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Supplemental Information

The Autism-Related Protein SETD5 Controls Neural Cell Proliferation through Epigenetic Regulation of rDNA Expression

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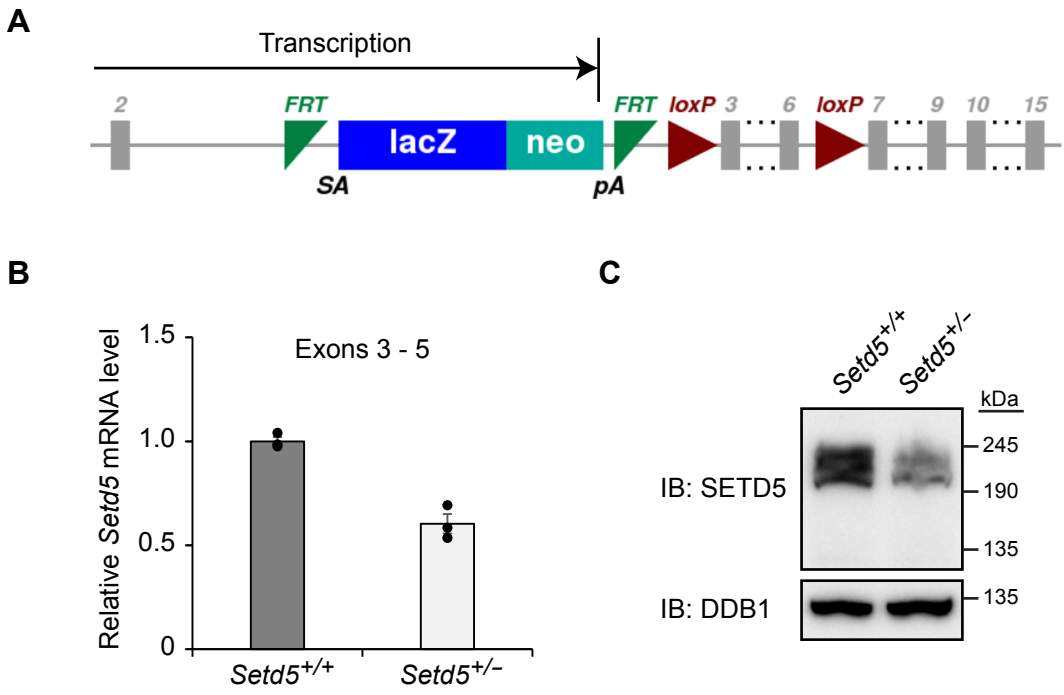
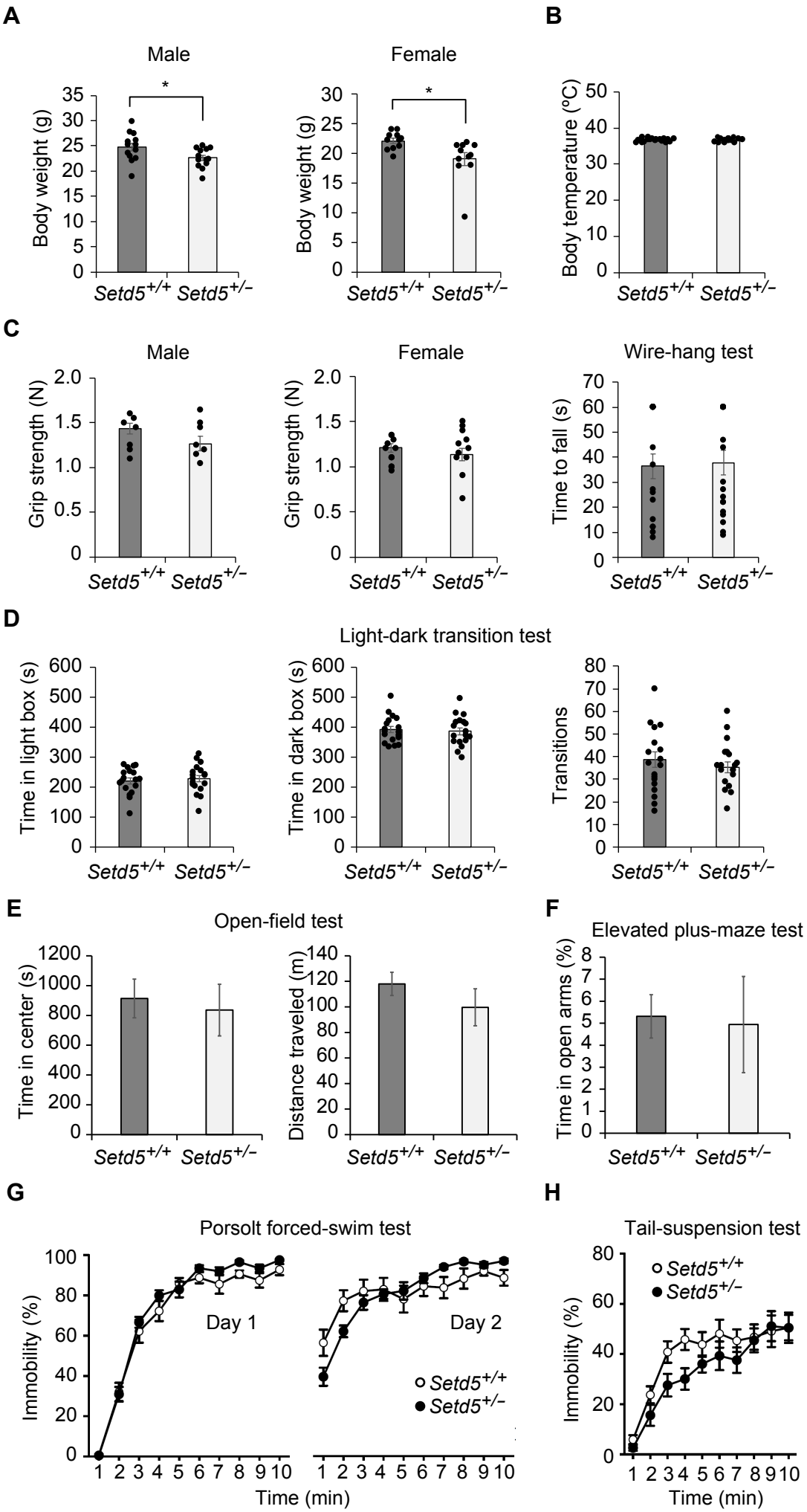
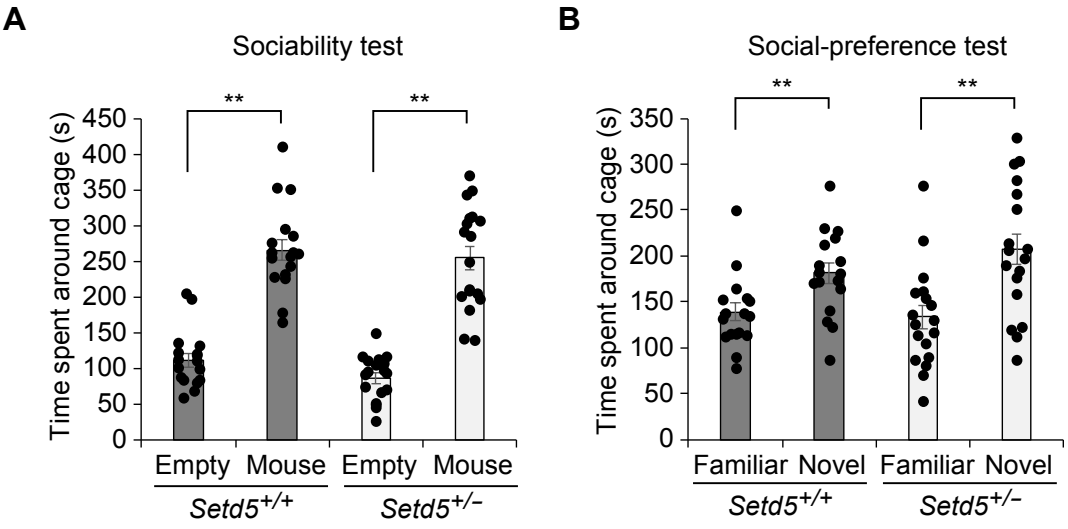
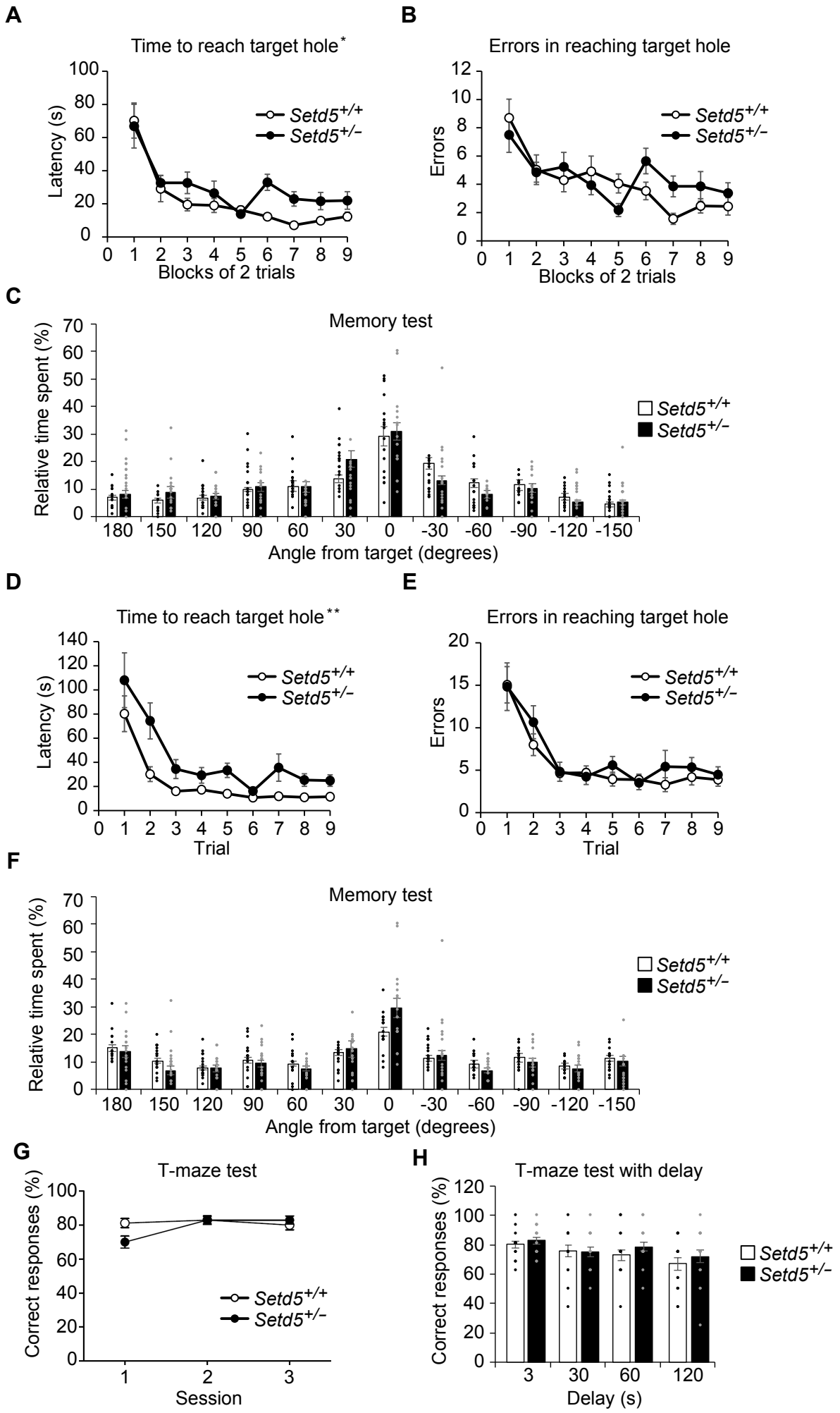

