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Supplemental Information

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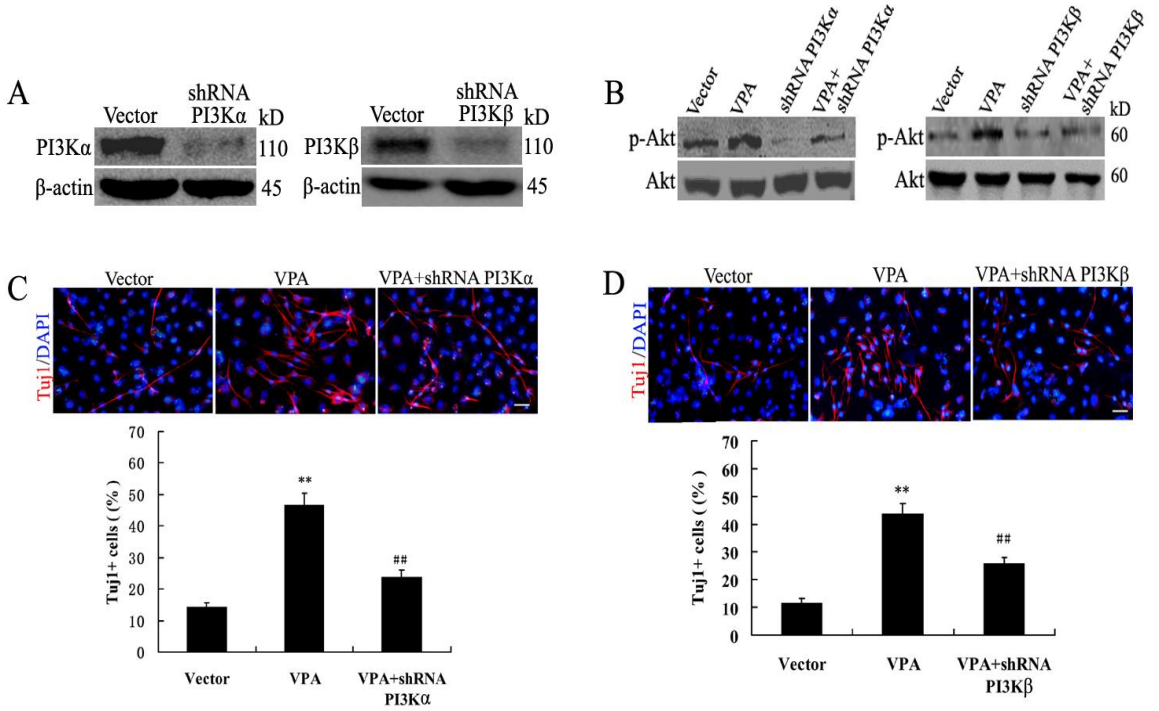

Figure S1

Knocking down PI3K p110α or β expression alleviated the neuronal differentiation of VPA-treated NSCs. Related to Figure 2

(**A**) NSCs were transfected with PI3K p110α or PI3K p110β or control shRNA for 48 h. Lysates from the cells were analyzed by immunoblotting for the levels of PI3K p110α, PI3K p110β and β-actin.

(**B**) PI3K p110α or PI3K p110β knockdown suppressed Akt activation in VPA-treated NSCs.

(**C**) PI3K p110α downregulation reduced the neuronal differentiation of VPA-treated NSCs (scale bar, 100μm). ** $p = 0.000 < 0.01$ compared with control (Vector); $^{\#\#}$ $p = 0.003 < 0.01$ compared with VPA (Vector+VPA).

(**D**) PI3K p110β downregulation decreased the neuronal differentiation of VPA-treated NSCs (scale

bar, 100μm). ** $p = 0.000 < 0.01$ compared with control (Vector); ## $p = 0.004 < 0.01$ compared with VPA (Vector+VPA). All data shown are from three independent experiments.

