons.**

(A-D) Immunofluorescent staining for SATB2 and CTIP2 on coronal cortical sections of E16.5 (A) and P0 (C) *Kdm2b<sup>CreERT2/+</sup>;Ai14* embryos. Tamoxifen (TAM, 100 mg/kg) was injected at E12.5 or E14.5 respectively. Boxed areas are enlarged at the bottom-right corners. Quantification of SATB2 or CTIP2 expression in tdTomato<sup>+</sup> recombined cells (B and D). A total of 3372 cells from 2 embryos were analyzed in (B), and 2282 cells from 2 animals in (D).

(E) Immunofluorescent staining for SOX2 and TUJ1 on head sections of E10.5 *Kdm2b<sup>CreERT2/+</sup>;Ai14* embryo. Boxed areas are enlarged at the bottom-right corners.

(F) Immunofluorescent staining for PAX6 (top) and TBR2 (bottom) on head sections of E13.5 *Kdm2b<sup>CreERT2/+</sup>;Ai14* embryo. Tamoxifen (TAM, 100 mg/kg) was injected at E12.5. Boxed areas are enlarged at the bottom-right corners.

(G-H) Immunofluorescent staining for SATB2 and CTIP2 on coronal cortical sections of P7 *Kdm2b<sup>CreERT2/+</sup>;Ai14* mouse brain. Boxed areas are enlarged at the bottom-right corners. Quantification of SATB2 or CTIP2 expression in tdTomato<sup>+</sup> recombined cells (H). A total of 373 cells were analyzed.

In (B), (D), and (H), quantification data are shown as mean + SEM.

LV, lateral ventricle; MB, midbrain. Scale bars, 50  $\mu$ m.

**Figure S3. *LncKdm2b* maintains *Kdm2b* transcription in *cis*.**

(A) Percentages of marker-expressing neuronal cells in adherent cultures derived from E12.5 cortices.

(B) RT-qPCR analysis of *LncKdm2b* and *Kdm2b* RNA levels in adherent cultures derived from E12.5 cortices. The cultures were treated with indicated ASOs. The y-axis represents relative expression normalized to *Gapdh*.

(C) RT-qPCR analysis of *Zfp292* mRNA levels in Neuro-2a cells treated with indicated *LncKdm2b* ASOs for three days. The y-axis represents relative expression normalized to *Gapdh*.

(D) Genotyping of NE-4C clones with *LncKdm2b*'s exon2 knocked out.

(E) RT-qPCR analysis of *Zfp292* mRNA levels in NE-4C clones with *LncKdm2b*'s exon2 knocked out. The y-axis represents relative expression normalized to *Gapdh*.

(F) RT-qPCR analysis for *LncKdm2b*, *Gapdh*, *Actb*, *Xist*, and *Neat2* from cytoplasmic and nuclear RNA fractions of primary E14.5 cortical neural progenitor cells (NPCs)

cultured *in vitro* for four days.

(G) Fluorescent *in situ* hybridization of *LncKdm2b* on cortical NPCs treated with or without RNase A. The nuclei were counter-stained with DAPI.

(H-I) RT-qPCR analysis of *LncKdm2b* and *Kdm2b* mRNA levels in Neuro-2a and NE-4C cells transfected with empty or *LncKdm2b*-expressing vectors for 48 hours. The y-axis represents relative expression normalized to *Gapdh* (n = 5).

(J) RT-qPCR analysis of *LncKdm2b* and *Kdm2b* mRNA levels in parental and *LncKdm2b*-KO NE-4C cells transfected with empty or *LncKdm2b*-expressing vectors for 48 hours. The y-axis represents relative expression normalized to *Gapdh*.

(K) RT-qPCR analysis of *LncKdm2b* and *Kdm2b* RNA levels in Neuro-2a cells treated for two days with Scramble ASOs or ASOs targeting *Kdm2b*.

In (A) and (H-I), quantification data are shown as mean + SEM. In (B-C), (E-F), and (J-K) quantification data are shown as mean + SD (n = 3 unless otherwise indicated).

In (B) and (H-I), statistical significance was determined using 2-tailed Student's *t* test.

In (C), (E), and (J-K), statistical significance was determined using 1-way ANOVA followed by the Turkey's *post hoc* test. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001, "NS" indicates no significance.

**Figure S4. *LncKdm2b* maintains *Kdm2b* transcription in *cis*.**

(A) Relative crosslinking frequency between the T5 and *Kdm2b*'s TSS measured by 3C-qPCR in Neuro-2a cells treated for two days with Scramble ASOs or ASOs

targeting *LncKdm2b*. The y-axis shows fold enrichment normalized to the scramble control.

(B) Luciferase activities in experiments where indicated vectors were transfected into HEK293T cells for 24 hours. 'Forward' and 'Reverse' indicate directions same as or opposite of *Kdm2b*'s transcription orientation.

(C) Genotyping of NE-4C cells with the T5 region knocked out.