A
Transcription
2
FRT
lacZ
neo
SA
pA
FRT
loxP
3
6
loxP
7
9
10
15
B
Exons 3 - 5
Relative Setd5 mRNA level
1.5
1.0
0.5
0
Setd5+/+
Setd5+/−
C
Setd5+/+
Setd5+/−
kDa
IB: SETD5
IB: DDB1
245
190
135
135


A
Male
Female
Body weight (g)
*
Setd5+/+
Setd5+/–
B
Body temperature (ºC)
C
Male
Female
Wire-hang test
Grip strength (N)
Time to fall (s)
D
Light-dark transition test
Time in light box (s)
Time in dark box (s)
Transitions
E
Open-field test
Time in center (s)
Distance traveled (m)
F
Elevated plus-maze test
Time in open arms (%)
G
Porsolt forced-swim test
Immobility (%)
Day 1
Day 2
Time (min)
H
Tail-suspension test


A
Sociability test
Time spent around cage (s)
450
400
350
300
250
200
150
100
50
0
**
**
Empty
Mouse
Empty
Mouse
Setd5+/+
Setd5+/−
B
Social-preference test
Time spent around cage (s)
350
300
250
200
150
100
50
0
**
**
Familiar
Novel
Familiar
Novel
Setd5+/+
Setd5+/−

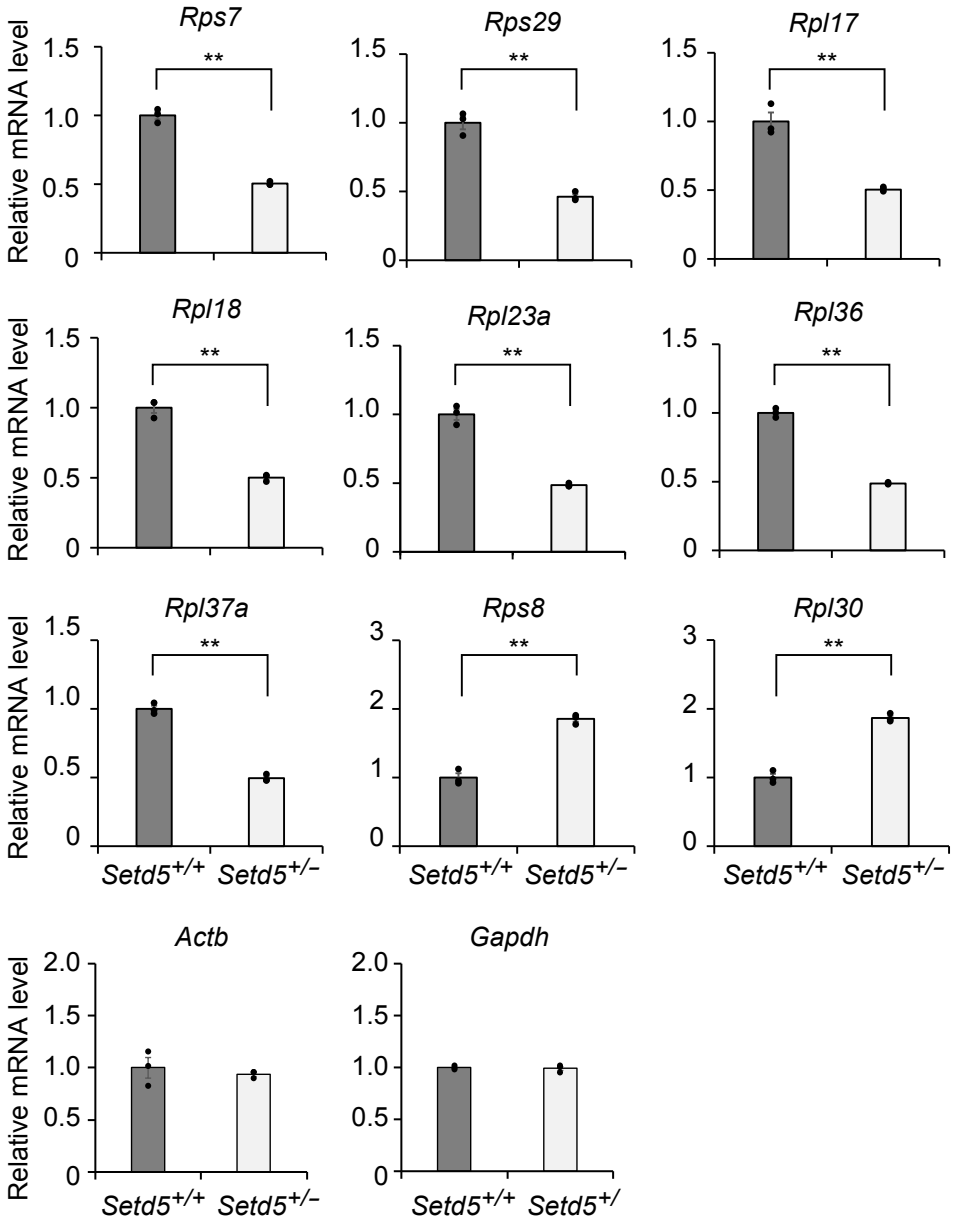

A
Time to reach target hole*
Latency (s)
Blocks of 2 trials
Setd5+/+
Setd5+/−
B
Errors in reaching target hole
Errors
Blocks of 2 trials
C
Memory test
Relative time spent (%)
Angle from target (degrees)
D
Time to reach target hole**
Latency (s)
Trial
E
Errors in reaching target hole
Errors
Trial
F
Memory test
Relative time spent (%)
Angle from target (degrees)
G
T-maze test
Correct responses (%)
Session
H
T-maze test with delay
Correct responses (%)
Delay (s)