Table S1

Sequences for shRNAs Related to Figure 7 and Figure S1.

sc-154204-V: Tet1 shRNA (m) Lentiviral Particles is a pool of 3 different shRNA plasmids
sc-154204-VA: Hairpin sequence: GATCCGAAGATGTACCCTCAACAATTCAAGAGATTGTTGAGGGTACATCTTCTTTTT Corresponding siRNA sequences (sc-154204A): • Sense: GAAGAUGUACCCUCAACAAtt • Antisense: UUGUUGAGGGUACAUCUUCtt sc-154204-VB: Hairpin sequence: GATCCCTACCTGTACGTACAGTAATTCAAGAGATTACTGTACGTACAGGTAGTTTTT Corresponding siRNA sequences (sc-154204B): • Sense: CUACCUGUACGUACAGUAAtt • Antisense: UUACUGUACGUACAGGUAGtt sc-154204-VC: Hairpin sequence: GATCCGGAAGCTGTTCGCTAACTATTCAAGAGATAGTTAGCGAACAGCTTCCTTTTT Corresponding siRNA sequences (sc-154204C): • Sense: GGAAGCUGUUCGCUAACUAtt • Antisense: UAGUUAGCGAACAGCUUCCtt Note: all sequences are provided in 5′ → 3′ orientation.
sc-154205-V: Tet2 shRNA (m) Lentiviral Particles is a pool of 3 different shRNA plasmids
sc-154205-VA: Hairpin sequence: GATCCGAGATGCCTTCACTACTAATTCAAGAGATTAGTAGTGAAGGCATCTCTTTTT Corresponding siRNA sequences (sc-154205A): • Sense: GAGAUGCCUUCACUACUAAtt • Antisense: UUAGUAGUGAAGGCAUCUCtt sc-154205-VB: Hairpin sequence: GATCCGGAAGAGTGCGGAAAGAATTTCAAGAGAATTCTTTCCGCACTCTTCCTTTTT Corresponding siRNA sequences (sc-154205B): • Sense: GGAAGAGUGCGGAAAGAAUtt • Antisense: AUUCUUUCCGCACUCUUCCtt sc-154205-VC: Hairpin sequence: GATCCGTTCCTACTTGTCGTCATATTCAAGAGATATGACGACAAGTAGGAACTTTTT Corresponding siRNA sequences (sc-154205C): • Sense: GUUCCUACUUGUCGUCAUAtt

• Antisense: UAUGACGACAAGUAGGAACtt Note: all sequences are provided in 5′ → 3′ orientation.
sc-154206-V: Tet3 shRNA (m) Lentiviral Particles is a pool of 3 different shRNA plasmids
sc-154206-VA: Hairpin sequence: GATCCGGGTTTGTCTATCTCCTTATTCAAGAGATAAGGAGATAGACAAACCCTTTTT Corresponding siRNA sequences (sc-154206A): • Sense: GGGUUUGUCUAUCUCCUUAtt • Antisense: UAAGGAGAUAGACAAACCCtt sc-154206-VB: Hairpin sequence: GATCCCTCTGTGCATTAAGCAGTATTCAAGAGATACTGCTTAATGCACAGAGTTTTT Corresponding siRNA sequences (sc-154206B): • Sense: CUCUGUGCAUUAAGCAGUAtt • Antisense: UACUGCUUAAUGCACAGAGtt sc-154206-VC: Hairpin sequence: GATCCCTGTCTCACAGCATACTAATTCAAGAGATTAGTATGCTGTGAGACAGTTTTT Corresponding siRNA sequences (sc-154206C): • Sense: CUGUCUCACAGCAUACUAAtt • Antisense: UUAGUAUGCUGUGAGACAGtt Note: all sequences are provided in 5′ → 3′ orientation.
sc-39128-V: PI3-kinase p110α shRNA (m) Lentiviral Particles is a pool of 3 different shRNA plasmids
sc-39128-VA: Hairpin sequence: GATCCCACAGACACTACTGCGTAATTCAAGAGATTACGCAGTAGTGTCTGTGTTTTT Corresponding siRNA sequences (sc-39128A): • Sense: CACAGACACUACUGCGUAAtt • Antisense: UUACGCAGUAGUGUCUGUGtt sc-39128-VB: Hairpin sequence: GATCCGAATCCTGCTCACCAACTATTCAAGAGATAGTTGGTGAGCAGGATTCTTTTT Corresponding siRNA sequences (sc-39128B): • Sense: GAAUCCUGCUCACCAACUAtt • Antisense: UAGUUGGUGAGCAGGAUUCtt sc-39128-VC: Hairpin sequence: GATCCGCACAAGAGTACACCAAGATTCAAGAGATCTTGGTGTACTCTTGTGCTTTTT Corresponding siRNA sequences (sc-39128C): • Sense: GCACAAGAGUACACCAAGAtt • Antisense: UCUUGGUGUACUCUUGUGCtt Note: all sequences are provided in 5′ → 3′ orientation.
sc-29447-V: PI3-kinase p110β shRNA (m) Lentiviral Particles is a pool of 4 different shRNA plasmids
sc-29447-VA: Hairpin sequence: GATCCGGAAGCAAGTTCACAACTATTCAAGAGATAGTTGTGAACTTGCTTCCTTTTT