(D) RT-qPCR analysis of *Zfp292* mRNA levels in NE-4C clones with the T5 region knocked out. The y-axis represents relative expression normalized to *Gapdh*.

(E) Genotyping of cortical cells subjected to Cas9-mediated knockout of the T5 region.

In (A-B) and (D), quantification data are shown as mean + SD (n = 3 unless otherwise indicated). In (A-B) and (D), statistical significance was determined using 1-way ANOVA followed by the Turkey's *post hoc* test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , "NS" indicates no significance.

### **Figure S5. Characterization of *LncKdm2b*-associated proteins.**

(A) The illustration describing the Gal4- $\lambda$ N/*BoxB* RNA tethering system.

(B-D) Relative luciferase activities in experiments where Neuro-2a cells were transfected with plasmids expressing *BoxB*-tagged *LacZ*, full-length *LncKdm2b*, 5' *LncKdm2b*, or 3' *LncKdm2b* along with Gal4- $\lambda$ N and 5xUAS-TK-Luciferase-expressing plasmids for 24 hours.

(E) RT-qPCR analysis of *Kdm2b* mRNA levels in Neuro-2a cells treated for three days

with scramble siRNA (siNC) or siRNA targeting indicated molecules. The y-axis represents relative expression normalized to *Gapdh*.

(F) Fluorescent *in situ* hybridization of *LncKdm2b* on cortical NPCs followed by co-staining of hnRNPAB and DAPI.

(G) RNA secondary structure prediction by *RNAfold* showed two putative stem-loop structures.

(H) CHIP-qPCR analysis of indicated primer sets enriched by anti-hnRNPAB antibodies in Neuro-2a cells. The y-axis shows fold enrichment normalized to the IgG control.

In (B–E) and (H), quantification data are shown as mean + SD (n = 3 unless otherwise indicated). In (B–E), statistical significance was determined using 1-way ANOVA followed by the Turkey's *post hoc* test. In (H), statistical significance was determined using 2-tailed Student's t test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , "NS" indicates no significance.

**Figure S6. *Kdm2b* promotes cortical neurogenesis.**

(A) Representative immunoblots of HEK293T cells transfected with empty vector or KDM2B-expressing vector for two days using antibodies against KDM2B and ACTIN.

(B) Immunoblotting of E15.5 embryonic cortices with indicated genotypes using antibodies against KDM2B and  $\beta$ -TUBULIN.

(C-D) Quantification of relative location (C) and percentiles (D) of NEUROD2<sup>+</sup> transduced cells in scramble or KDM2B shRNA electroporated sections. Three embryos each.

(E-F) E13.5 mouse cortices were electroporated with indicated combination of vectors, with transduced cells labeled with EGFP. Embryos were sacrificed at E16.5 for immunofluorescent analysis. Representative VZ/SVZ images of sections immunostained with PAX6 (E) and quantification of PAX6<sup>+</sup> transduced cells (F) were shown. Three embryos in control and shKDM2B, five embryos in shKDM2B plus KDM2B. Scale bars, 50  $\mu$ m.

(G) Quantification of Cleaved Caspase3<sup>+</sup> transduced cells in scramble or KDM2B shRNA electroporated sections. Three embryos each.

In (C-D) and (F-G), quantification data are shown as mean + SEM. In (C-D) and (G), statistical significance was determined using 2-tailed Student's *t* test. In (F), statistical significance was determined using 1-way ANOVA followed by the Turkey's *post hoc* test. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001, "NS" indicates no significance.

VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate.

**Figure S7. *LncKdm2b* maintains mouse cortical neurogenesis through KDM2B.**

(A) E13.5 mouse cortices were electroporated with Scramble ASO or ASOs targeting *LncKdm2b*, with transduced cells labeled with EGFP. RT-qPCR analysis of *LncKdm2b*

and *Kdm2b* mRNA levels in EGFP<sup>+</sup> and EGFP<sup>-</sup> cells from E15.5 electroporated cortices. The y-axis represents relative expression normalized to *Gapdh*.

(B) Quantification of Cleaved Caspase3<sup>+</sup> transduced cells in Scramble or *LncKdm2b* ASO electroporated cortices. Three embryos each.

(C-E) E13.5 mouse cortices were electroporated with indicated siRNAs, with transduced cells labeled with EGFP. Embryos were sacrificed at E16.5 for PAX6 immunofluorescent stainings (C). The relative location of EGFP<sup>+</sup> cells (D) and percentiles of PAX6<sup>+</sup> transduced cells (E) were quantified. Three embryos in control (siNC), five embryos in sihnRNPA2B1. Scale bars, 50  $\mu$ m.

(F-G) E13.5 mouse cortices were electroporated with empty or *LncKdm2b*-expressing vector, along with mCherry-expressing vector to label transduced cells. Embryos were sacrificed at E15.5 followed by DAPI staining of coronal sections (F). The locations of mCherry<sup>+</sup> cells were quantified (G). Seven embryos each. Scale bars, 50  $\mu$ m.