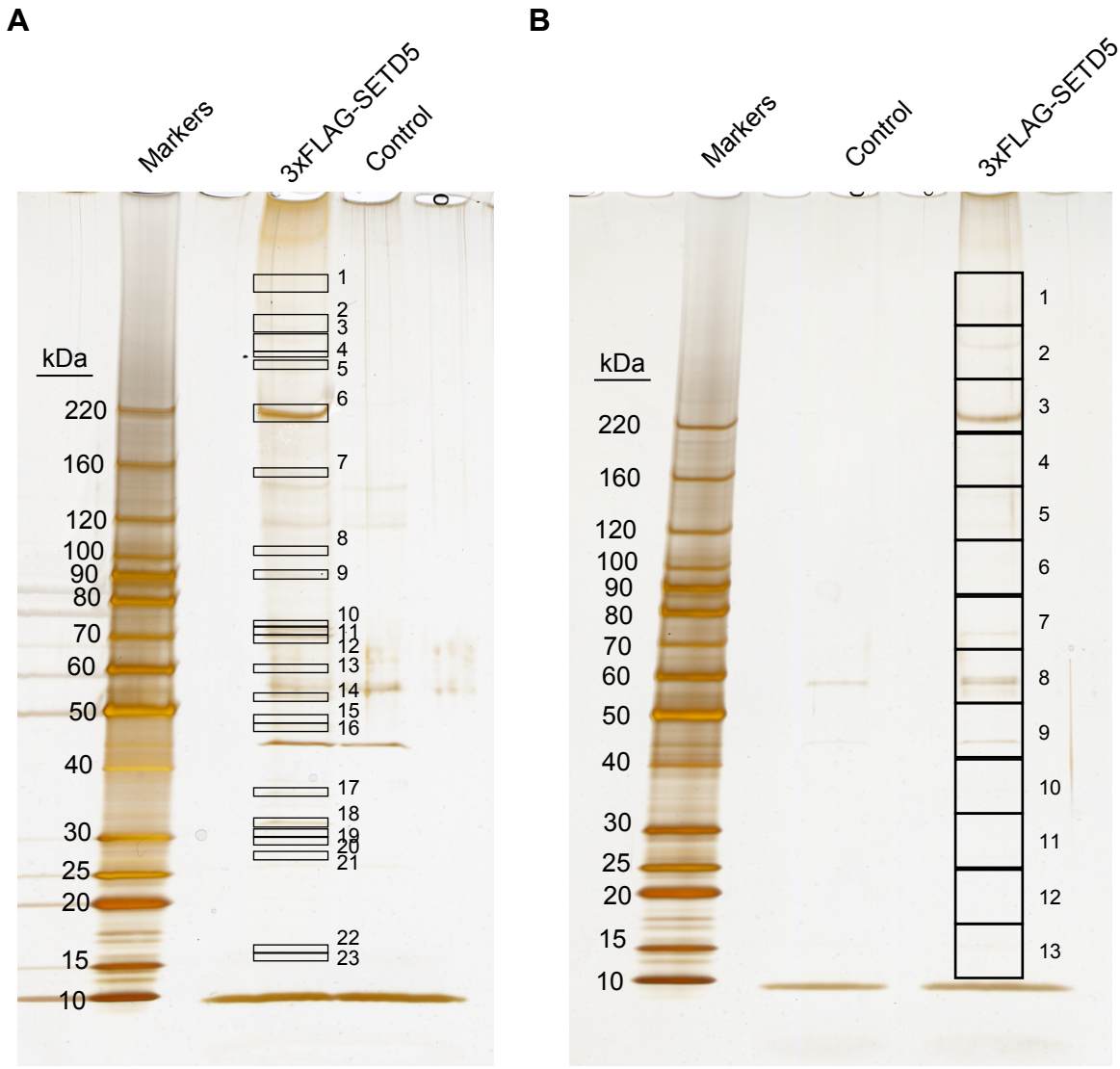
A

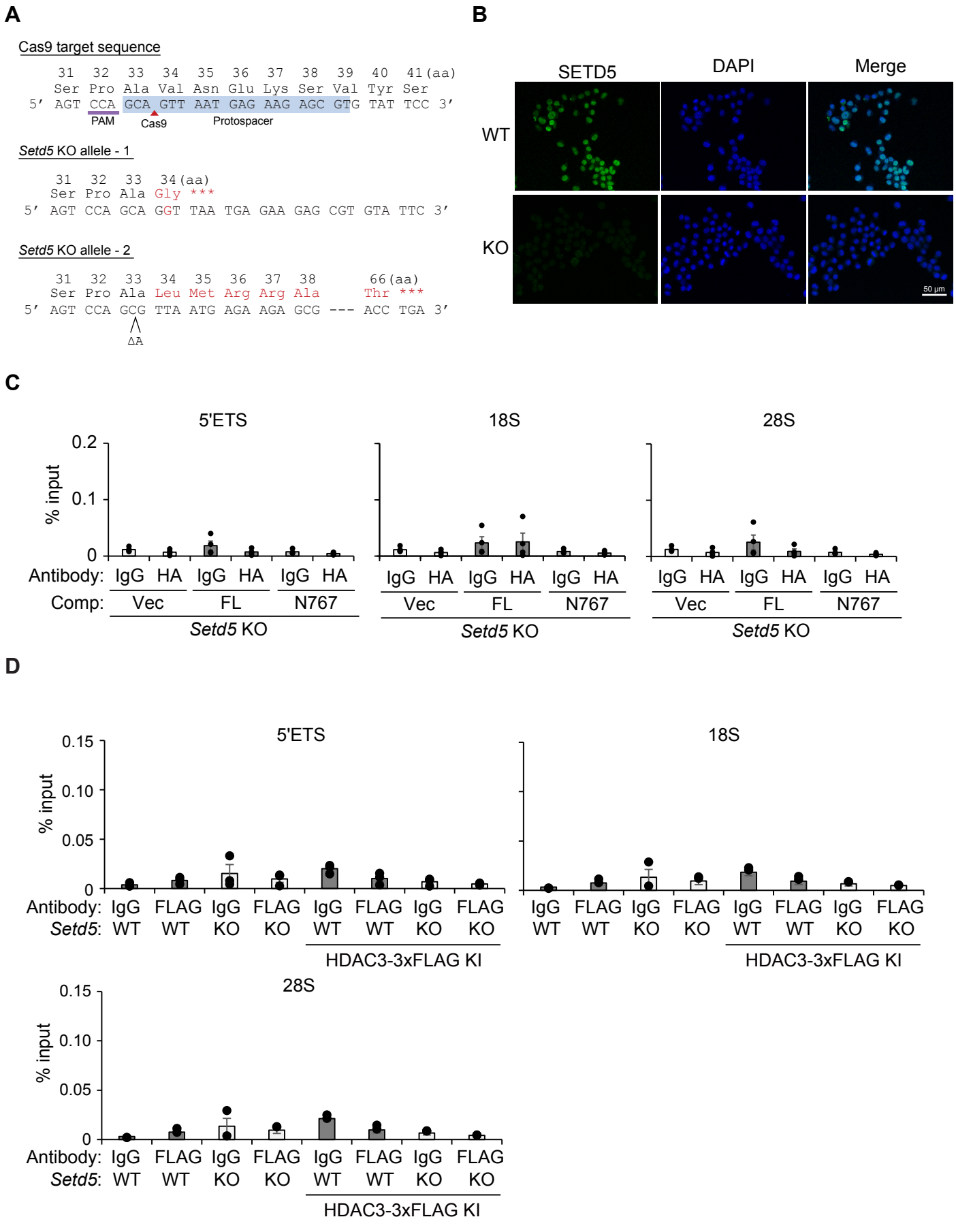
Up-regulated genes in *Setd5*$^{+/-}$ brain (top 5%)

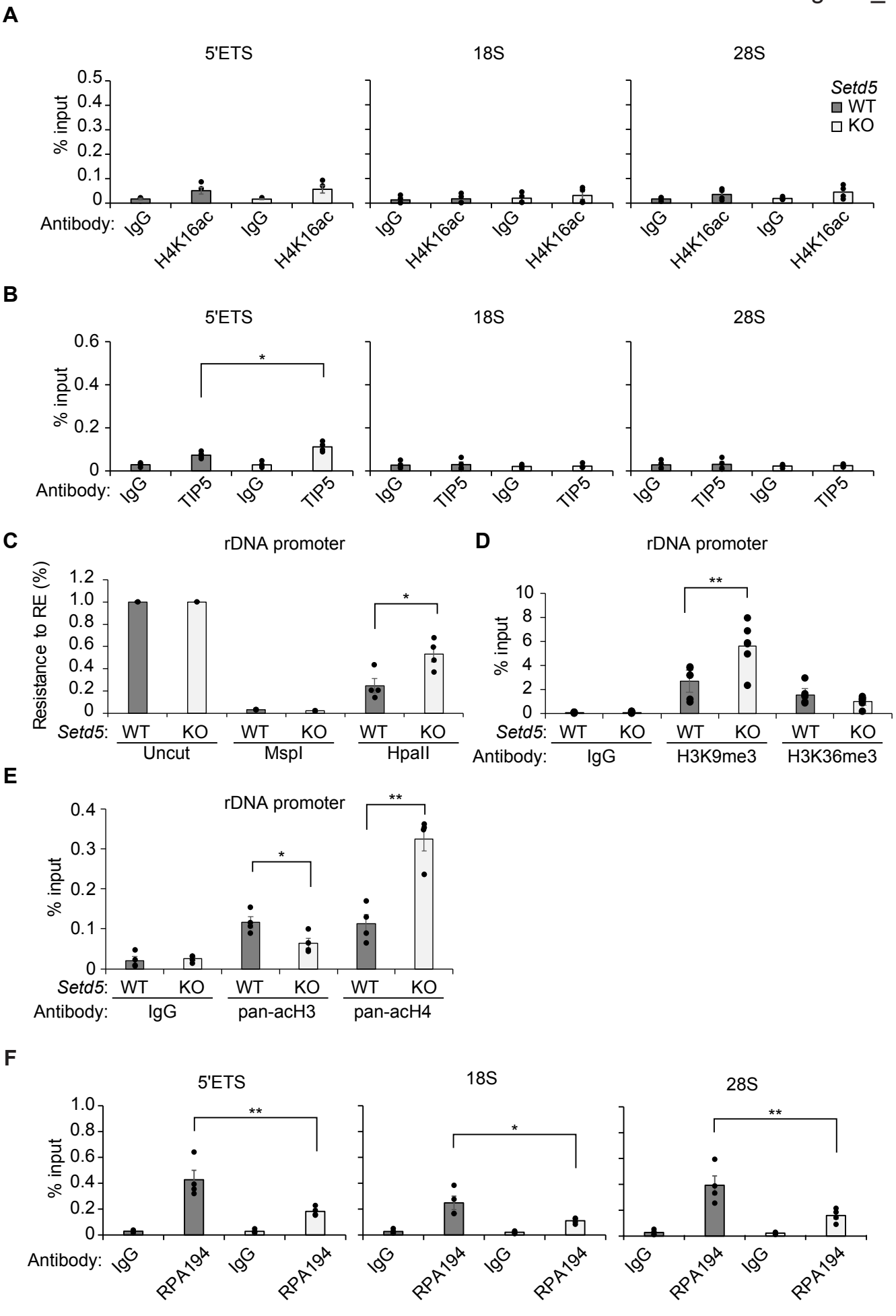
Term name	p value	FDR
GO Molecular Function		
metal ion binding	1.10E-10	6.21E-08
RNA polymerase II regulatory region sequence-specific DNA binding	2.82E-06	4.55E-04
transcription factor binding	5.63E-06	8.19E-04
core promoter binding	9.92E-06	1.21E-03
transcriptional activator activity, RNA polymerase II proximal promoter sequence-specific DNA binding	1.80E-05	1.98E-03
GO Cellular Component		
integral component of plasma membrane	6.37E-09	2.40E-06
cell junction	1.39E-05	1.31E-03
postsynaptic membrane	1.60E-05	1.44E-03
dendrite	7.46E-05	5.41E-03
caveola	1.33E-04	8.38E-03