In (A), quantification data are shown as mean + SD. In (B), (D-E), and (G), quantification data are shown as mean + SEM. In (A), statistical significance was determined using 2-way ANOVA followed by the Bonferroni's *post hoc* test. In (B), (D-E), and (G), statistical significance was determined using 2-tailed Student's *t* test. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001, "NS" indicates no significance.

VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate.

**Figure S8. *LncKdm2b* regulates cortical neuronal differentiation and migration.**

(A) A schematic illustration of the piggyBac-CRISPR/Cas9 toolkit for *LncKdm2b* knockout by *in utero* electroporation. Two sgRNAs were used for deletion of the *LncKdm2b*'s second exon.

(B) Quantification of neuron to glia ratio in brains at P10. Four brains each.

(C) Quantification of the distribution of SATB2<sup>+</sup>EGFP<sup>+</sup> cells in cortices at P10. Four brains each.

(D) Quantification of Cleaved Caspase3<sup>+</sup> transduced cells. Three brains each.

In (B-D), quantification data are shown as mean + SEM. In (B-D), statistical significance was determined using 2-tailed Student's *t* test. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001, "NS" indicates no significance.

**REFERENCES**

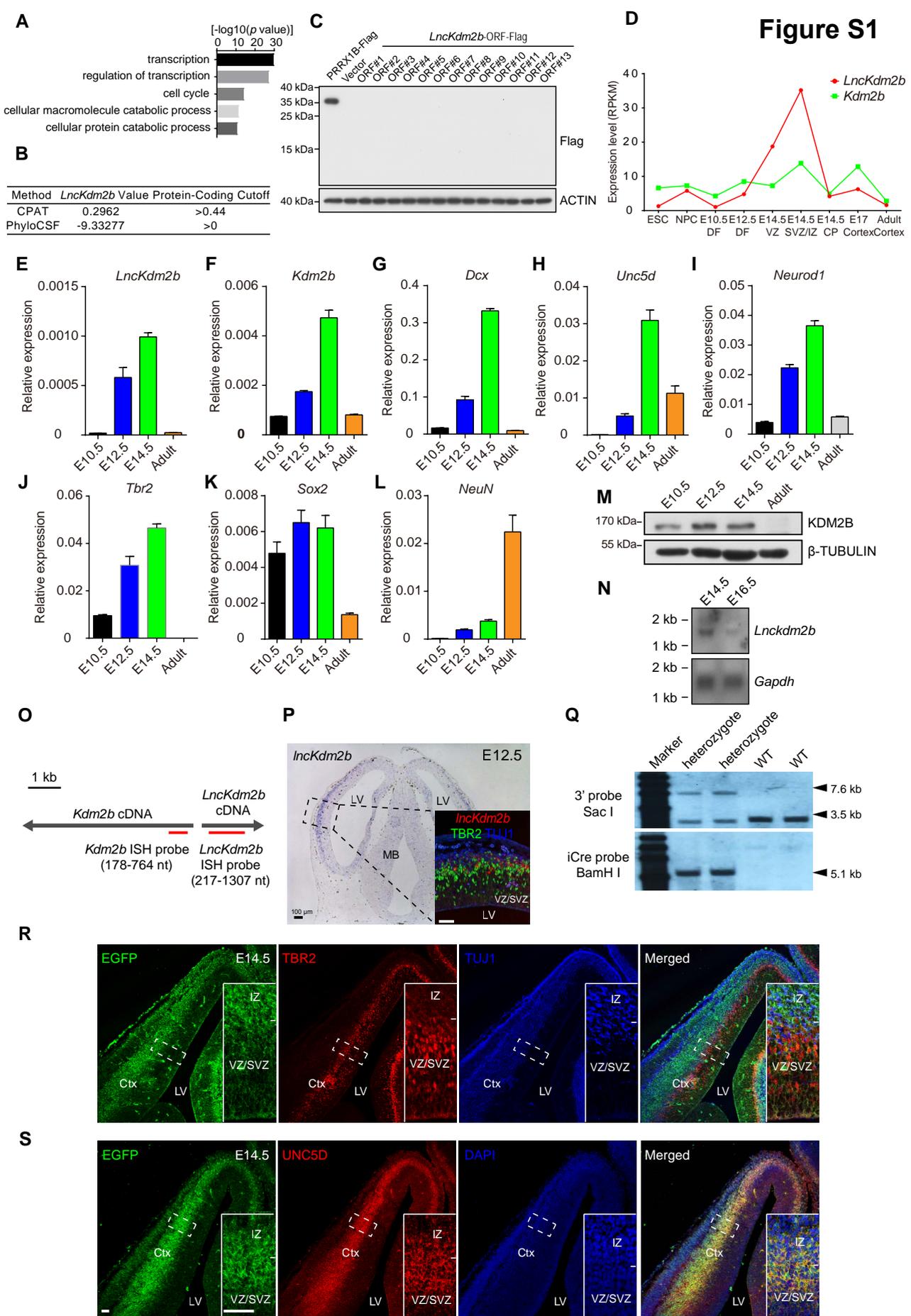
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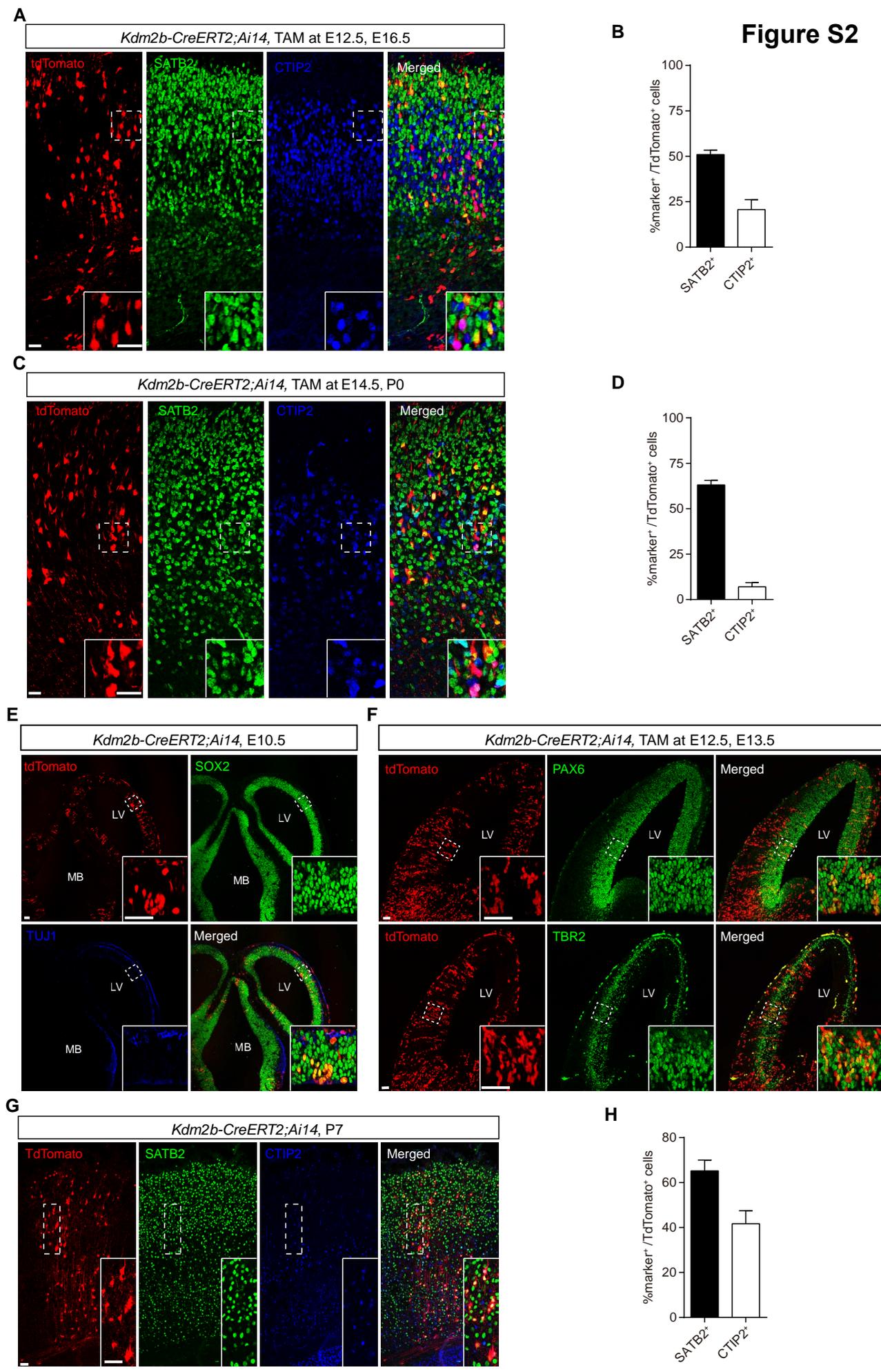
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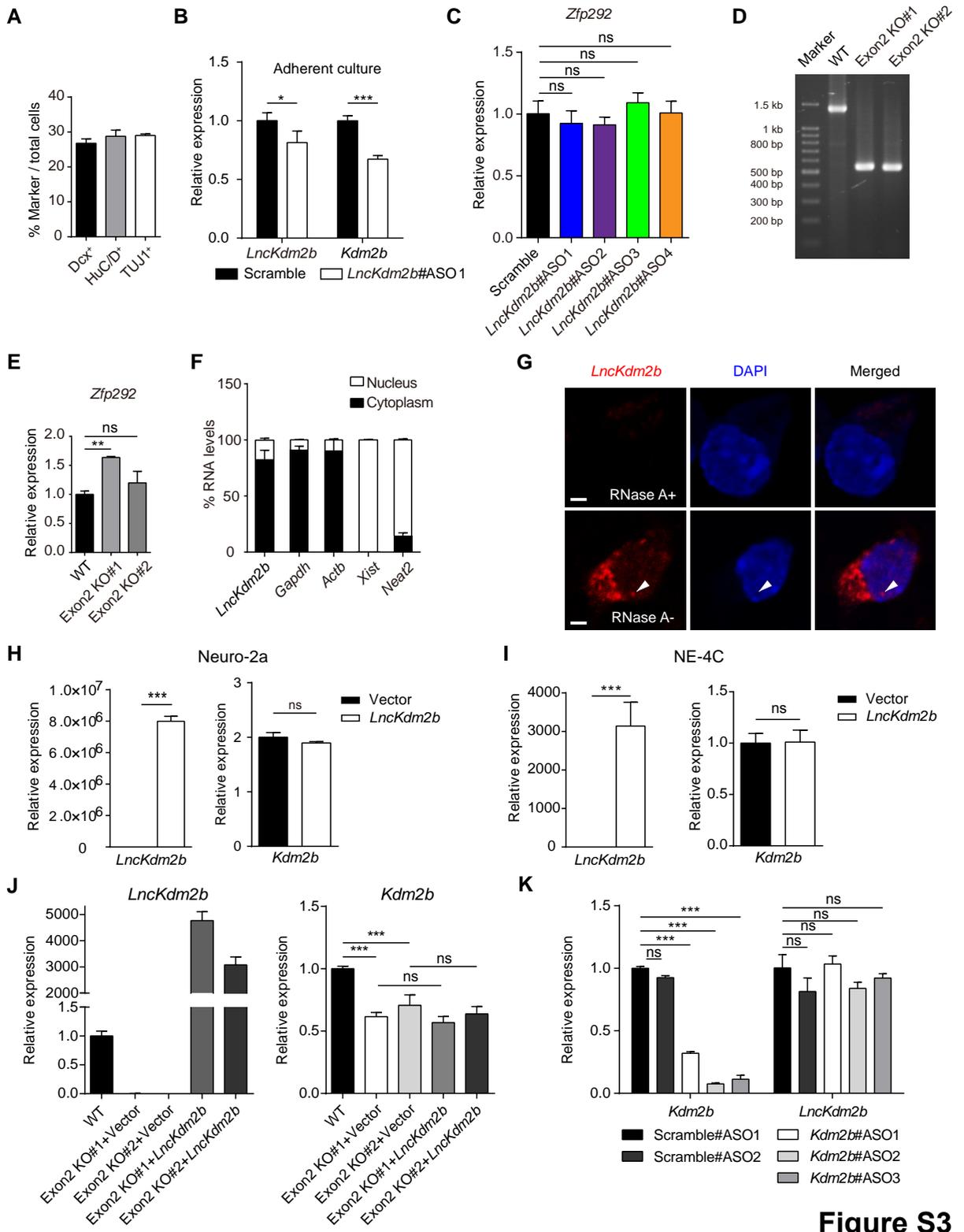
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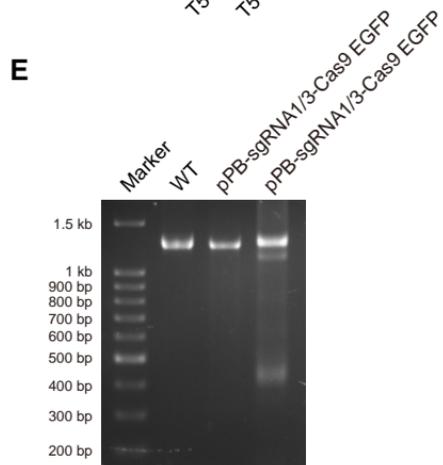
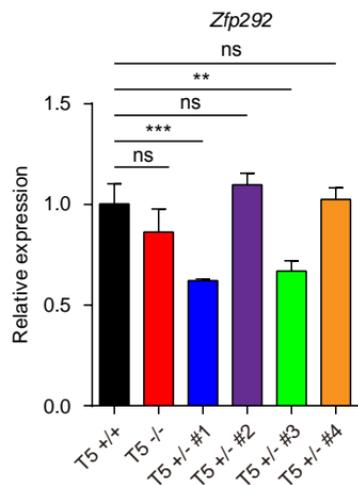
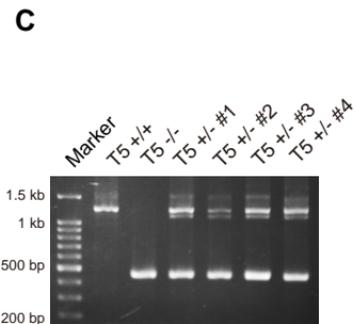
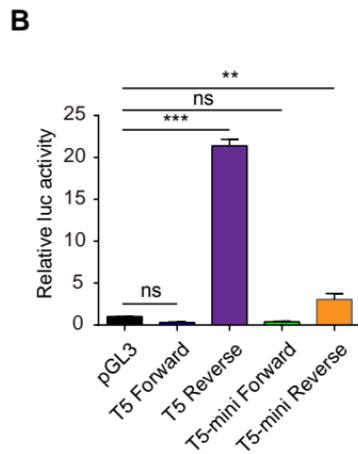
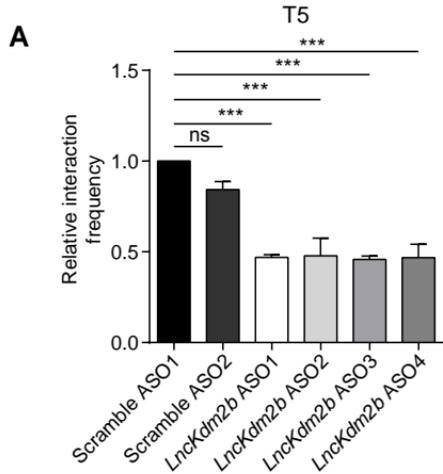
Wang, L., Park, H.J., Dasari, S., Wang, S., Kocher, J.P., and Li, W. (2013) CPAT: Coding-Potential Assessment Tool using an alignment-free logistic regression model. Nucleic Acids Res 41:e74