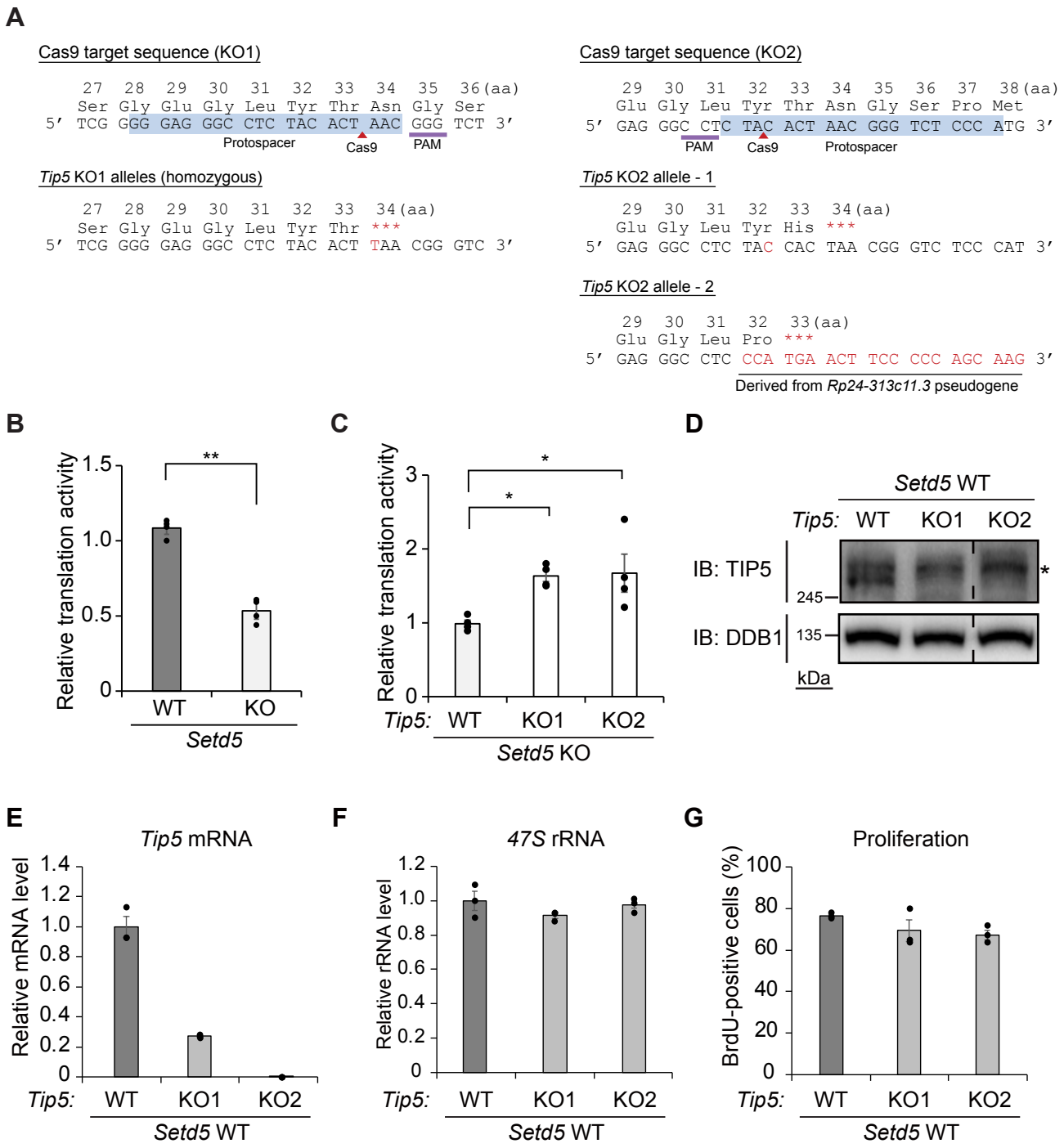
B

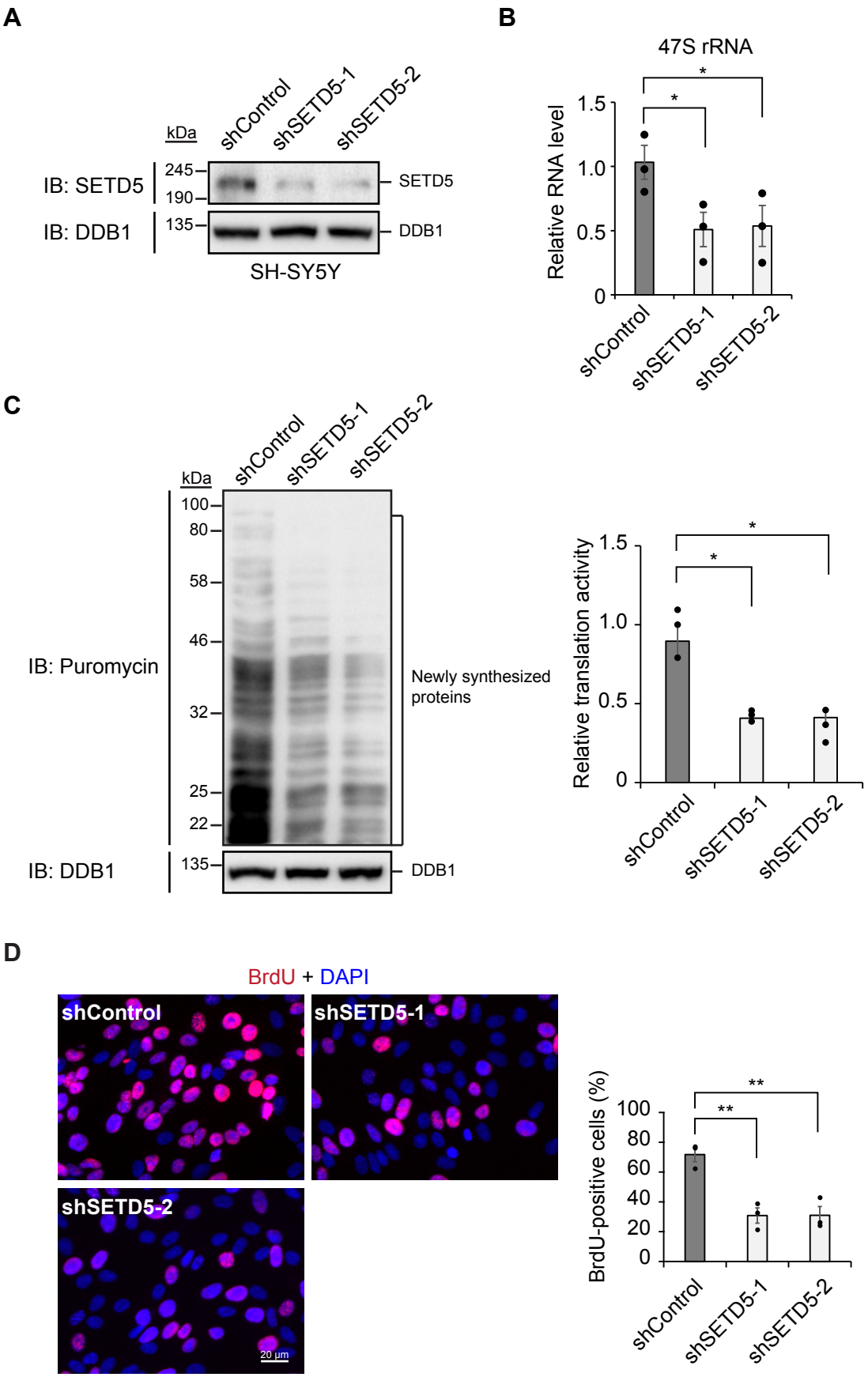



A
Markers
3xFLAG-SETD5
Control
kDa
220
160
120
100
90
80
70
60
50
40
30
25
20
15
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
HEK293T
B
Markers
Control
3xFLAG-SETD5
kDa
220
160
120
100
90
80
70
60
50
40
30
25
20
15
10
1
2
3
4
5
6
7
8
9
10
11
12
13
SH-SY5Y


A
Cas9 target sequence
31 32 33 34 35 36 37 38 39 40 41(aa)
Ser Pro Ala Val Asn Glu Lys Ser Val Tyr Ser
5′ AGT CCA GCA GTT AAT GAG AAG AGC GTG TAT TCC 3′
PAM
Cas9
Protospacer
Setd5 KO allele - 1
31 32 33 34(aa)
Ser Pro Ala Gly ***
5′ AGT CCA GCA GGT TAA TGA GAA GAG CGT GTA TTC 3′
Setd5 KO allele - 2
31 32 33 34 35 36 37 38 66(aa)
Ser Pro Ala Leu Met Arg Arg Ala Thr ***
5′ AGT CCA GCG TTA ATG AGA AGA GCG --- ACC TGA 3′
ΔA
B
SETD5
DAPI
Merge
WT
KO
50 μm
C
5'ETS
18S
28S
% input
0.2
0.1
0
Antibody: IgG HA IgG HA IgG HA
Comp: Vec FL N767
Setd5 KO
D
5'ETS
18S
28S
% input
0.15
0.10
0.05
0
Antibody: IgG FLAG IgG FLAG IgG FLAG IgG FLAG
Setd5: WT WT KO KO WT WT KO KO
HDAC3-3xFLAG KI


A
5'ETS
18S
28S
% input
Setd5
WT
KO
Antibody:
IgG
H4K16ac
B
TIP5
C
rDNA promoter
Resistance to RE (%)
Setd5:
Uncut
MspI
HpaII
D
H3K9me3
H3K36me3
E
pan-acH3
pan-acH4
F
RPA194


A
Cas9 target sequence (KO1)
27 28 29 30 31 32 33 34 35 36(aa)
Ser Gly Glu Gly Leu Tyr Thr Asn Gly Ser
5′ TCG GGG GAG GGC CTC TAC ACT AAC GGG TCT 3′
Protospacer
Cas9
PAM
Tip5 KO1 alleles (homozygous)
27 28 29 30 31 32 33 34(aa)
Ser Gly Glu Gly Leu Tyr Thr ***
5′ TCG GGG GAG GGC CTC TAC ACT TAA CGG GTC 3′
Cas9 target sequence (KO2)
29 30 31 32 33 34 35 36 37 38(aa)
Glu Gly Leu Tyr Thr Asn Gly Ser Pro Met
5′ GAG GGC CTC TAC ACT AAC GGG TCT CCC ATG 3′
PAM
Cas9
Protospacer
Tip5 KO2 allele - 1
29 30 31 32 33 34(aa)
Glu Gly Leu Tyr His ***
5′ GAG GGC CTC TAC CAC TAA CGG GTC TCC CAT 3′
Tip5 KO2 allele - 2
29 30 31 32 33(aa)
Glu Gly Leu Pro ***
5′ GAG GGC CTC CCA TGA ACT TCC CCC AGC AAG 3′
Derived from Rp24-313c11.3 pseudogene
B
Relative translation activity
1.5
1.0
0.5
0
**
WT
KO
Setd5
C
Relative translation activity
3
2
1
0
*
*
Tip5: WT KO1 KO2
Setd5 KO
D
Setd5 WT
Tip5: WT KO1 KO2
IB: TIP5
IB: DDB1
245
135
kDa
*
E
Tip5 mRNA
Relative mRNA level
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Tip5: WT KO1 KO2
Setd5 WT
F
47S rRNA
Relative rRNA level
1.2
1.0
0.8
0.6
0.4
0.2
0
Tip5: WT KO1 KO2
Setd5 WT
G
Proliferation
BrdU-positive cells (%)
100
80
60
40
20
0
Tip5: WT KO1 KO2
Setd5 WT


A
shControl
shSETD5-1
shSETD5-2
kDa
IB: SETD5
245
190
SETD5
IB: DDB1
135
DDB1
SH-SY5Y
B
47S rRNA
Relative RNA level
1.5
1.0
0.5
0
shControl
shSETD5-1
shSETD5-2
C
kDa
100
80
58
46
32
25
22
IB: Puromycin
Newly synthesized proteins
IB: DDB1
135
DDB1
Relative translation activity
D
BrdU + DAPI
shControl
shSETD5-1
shSETD5-2
20 μm
BrdU-positive cells (%)
100
80
60
40
20
0

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Deletion of *Setd5* in Mice, Related to Figure 1

(A) Schematic diagram of the disrupted *Setd5* allele, showing insertion of a lacZ-neo cassette flanked by FRT sequences in intron 2 as well as loxP sites flanking exons 3 to 6. Transcription is terminated by a poly(A) addition sequence (pA) located in the neo gene. SA, splicing acceptor.

(B) Reverse transcription (RT) and quantitative polymerase chain reaction (qPCR) analysis of *Setd5* mRNA in adult $Setd5^{+/+}$ or $Setd5^{+/-}$ mouse brain. PCR primers were targeted to exons 3 to 5. Data are means ± SEM (n = 3 per genotype).

(C) Immunoblot (IB) analysis of SETD5 protein and DDB1 (loading control) in adult $Setd5^{+/+}$ or $Setd5^{+/-}$ mouse brain.

Figure S2. General Health of and Absence of Anxiety- or Depression-like Behavior in $Setd5^{+/-}$ Mice, Related to Figure 1

(A) Body weight at 10 weeks of age. A significant sex difference ($p < 0.01$, one-way ANOVA) in body weight was detected, and so male and female mutant mice were compared separately with WT controls. Data are means ± SEM (n = 13 males and n = 11 females for each genotype). *$p < 0.05$ (one-way ANOVA).

(B) Body temperature at 10 weeks of age. Data are means ± SEM (n = 18 for each genotype).

(C) Grip strength and latency to falling in the wire-hang test. A significant sex difference in grip strength was detected ($p < 0.05$, one-way ANOVA), and so male and female mutant mice were compared separately with WT controls. Data are means ± SEM (total of n = 18 for each genotype).

(D) Light-dark transition test. Time spent in the light box, time spent in the dark box, and the number of light-dark transitions were measured. Data are means ± SEM (n = 18 for each genotype).

(E) Open-field test. Time spent in the center field and distance traveled were measured. Data are means ± SEM (n = 18 for each genotype).

(F) Elevated plus-maze test. Time spent in the open arms was measured. Data are means ± SEM (n = 18 for each genotype).

(G) Porsolt forced-swim test. Immobility in consecutive blocks of 1 min in trials on day 1 and day 2 was measured. Data are means ± SEM (n = 17 for each genotype).

(H) Tail-suspension test. Immobility was calculated in consecutive blocks of 1 min. Data are means ± SEM (n = 17 for each genotype).

Figure S3. Three-Chamber Tests of Sociability and Social Preference of *Setd5*$^{+/-}$ Mice, Related to Figure 1

(A) *Setd5*$^{+/+}$ or *Setd5*$^{+/-}$ mice were given access to an empty cage and a cage containing a stranger mouse of the same sex, and the time spent around each cage was measured. Data are means ± SEM (n = 17, *Setd5*$^{+/+}$; n = 18, *Setd5*$^{+/-}$). **p < 0.01 (paired t test).

(B) Test mice were given access to a cage containing a familiar mouse of the same sex and a cage containing a novel mouse of the same sex, and the time spent around each cage was measured. Data are means ± SEM (n = 17, *Setd5*$^{+/+}$; n = 18, *Setd5*$^{+/-}$). **p < 0.01 (paired t test).

Figure S4. Memory Formation in *Setd5*$^{+/-}$ Mice Assessed by the Barnes Maze Test and the T-Maze Test, Related to Figure 1

(A–C) Time spent to reach the target hole (A) and number of errors committed before reaching the target hole (B) in training trials as well as the time spent at each hole in the probe test (C) for *Setd5*$^{+/+}$ and *Setd5*$^{+/-}$ mice in the Barnes maze test. Data are means ± SEM (n = 17 for each genotype). *p < 0.05 (two-way repeated-measures ANOVA).

(D–F) Time spent to reach the target hole (D) and the number of errors committed before reaching the target hole (E) in training trials as well as the time spent at each hole

in the probe test (F) for the reversal phase of the Barnes maze test. Data are means ± SEM (n = 17 for each genotype). **p < 0.01 (two-way repeated-measures ANOVA).

(G and H) Percentage of correct responses for mice during the first three sessions (G) and during subsequent consecutive sessions with various delay times (H) in the T-maze spontaneous alternation task. Data are means ± SEM (n = 17 for each genotype).

Figure S5. Analysis of Gene Expression in the Brain of *Setd5*$^{+/-}$ Mice, Related to Figure 2

(A) GO analysis of molecular function and cellular component for the 617 genes (5%) showing the highest level of up-regulation in the *Setd5*$^{+/-}$ mouse brain compared with the WT mouse brain as performed with the PANTHER overrepresentation test (Mi et al., 2013).

(B) RT-qPCR analysis of ribosomal protein gene expression in the brain of adult *Setd5*$^{+/+}$ and *Setd5*$^{+/-}$ mice. *Actb* and *Gapdh* mRNAs were examined as internal controls. Data are means ± SEM (n = 3 for each genotype). **p < 0.01 (Student's t test).

Figure S6. Identification of Proteins Associated with SETD5, Related to Figure 3

(A) Silver staining of an SDS-PAGE gel loaded with an immunoprecipitate of 3×FLAG-SETD5 expressed in HEK293T cells. Band slices subjected to LC–MS/MS analysis are indicated.

(B) Silver staining of an SDS-PAGE gel loaded with an immunoprecipitate of 3×FLAG-SETD5 expressed in SH-SY5Y cells. Band slices subjected to LC–MS/MS analysis are indicated.

Figure S7. Generation of *Setd5* KO Neuro2a Cells and Lack of Association of SETD5 and HDAC3 with the rDNA Gene Body, Related to Figure 4

(A) Strategy for mutagenesis of mouse *Setd5* with the CRISPR/Cas9 system, and Sanger genomic sequencing results for *Setd5* KO Neuro2a cells. The protospacer sequence is highlighted in blue, the protospacer-adjacent motif (PAM) is underlined in purple, and the cleavage site is indicated by the red arrowhead. The KO cells harbor one *Setd5* allele with a 1-nucleotide insertion that results in protein truncation at amino acid (aa) position 34 and one allele with a 1-nucleotide deletion that results in a codon frameshift after codon 33.

(B) Immunofluorescence analysis of SETD5 in WT and *Setd5* KO Neuro2a cells. Nuclei were stained with DAPI. Scale bar, 50 μm.

(C) ChIP-qPCR analysis of HA-SETD5 binding to the rDNA gene body in *Setd5* KO Neuro2a cells complemented (Comp) with FL or N767 mutant forms of HA-SETD5 as in Figure 4D. ChIP was performed with antibodies to HA and with control IgG. Data are means ± SEM (n = 4 independent experiments). Scales are identical to that in Figure 4F, so that direct comparison of protein levels at the rDNA promoter and gene body is possible.

(D) ChIP-qPCR analysis of endogenous HDAC3-3×FLAG binding to the rDNA gene body in *Setd5* WT or KO Neuro2a cells engineered as in Figure 4G. ChIP was performed with antibodies to FLAG and with control IgG. Data are means ± SEM (n = 3 independent experiments). Scales are identical to that in Figure 4H, so that direct comparison of protein levels at the rDNA promoter and gene body is possible.

Figure S8. Epigenetic Modifications and Binding of TIP5 and RPA194 at rDNA in *Setd5* KO Neuro2a Cells, Related to Figure 5

(A and B) ChIP-qPCR analysis of H4K16ac (A) and TIP5 (B) at the rDNA gene body in WT or *Setd5* KO Neuro2a cells. Data are means ± SEM (n = 4 independent experiments). *p < 0.05 (one-way ANOVA followed by Tukey's test). Scales are

identical to those in Figure 5A (H4K16ac) and Figure 5B (TIP5), so that direct comparison of protein levels at the rDNA promoter and gene body is possible.

(C) DNA methylation at the rDNA promoter in WT or *Setd5* KO Neuro2a cells. Genomic DNA was cut with MspI (which is methylation insensitive) or HpaII (which is methylation sensitive), and resistance to restriction enzyme (RE) digestion was determined by qPCR amplification of the rDNA promoter region. Data are means ± SEM (n = 4 independent experiments). *p < 0.05 (Student's t test).

(D and E) ChIP-qPCR analysis of H3K9me3 and H3K36me3 (D) as well as of pan (K9+K14+K18+K23+K27)–acetylated histone 3 (pan-acH3) and pan (K5+K8+K12)–acetylated histone 4 (pan-acH4) at the rDNA promoter of WT or *Setd5* KO Neuro2a cells. Data are means ± SEM (n = 4 independent experiments). *p < 0.05, **p < 0.01 (one-way ANOVA followed by Tukey's test).

(F) ChIP-qPCR analysis of RPA194 at the rDNA gene body in WT or *Setd5* KO Neuro2a cells. Data are means ± SEM (n = 4 independent experiments). *p < 0.05, **p < 0.01 (one-way ANOVA followed by Tukey's test). Scales are identical to that in Figure 5C, so that direct comparison of protein levels at the rDNA promoter and gene body is possible.

Figure S9. Generation and Translational Activity of *Tip5;Setd5* Double-KO Neuro2a Cells as well as Generation, rDNA expression and Proliferation of *Tip5* single-KO Neuro2a Cells, Related to Figure 5

(A) Strategy for mutagenesis of mouse *Tip5* with the CRISPR/Cas9 system, and Sanger genomic sequencing results for *Tip5* KO Neuro2a cells. The protospacer sequence is highlighted in blue, the PAM is underlined in purple, and the cleavage site is indicated by the red arrowhead. *Tip5* KO1 Neuro2a cells harbor a 1-nucleotide insertion in both alleles of *Tip5* that generates a stop codon at position 34. *Tip5* KO2 Neuro2a cells harbor one allele of *Tip5* with a 1-nucleotide insertion that results in protein truncation at amino acid (aa) position 33, and one allele with an insertion likely derived from the *Rp24-313c11.3* pseudogene that generates a stop codon at position 33.

(B and C) Relative translational activity measured as in Figure 5F and 5G, respectively. Data are means ± SEM (n = 4 independent experiments). *p < 0.05, **p < 0.01 by Student's t test (E) or one-way ANOVA followed by Tukey's test (F).

(D) Immunoblot analysis of TIP5 in WT and *Tip5* KO Neuro2a cells. The Cas9 targets are the same as in *Tip5* KO cells on the *Setd5* KO background (Figure S9A). * indicates non-specific band.

(E) RT-qPCR analysis of *Tip5* mRNA. The reduction in *Tip5* mRNA abundance in the *Tip5* KO cells is most likely mediated by nonsense mRNA decay. Data are means ± SEM (n = 3 independent experiments).

(F) RT-qPCR analysis of 47S rRNA. Data are means ± SEM (n = 3 independent experiments).

(G) Cell proliferation based on BrdU staining. Data are means ± SEM (n = 3 independent experiments).

Figure S10. Depletion of SETD5 Impairs rDNA Expression, Translational Activity, and Cell Proliferation in SH-SY5Y Cells, Related to Figures 4 and 5

(A) Immunoblot analysis of SETD5 in SH-SY5Y cells infected with lentiviruses encoding doxycyclin-inducible control (shControl) or SETD5 (shSETD5-1 or -2) short hairpin RNAs.

(B) RT-qPCR analysis of 47S rRNA in cells as in (A). Data are means ± SEM (n = 3 independent experiments). *$p < 0.05$ (one-way ANOVA followed by Tukey's test).

(C) Immunoblot analysis of translational activity on the basis of puromycin incorporation into newly synthesized proteins in cells as in (A). Relative translational

activity was determined as mean ± SEM values (n = 3 independent experiments). *p < 0.05 (one-way ANOVA followed by Tukey's test).

(D) Immunofluorescence analysis of BrdU incorporation in cells as in (A). Nuclei were stained with DAPI. Scale bar, 20 μm. The percentage of BrdU-positive cells was determined for each condition as the mean ± SEM (n = 3 independent experiments). **p < 0.01 (one-way ANOVA followed by Tukey's test).