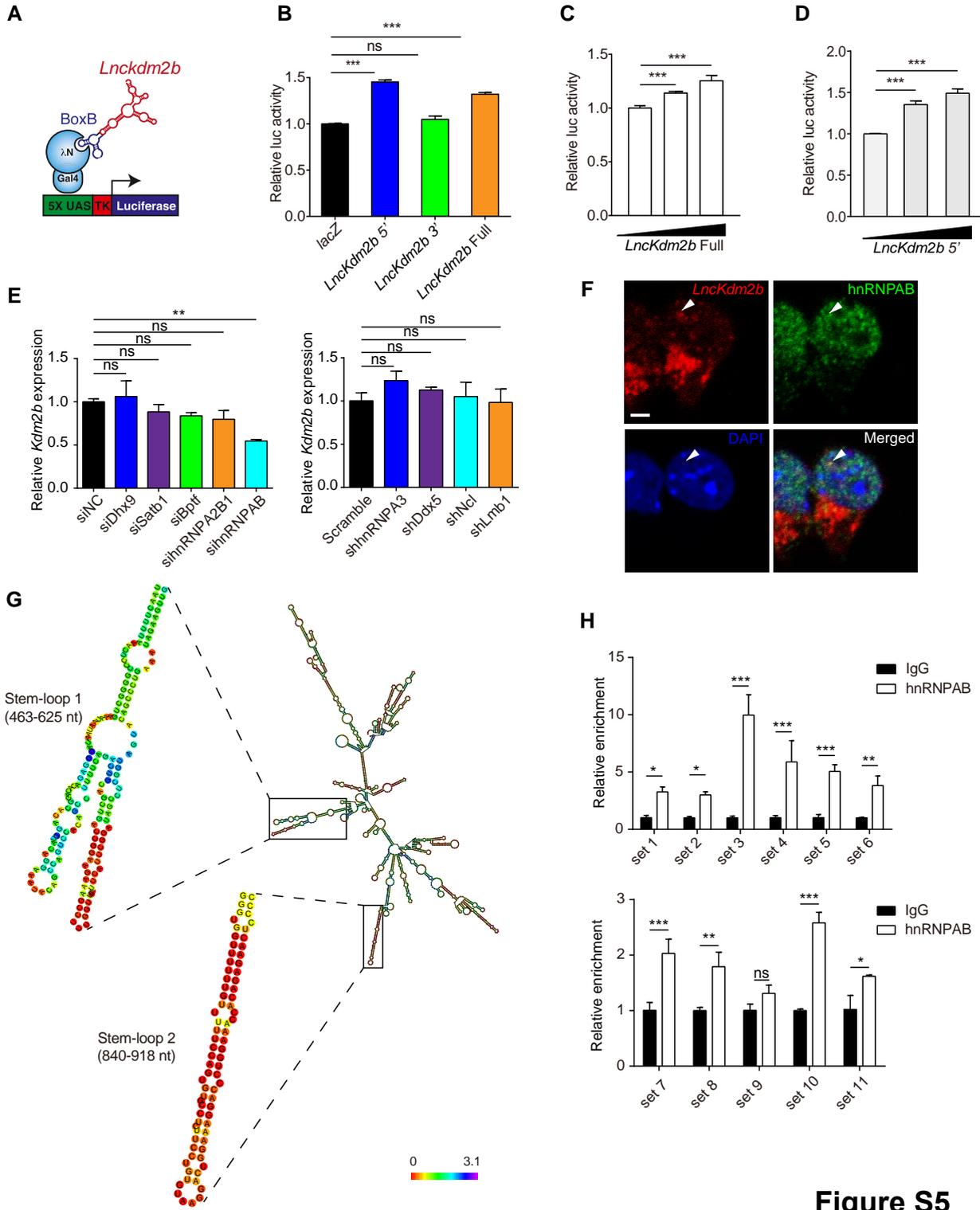
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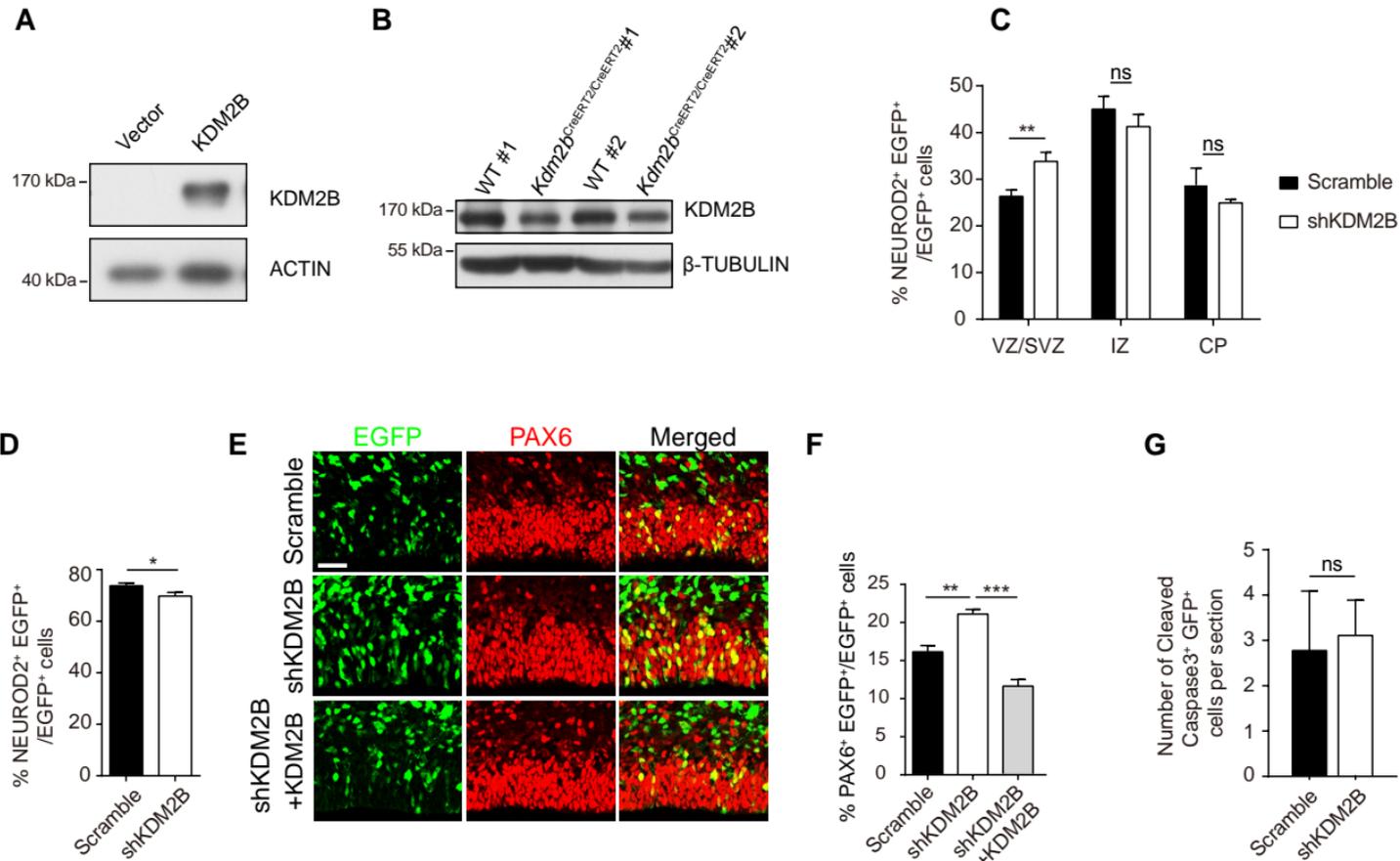
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