TRANSPARENT METHODS

Mouse Maintenance

Setd5$^{+/-}$ mice on the C57BL/6N background were obtained from the Wellcome Trust Sanger Institute (Bradley et al., 2012; Pettitt et al., 2009; Skarnes et al., 2011; White et al., 2013) and were maintained in a specific pathogen–free facility at the Institute of Animal Experimentation, Tohoku University Graduate School of Medicine. They were provided with water and rodent chow ad libitum and were treated according to the Standards for Humane Care and Use of Laboratory Animals of Tohoku University and the Guidelines for Proper Conduct of Animal Experiments of the Ministry of Education, Culture, Sports, Science, and Technology of Japan. The behavioral experimental protocols were approved by the Animal Care and Use Committee of Fujita Health University.

Behavioral Analyses of Mice

Setd5$^{+/-}$ mice or their WT littermates were group-housed (three or four animals per cage) in a room with a 12-h-light, 12-h-dark cycle (lights on at 0700 hours) and with access to food and water ad libitum. Behavioral tests were performed at 10 to 32 weeks of age between 0900 and 1800 hours unless indicated otherwise. Each apparatus was

cleaned with dilute sodium hypochlorite solution and 70% ethanol before testing of each animal in order to prevent bias due to olfactory cues. With the exception of the ultrasonic vocalization test, behavioral analyses were initiated with 7 male and 11 female mice of each genotype. During the analyses, one *Setd5*$^{+/-}$ mouse and one WT mouse died of unknown causes. Differences in data between male and female mice were assessed with two-way ANOVA; if the p value was <0.05, we presented the male and female results separately. Otherwise, data for the two sexes were combined. Behavioral data were obtained automatically with the use of ImageJ-based programs developed and modified by T.M. (available through O'Hara & Co.). Statistical analysis was performed with the use of StatView (SAS Institute).

Ultrasonic Vocalization

Mice at postnatal day 6 were isolated from their mother and placed in a recording chamber to record ultrasonic vocalizations over 5 min. The vocalizations were recorded through a 0.25-inch microphone and processed with a preamplifier and main amplifier (Brüel and Kjaer, Copenhagen, Denmark). Signals were filtered from 1 Hz to 100 kHz and digitized with a sampling frequency of 250 kHz, at 16 bits per sample, with the use of a 1000-Hz high-pass digital filter (model 1322A, Axon Instruments). Sound

recordings were processed with a custom Matlab program. Short-time Fourier transform analysis was performed to obtain sonograms (1024 samples/block and 1/4 overlap, resulting in a time resolution of 1.02 ms and a frequency resolution of 0.45 kHz). Frequencies of <35 kHz were filtered out to reduce background white noise and audible squeaking from females. Consecutive powers of 10 s without ultrasonic vocalizations were sampled and averaged over time to obtain a basal power spectrum, and 1.2 times the basal power spectrum was subtracted from each experimental power spectrum and frequencies with a power of less than zero were set to zero. This procedure was used to reduce the background white noise.

Wire-Hang Test

A wire-hang test apparatus (O'Hara & Co.) was used to assess balance and grip strength. The apparatus consists of a box (21.5 by 23 by 32 cm) with a wire-mesh grid (10 by 10 cm) on top that can be inverted. Mice at 10 weeks of age were placed on the wire mesh, which was then inverted, causing the animal to grip the wire. The latency to the mouse falling was recorded, with a 60-s cutoff time.

Open-Field Test

Each mouse at 10 to 11 weeks of age was placed in the corner of an open-field apparatus (40 by 40 by 30 cm, Accuscan Instruments), which was illuminated at 100 lux. Total distance traveled and time spent in the central area (20 by 20 cm) were recorded over 120 min.

Light-Dark Transition Test

The apparatus for the light-dark transition test consisted of a cage (21 by 42 by 25 cm) that was divided into two sections of equal size by a partition with a door (O'Hara & Co.). One chamber was made of white plastic and brightly illuminated, whereas the other was black and dark. Mice at 10 weeks of age were placed in the dark side and allowed to move freely between the two chambers with the door open for 10 min. The number of transitions between the two compartments and time spent in each chamber were recorded with the use of ImageLD software.

Elevated Plus-Maze Test

The apparatus consisted of two open arms (25 by 5 cm) and two enclosed arms of the same size with 15-cm-high transparent walls (O'Hara & Co.). The arms and central square were made of white plastic plates and were elevated to a height of 55 cm above

the floor. The likelihood of animals falling from the apparatus was minimized by attachment of 3-mm-high plastic ledges to the open arms. Arms of the same type were arranged on opposite sides. Each mouse at 11 weeks of age was placed in the central square of the maze (5 by 5 cm) facing one of the closed arms, and its behavior was recorded over 10 min. The percentage of time spent in the open arms was measured with the use of ImageEP software.

T-Maze Test

The spontaneous alternation task was conducted with an automatic T-maze apparatus (O'Hara & Co.) constructed of white plastic runways with 25-cm-high walls. The maze is partitioned into six areas by sliding doors that can be opened downward. The stem of the T constitutes area S2 (13 by 24 cm), and the arms of the T constitute areas A1 and A2 (11.5 by 20.5 cm). Areas P1 and P2 correspond to connecting passageways from the respective arms (area A1 or A2) to the start compartment (area S1). Mice were subjected to a spontaneous alternation protocol for 3 days (one session consisting of 10 trials per day; cutoff time, 50 min). Each trial had first and second runs. On the first run, the mouse was forced to choose one of the arms of the T (area A1 or A2). After the mouse stayed for >10 s, the door that separated the arm (area A1 or A2) and the

connecting passageway (area P1 or P2) would be opened, and the mouse could return to the starting compartment (area S1) via the connecting passageway. After a 3-s delay in area S1, the mouse was given a free choice between the two T arms. The percentage of trials in which mice entered the arm opposite to that of the forced-choice run during the free-choice run was calculated. The location of the first arm (left or right) was changed in a pseudorandom manner across trials so that mice received equal numbers of left and right presentations. On days 4 to 7, a delay of 3, 30, 60, or 120 s, respectively, between the first and second runs of each trial was applied. Data acquisition, control of the sliding doors, and data analysis were performed with ImageTM software.

Social-Interaction Test in a Novel Environment

Two mice of the same sex and genotype at 11 weeks of age that had been housed in different cages were placed together in a box (40 by 40 by 30 cm) and allowed to explore freely for 10 min. Analysis was performed automatically with the use of ImageSI software. Images were captured at a rate of three frames per second. Mean duration per contact and total duration per contact were recorded by the software.

Sociability and Social-Novelty Preference Test

The testing apparatus consisted of a rectangular, three-chambered box with a lid fitted with a video camera (O'Hara & Co.). Each chamber measured 20 by 40 by 47 cm, and the dividing walls were made of clear Plexiglas and had a small rectangular opening (5 by 3 cm) that allowed access into each chamber. Small round wire cages (11 cm in height, with a bottom diameter of 9 cm and vertical bars 0.5 cm apart) were located in corners of the left and right chambers. The subject mouse was first placed in the middle chamber and allowed to explore the entire test box for 10 min. It was then immediately transferred to a clean holding cage, and an unfamiliar mouse of the same sex (stranger 1) that had had no prior contact with the subject mouse was placed in one of the side cages. The location of stranger 1 in the left versus right side chamber was systematically alternated between trials. The cage containing the stranger mouse allowed nose contact with the subject mouse between the bars but prevented fighting. The subject mouse was then returned to the middle chamber and allowed to explore for 10 min (sociability test). The amount of time spent around each cage was measured with the aid of the camera fitted on top of the box in order to quantify sociability with regard to stranger 1. After the sociability test, the subject mouse was again transferred to the holding cage, and a second unfamiliar mouse of the same sex (stranger 2) was placed in the cage that had been empty during the first session. The test mouse was then returned to the middle

chamber and had a choice between the first, already-investigated unfamiliar mouse (stranger 1) and the novel unfamiliar mouse (stranger 2). The amount of time spent around each cage during a second 10-min session was measured as before (social-novelty preference test). All the mice used in these tests were 12 weeks of age. Data acquisition and analysis were performed automatically with the use of ImageCSI software.

Social-Preference Test

This test was conducted in a manner similar to that for the sociability and social-novelty preference tests. An unfamiliar mouse of the same sex and genotype (stranger) that had had no prior contact with the subject mouse as well as a cage mate of the subject mouse were placed in the cages of the side chambers. The test mouse thus had a choice between an unfamiliar mouse (stranger) and a familiar mouse (cage mate). All the mice used in this test were 47 to 50 weeks of age.

Barnes Maze Test

The Barnes maze test was performed on "dry land," a white circular surface with a diameter of 1.0 m and with 12 holes equally spaced around the perimeter (O'Hara &

Co.). The circular open field was elevated 75 cm from the floor. A black Plexiglas escape box (17 by 13 by 7 cm) containing paper cage-bedding on its floor was located under one of the holes. The hole above the escape box represented the target, analogous to the hidden platform in the Morris task. The location of the target was consistent for a given mouse but was randomized across mice. The maze was rotated daily, with the spatial location of the target unchanged with respect to visual room cues, in order to prevent bias based on olfactory or proximal cues within the maze. One or two trials per day were conducted. A probe trial was performed without the escape box at 1 day after the last training trial to confirm that this spatial task was dependent on navigation based on distal environmental cues in the room. The location of the target for each mouse was then shifted to the opposite side of the circular surface, and the same protocol for training and probe trials was followed. All the mice used in this test were 15 to 19 weeks of age. Behavior was recorded with the use of ImageBM software.

Porsolt Forced-Swim Test

A transparent plastic cylinder (20 cm in height with a diameter of 10 cm) filled with water (21°–23°C) up to a height of 7.5 cm was placed in a white plastic chamber (32 by 44 by 49 cm) (O'Hara & Co.). The mouse was placed in the cylinder, and the time

during which the animal was immobile was recorded over a 10-min test period. Images were captured at two frames per second. For each pair of successive frames, the area (pixels) within which the mouse moved was measured. When the area was below a certain threshold, the mouse behavior was classified as "immobility." When the area equaled or exceeded the threshold, the mouse was classified as "moving." The optimal threshold for the classification was determined on the basis of human observation. Immobility lasting for <2 s was not included in the analysis. Data acquisition and analysis were performed automatically with ImagePS software.

Tail-Suspension Test

Mice were suspended 30 cm above the floor of a white plastic chamber (39 by 32 by 44 cm) (O'Hara & Co.) in a visually isolated area by adhesive tape placed ~1 cm from the base of the tail, and behavior was recorded over a 10-min test period. Images were captured at two frames per second. Similar to the Porsolt forced-swim test, immobility was judged by the application program according to a certain threshold. Immobility lasting for <2 s was not included in the analysis. Data acquisition and analysis were performed automatically with ImageTS software.

Cued Fear-Conditioning Test

Each mouse was placed in a transparent acrylic chamber (33 by 25 by 28 cm) with a stainless-steel grid floor made of rods 0.2 cm in diameter spaced 0.5 cm apart (O'Hara & Co.). The chamber was illuminated at 100 lux, and the mouse was allowed to explore freely for 2 min. White noise of 55 dB, which served as the conditioned stimulus (CS), was presented for 30 s, followed by a mild foot shock (0.3 mA for 2 s), which served as the unconditioned stimulus (US). Two more CS-US pairings were presented with a 2-min interstimulus interval. One day after conditioning, a cued test with altered context was conducted in a triangular box (33 by 29 by 32 cm) that was made of white Plexiglas and located in a different room. The chamber was illuminated at 30 lux. The mouse was allowed to explore the chamber for 180 s, after which the CS was presented for 180 s. Data acquisition, control of stimuli (white noise and foot shock), and data analysis were performed automatically with ImageFC software. Images were captured at one frame per second. For each pair of successive frames, the area (pixels) in which the mouse moved was measured. When this area was below a certain threshold, the behavior was judged as "freezing." When the area equaled or exceeded the threshold, the behavior was considered to be "nonfreezing." The optimal threshold (number of pixels) for such

classification was determined on the basis of human observation. Freezing that lasted for less than the defined time threshold (2 s) was not included in the analysis.

Home cage monitoring

For monitoring of behavior in a familiar environment, two genetically identical mice that had been housed separately were placed together in a home cage (29 by 18 by 12 cm). The social behavior was then monitored for 7 days with a video camera, the output of which was fed into a computer. Images were captured at a rate of one frame per second with ImageHA software.

Immunofluorescence Staining of Mouse Brain

The embryonic brain was dissected, immersed overnight in 1% paraformaldehyde in phosphate-buffered saline (PBS), and transferred to 15% sucrose in PBS overnight and then to 30% sucrose in PBS for at least 2 days for cryoprotection. The brain tissue was frozen in Tissue-Tek OTC compound (Miles) and cut into 10-μm-thick sections with a cryostat (Leica Microsystems). The cryostat sections were incubated with primary antibodies at 4°C for 16 h. After two washes with PBS containing 0.1% Tween-20 (PBS-T), the sections were incubated with AlexaFluor-conjugated secondary antibodies

at room temperature for 45 min, washed twice with PBS-T and twice with PBS, and mounted with the use of SlowFade Gold antifade reagent with DAPI (Thermo Fisher Scientific). Fluorescence signals were detected with a BZ-9000 microscope (Keyence).

Cell Culture

HEK293T (ATCC, CRL-3216), SH-SY5Y (ATCC, CRL-2266), Neuro2a (ATCC, CCL-131), and Plat-E (Gift from Toshio Kitamura' lab) cells were cultured under 5% CO_2 at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin (50 U/ml), streptomycin (50 μg/ml), 2 mM L-glutamine, 1% MEM–nonessential amino acids, and 1% sodium pyruvate (Thermo Fisher Scientific).

Cell Transfection

HEK293T and Neuro2a cells were transfected with expression plasmids with the use of polyethylenimine (Polysciences) or Lipofectamine 2000 (Thermo Fisher Scientific), respectively. For retrovirus-mediated gene transfer, Plat-E cells were transfected with retroviral vectors (pMX). For lentivirus-mediated gene transfer, HEK293T cells were transfected with lentiviral vectors (pSLIK, Addgene #25735), psPAX2 (Addgene #12260), and pMD2.G (Addgene #12259) (Shin et al., 2006). Retrovirus- or lentivirus-

containing medium was collected 48 h after transfection and supplemented with polybrene (Sigma) at 4 μg/ml. Neuro2a or SH-SY5Y cells were infected for 24 h by exposure to the virus-containing medium. Selection was performed with puromycin or G418. SH-SY5Y cells infected with lentiviruses were exposed to doxycycline (1 μg/ml) for 2 days (for overexpression of 3×FLAG-SETD5) or 5 days (for knockdown of endogenous SETD5). miRNA targeting sequences for human SETD5 knockdown are GCUUCUGGAUUUGGGCAAACA and GGAGAAAGCUGUAAAUCUUGU.

Establishment of *Setd5* and *Tip5* KO Cells

Frameshift mutations in *Setd5* or *Tip5* were induced by introduction of pSpCas9(BB)-2A-Puro–based plasmids (Addgene #48139) into Neuro2a cells by transient transfection (Ran et al., 2013). The cells were then cloned by limiting dilution in 96-well plates, and the resulting single cell–derived clones were screened for frameshift mutations by genomic PCR and sequencing. Protospacer sequences targeted by SpCas9 are as follows; ACGCTCTTCTCATTAACTGC for mouse Setd5, GGGAGGGCCTCTACACTAAC and TGGGAGACCCGTTAGTGTAG for mouse Tip5.

Generation of HDAC3-3×FLAG Knock-in Cells

A targeting vector (pBS-mHDAC3-3FLAG-KI TV) was constructed by PCR-mediated cloning of the 1-kbp region immediately upstream of the stop codon of mouse *Hdac3* (left arm) and the 1-kbp region immediately downstream of the stop codon (right arm), followed by insertion of these fragments into the pBS-3FLAG-LNL (loxP-neo-loxP) vector. Neuro2a cells were transfected with the linearized targeting vector and pSpCas9-HDAC3, which induces the formation of double-strand breaks in the 3' untranslated region of *Hdac3*. After selection in the presence of G418 (1000 μg/ml) for 7 days, the cells were transfected with pMX-puro-Cre, selected with puromycin (5 μg/ml) for 2 days, and then cloned by limiting dilution in 96-well plates. The resulting single cell–derived clones were screened for biallelic 3×FLAG knock-in by genomic PCR and sequencing as well as by immunoblot analysis.

Plasmids

A cDNA encoding SETD5 was amplified from HEK293T cells, cloned into pENTR (Thermo Fisher Scientific), and verified by sequencing. The pENTR plasmid was recombined with destination plasmids (Nakagawa et al., 2015) with the use of LR

clonase II (Thermo Fisher Scientific). Deletion mutants were prepared by PCR-based mutagenesis.

RNA Isolation and RT-qPCR Analysis

RNA was isolated with the use of an SV Total RNA Isolation System (Promega), and it was subjected to RT with a PrimeScript RT reagent kit (Takara Bio) followed by real-time PCR analysis with a StepOnePlus Real Time PCR System (Life Technologies) and Fast SYBR Green Master Mix (Life Technologies). Data were analyzed according to the $2^{-\Delta\Delta Ct}$ method and were normalized by the amount of *Cul1* mRNA. Oligonucleotide sequences of RT-qPCR primers are presented in Table S4.

RNA-seq Analysis

Total RNA was extracted from the hemispheres of 2-month-old female littermates (*Setd5*$^{+/+}$ and *Setd5*$^{+/-}$, n = 1 for each genotype) for RNA-seq analysis. RNA-seq libraries were prepared with a TruSeq Standard mRNA LT Sample Prep Kit (Illumina). Libraries were clonally amplified in the flow cell of an Illumina HiSeq 2500 instrument and sequenced (51-nucleotide paired end). Paired-end reads were mapped to the mouse genome (UCSC mm9 and RefSeq) with TopHat (Trapnell et al., 2009). Cufflinks was

used to estimate gene expression level on the basis of FPKM (Trapnell et al., 2013). FPKM data are presented in Table S2. GO analysis of molecular function and cellular component for the 617 (5%) most up-regulated or down-regulated genes in the *Setd5*$^{+/-}$ mouse brain was performed with the use of the PANTHER overrepresentation test (Mi et al., 2013). No statistics were applied to identify the most up- and down-regulated genes.

Immunoprecipitation and Immunoblot Analysis

With the exception of cell lysis for the identification of SETD5 binding proteins, cells were lysed for immunoprecipitation at 4°C for 10 min in NP-40 lysis buffer [0.5% Nonidet P-40, 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 10% glycerol, aprotinin (10 μg/ml), leupeptin (10 μg/ml), 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.4 mM sodium orthovanadate, 0.4 mM EDTA, 10 mM NaF, 10 mM sodium pyrophosphate]. Crude lysates were cleared of debris by centrifugation at 20,000 × *g* for 15 min at 4°C, and the resulting supernatants were incubated with Dynabeads Protein G (Thermo Fisher Scientific) that had been conjugated with appropriate antibodies. The immune complexes were washed three times with wash buffer (0.1% Triton X-100 and 10% glycerol in PBS) and then subjected to SDS–polyacrylamide gel electrophoresis

(PAGE) followed by immunoblot analysis with appropriate antibodies. For direct immunoblot analysis of cultured cells, total cell extracts were prepared with RIPA buffer [50 mM Tris-HCl (pH 8.0), 0.1% SDS, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate] and cleared of debris by centrifugation at 20,000 × *g* for 15 min at 4°C. The resulting supernatants were subjected to SDS-PAGE. For immunoblot assay of embryonic and adult mouse brains, extracts were prepared by homogenization of tissue in a solution containing 50 mM Tris-HCl (pH 7.5), 250 mM sucrose, 1 mM EDTA, a protease inhibitor cocktail, and phosphatase inhibitor cocktail [(0.4 mM Sodium orthovanadate (Wako), 0.4 mM EDTA (Dojindo), 10 mM NaF (Wako) and 10 mM sodium pyrophosphate (Sigma)]. The homogenate was then mixed with an equal volume of 2× radioimmunoprecipitation assay (RIPA) buffer. Band intensities were quantified with Image J software.

Puromycin Incorporation Assay

Cells were treated with puromycin (20 μg/ml) for 30 min before lysis for immunoblot analysis with antibodies to puromycin.

Identification of SETD5 Binding Proteins by LC–MS/MS

HEK293T or SH-SY5Y cells expressing 3×FLAG-SETD5 were lysed in digitonin buffer [1% digitonin, 150 mM NaCl, 50 mM Tris-HCl (pH 7.4), aprotinin (10 μg/ml), leupeptin (10 μg/ml), 1 mM PMSF, 0.4 mM sodium orthovanadate, 0.4 mM EDTA, 10 mM NaF, 10 mM sodium pyrophosphate] and then subjected to immunoprecipitation as described above with antibodies to FLAG. After three washes with wash buffer (0.1% Triton X-100 and 10% glycerol in PBS), the immunoprecipitated proteins were eluted with elution buffer [0.1% Triton X-100, 10% glycerol, and 3×FLAG peptide (500 μg/ml) in PBS] and subjected to SDS-PAGE on a 4% to 20% gradient gel followed by silver staining. The gel was cut into slices as indicated in Figure S6, and proteins in each gel slice were digested with trypsin. The obtained peptides were dried, dissolved in a solution containing 0.1% trifluoroacetic acid and 2% acetonitrile, and then subjected to nanoscale LC and MS/MS analysis with a system consisting of an LTQ mass spectrometer (Thermo Fisher Scientific) coupled with a nanoLC instrument (Paradigm MS4, Michrom BioResources) and HTC-PAL autosampler (CTC Analytics). Peptide separation was performed with an in-house pulled fused-silica capillary packed with 3-μm C18 L-column resin (Chemicals Evaluation and Research Institute, Japan). The mobile phases consisted of 0.1% formic acid/2% acetonitrile and 0.1% formic acid/90% acetonitrile, and peptides were eluted with a linear gradient. Collision-induced

dissociation spectra were acquired automatically in the data-dependent scan mode with the dynamic exclusion option. The peak lists were generated by MSn.exe (Thermo Fisher Scientific) with a minimum scan/group value of 1 and were compared with the ipi_HUM_NEW database with the use of the MASCOT algorithm (ver. 2.4.1). Identified peptides are presented in Table S3.