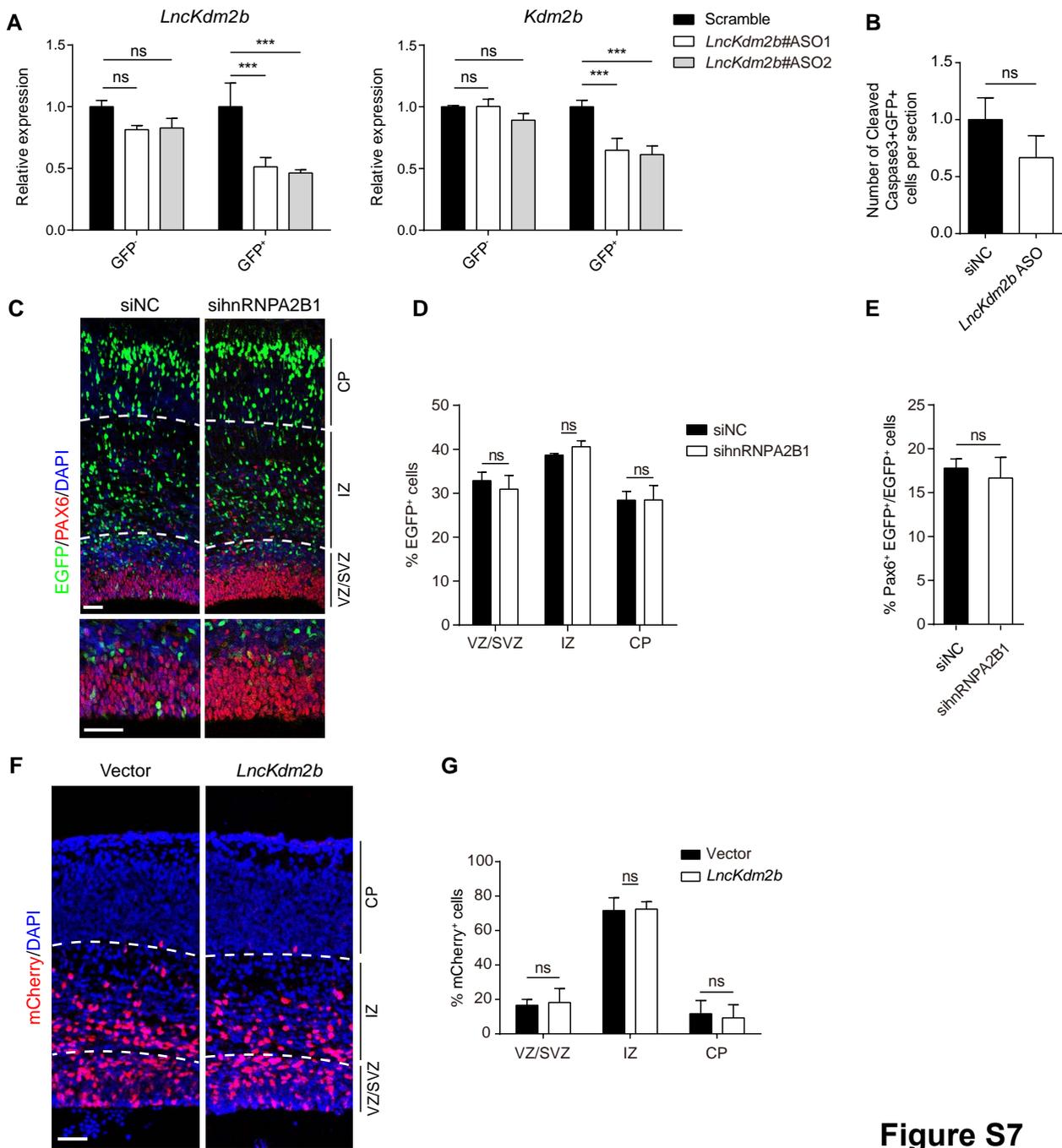
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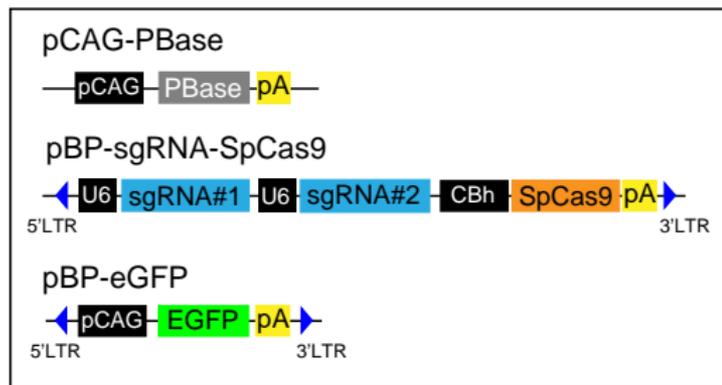
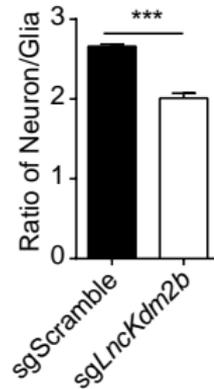
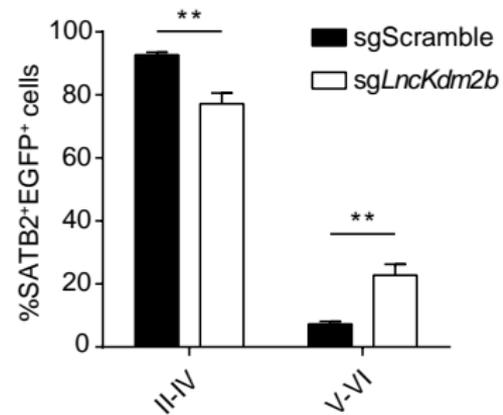
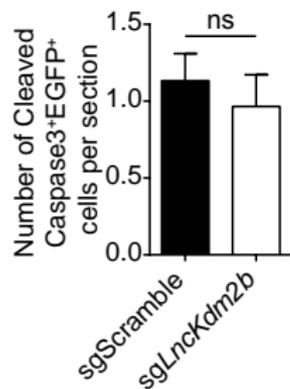
**Figure S5**



**Figure S6**



**Figure S7**

**A****B****C****D****Figure S8**