Gel Filtration

Cell lysates (500 µl) prepared with NP-40 lysis buffer containing 250 U of benzonase (Novagen) were subjected to gel filtration on Superose 6 10/300 GL (GE Healthcare) with the use of an AKTA Purifier (GE Healthcare) LC system. Elution was performed with PBS at a flow rate of 0.5 ml/min. Fractions (1 ml) were collected from 6 to 17 ml and were subjected to immunoblot analysis. Approximate molecular size was calculated after calibration of the column with a Gel Filtration Calibration Kit LMW HMW (GE Healthcare).

ChIP Analysis

Cells were fixed with 1% paraformaldehyde for 10 min, exposed to glycine at a final concentration of 0.125 M in PBS to terminate fixation, and centrifuged at 1000 × *g* for 2

min at 4°C. The cell pellets were suspended in 500 μl of nuclear extraction buffer [50 mM Tris-HCl (pH 7.4), 10 mM potassium acetate, 15 mM magnesium acetate, 1% Nonidet P-40, aprotinin (10 μg/ml), leupeptin (10 μg/ml), 1 mM PMSF], incubated for 15 min on ice, and centrifuged again at 1000 × *g* for 5 min at 4°C. The resulting pellets were suspended in 500 μl of Buf NUC solution [15 mM HEPES-NaOH (pH 7.5), 60 mM KCl, 15 mM NaCl, 0.32 mM sucrose, aprotinin (10 μg/ml), leupeptin (10 μg/ml), 1 mM PMSF], mixed with 1.5 μl of 1 M $CaCl_2$, and digested with 2000 U of micrococcal nuclease (New England Biolabs) for 20 min at 37°C. After the addition of 500 μl of 2× sonication buffer [90 mM HEPES-NaOH (pH 7.8), 220 mM NaCl, 10 mM EDTA, 1% Nonidet P-40, 0.2% sodium deoxycholate, 0.2% SDS] to stop the reaction, the suspension was subjected to ultrasonic treatment with the use of a Cosmo Bio Bioruptor UCD-250 (seven cycles of 30 s on and 30 s off, level H) and then centrifuged at 13,000 × *g* for 5 min at 4°C. The resulting supernatants were incubated with rotation overnight at 4°C with the indicated antibodies conjugated to magnetic beads. Bead-bound proteins were washed consecutively with buffer A [20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS], buffer B [20 mM Tris-HCl (pH 8.0), 500 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS], buffer C [20 mM Tris-HCl (pH 8.0), 250 mM LiCl, 1 mM

EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate], and Tris-EDTA buffer, and they were then eluted by consecutive exposure to RNase A (100 µg/ml) for 30 min at 50°C, proteinase K (200 µg/ml) for 30 min at 50°C, and 350 mM NaCl overnight at 65°C, each of which was dissolved in ChIP elution buffer [50 mM Tris-HCl (pH 8.0), 1 mM EDTA, 1% SDS]. After incubation of the samples with proteinase K (200 ug/ml) for 60 min at 50°C, DNA was purified with the use of AMpure XP beads in 2.5 M NaCl containing 20% polyethylene glycol, washed with 80% ethanol, eluted with EB buffer (Qiagen), and subjected to real-time PCR analysis. Oligonucleotide sequences of primers for real-time PCR analysis are presented in Table S4.

Analysis of DNA Methylation at the rDNA Promoter

CpG methylation was assayed by digestion with HpaII (a methylation-sensitive restriction enzyme) and MspI (a methylation-insensitive restriction enzyme) (Murano et al., 2014; Santoro et al., 2002). Genomic DNA (500 ng) was digested with 20 U of HpaII or MspI for 1 h at 37°C, and resistance to the restriction enzymes was determined by qPCR analysis with the primer set 5'-AAGCCCTCTCTGTCCCTGTCAC-3' and 5'-GGAGAACTGATAAGACCGACAGGT-3'.

BrdU Incorporation Assay

Cells exposed to 10 μM BrdU for 24 h were fixed with 1% paraformaldehyde, permeabilized with 0.5% Triton X-100 in PBS, denatured with 2 M HCl, washed with PBS-T, and exposed to 1% bovine serum albumin in PBS before incubation with antibodies to BrdU. The cells were then washed with PBS-T, incubated with AlexaFluor 555–labeled secondary antibodies (Thermo Fisher Scientific), washed again with PBS-T, treated with DAPI (1 μg/ml), and examined with a BZ-9000 microscope (Keyence).

Cell Cycle Profiling

Neuro2a cells (1×10^5) were incubated with 10 μM BrdU for 30 min, fixed with ice-cold 70% ethanol for 30 min, and treated with a denaturing solution (2 M HCl containing 0.5% Triton X-100) for another 30 min at 37ºC. After exposure to 0.1 M sodium tetraborate decahydrate (pH 8.5) for 2 min at 37ºC, the cells were stained with fluorescein isothiocyanate–labeled antibodies to BrdU and PI for 30 min at 37ºC and were then analyzed with a FACSCanto II flow cytometer (BD Biosciences).

Immunofluorescence Staining of Neuro2a Cells

Neuro2a cells grown on glass coverslips were fixed in 1% paraformaldehyde for 10 min, washed with PBS, and permeabilized for 10 min with PBS containing 0.5% Triton X-100. They were then exposed to 5% nonfat milk in PBS before incubation with primary antibodies for 16 h at 4°C. After three washes with PBS-T, the cells were incubated with AlexaFluor-conjugated secondary antibodies for 45 min at room temperature, washed with PBS-T, exposed to DAPI (5 μg/ml) for 1 min, and then examined with an LSM780 confocal microscope (Zeiss).

Neurosphere Formation

Neurosphere was generated by the protocol of Larysa H. Pevny laboratory (Hutton and Pevny, 2008). Periventricular tissues of the 2-month-old adult brain were taken out, cut into smaller pieces, and incubated with 20 ml of enzyme solution [98 mM Na_2SO_4, 30 mM K_2SO_4, 5.8 mM $MgCl_2$, 0.25 mM $CaCl_2$, 1 mM HEPES-NaOH (pH 7.4), 20 mM Glucose, 0.001% Phenol Red, 2 mM Cysteine and 60 U Papain] for 40 min at 37°C. After removal of enzyme solution, samples were then sequentially incubated with 9 ml of light inhibitory solution [98 mM Na_2SO_4, 30 mM K_2SO_4, 5.8 mM $MgCl_2$, 0.25 mM $CaCl_2$, 1 mM HEPES-NaOH (pH 7.4), 20 mM Glucose, 0.001% Phenol Red, 0.1% BSA and 0.1% trypsin inhibitor] for 4 min at 37°C, 6 ml of heavy inhibitory solution [98 mM

Na_2SO_4, 30 mM K_2SO_4, 5.8 mM $MgCl_2$, 0.25 mM $CaCl_2$, 1 mM HEPES-NaOH (pH 7.4), 20 mM Glucose, 0.001% Phenol Red, 1% BSA and 1% trypsin inhibitor] for 2 min at 37°C, 5 ml of NEP basal medium (Neurobasal medium with B-27 and N-2) for 2 min at room temperature, and 2 ml of NEP complete medium (NEP basal medium with 100 ng/ml EGF and 10 ng/ml bFGF) at room temperature. Cells were then seeded in 1 well (9.5 cm^2) of ultra-low attachment 6-well plate (Corning) and were cultured under 5% CO_2 at 37°C. Neurosphere formed was counted using a hemacytometer at day 10.

Polysome Analysis

Neuro2a cells (1×10^7) were harvested, and cell extracts were layered onto linear sucrose density gradients [10–50% sucrose in 20 mM HEPES-NaOH (pH 7.6), 100 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, and cycloheximide (100 μg/ml)] that had been prepared in open-top polyclear tubes (Seton) with the use of a Gradient Master. Samples were centrifuged at 131,000 × *g* for 3 h at 4°C in a P28S rotor (Hitachi Koki). Gradients were then fractionated (Towa). Polysome profiles were generated by continuous measurement of absorbance at 254 nm with a single-path UV-1 optical unit (AC-5200, ATTO) connected to a chart recorder (ATTO). Fractions of equal volume were collected and processed for RT-qPCR analysis.

Antibody

Anti-SETD5 (ab204363), anti-BrdU (ab6326), anti-Ki67 (ab15580), anti-H3K9me3 (ab8898), anti-H3K36me3 (ab9050), and anti-histone H3 (acetyl K9+K14+K18+K23+K27) (ab47915) antibodies were purchased from Abcam. Anti-DDB1 (5428), anti-HDAC3 (3949) and anti-PAF1 (12883) antibodies were from Cell Signaling Technology. Anti-SOX2 (sc-17320), anti-UBF (sc-13125), anti-RPA194 (sc-48385), anti-Cyclin D1 (sc-450), anti-Cyclin E1 (sc-481), and anti-Cyclin E2 (sc-28351) were from Santa Cruz Biotechnology. Anti-FLAG (F1804) and anti-Tubulin (T6074) were from Sigma-Aldrich. Anti-HA (11867423001) was from Roche. Anti-H4K16ac (07-329), anti-histone H4 (acetyl K5+K8+K12) (04-557), and anti-puromycin (MABE343) antibodies were from Millipore. Anti-TIP5 (100-401-v86) antibody was from Rockland. FITC-conjugated anti-BrdU (364103) was from Becton Dickinson. Alexa-555 conjugated anti-Rat IgG (A-21434) was from Thermo Fisher Scientific. HRP-conjugated anti-mouse IgG (W4021) and HRP-conjugated anti-rabbit IgG (W4011) were from Promega.

Statistical Analysis

Data are presented as means ± SEM and were analyzed by Student's t test, the paired t test, one-way ANOVA with or without Tukey's post hoc test, two-way ANOVA, or two-way repeated-measures ANOVA as indicated. A p value of <0.05 was considered statistically significant.

Data and Software Availability

Raw FASTQ files of RNA-seq data obtained in this study have been deposited in the DRA database (DRA009877).

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