Corresponding siRNA sequences (sc-29447A):
• Sense: GGAAGCAAGUUCACAACUAtt
• Antisense: UAGUUGUGAACUUGCUUCCtt

sc-29447-VB:
Hairpin sequence:
GATCCGAACGACCATATTTGGAATTTCAAGAGAATTCCAAATATGGTCGTTCTTTTT
Corresponding siRNA sequences (sc-29447B):
• Sense: GAACGACCAUAUUUGGAAUtt
• Antisense: AUUCCAAAUAUGGUCGUUCtt

sc-29447-VC:
Hairpin sequence:
GATCCCTACTCTTGTCAATCAAGTTTCAAGAGAACTTGATTGACAAGAGTAGTTTTT
Corresponding siRNA sequences (sc-29447C):
• Sense: CUACUCUUGUCAAUCAAGUtt
• Antisense: ACUUGAUUGACAAGAGUAGtt

sc-29447-VD:
Hairpin sequence:
GATCCCAGACTCGCTGAGAATCTATTCAAGAGATAGATTCTCAGCGAGTCTGTTTTT
Corresponding siRNA sequences (sc-29447D):
• Sense: CAGACUCGCUGAGAAUCUAtt
• Antisense: UAGAUUCUCAGCGAGUCUGtt
Note: all sequences are provided in 5′ → 3′ orientation.

Table S2

Antibodies Related to Figures 1-7 and Figure S1.

Name	Catalogue number	supplier
NeuN	ab104224	Abcam, UK
Doublecortin (DCX)	sc-271390	Santa cruz, USA
GFAP	ab7260	Abcam, UK
beta III Tubulin	ab78078	Abcam, UK
Neurogenin 1(Ngn1)	ab89461	Abcam, UK
Tet1	sc-163443	Santa cruz, USA
Tet2	sc-398535	Santa cruz, USA
Tet3	ABE383	Millipore, USA
5-mC	GTX21884	GeneTex, USA
5-hmC	MABE176	Millipore, USA
p-p70S6K	9204	CST, USA
p70S6K	2708	CST, USA
p-mTOR	2971	CST, USA
mTOR	2972	CST, USA
p-Akt	13038	CST, USA
Akt	9272	CST, USA
Acetyl-H3	7627	CST, USA
H3	14269	CST, USA
Acetyl-H4	2594	CST, USA
H4	2592	CST, USA

Dnmt1	ab13537	Abcam, UK
Dnmt3a	ab2850	Abcam, UK
Dnmt3b	ab79822	Abcam, UK
Dnmt3L	ab3493	Abcam, UK
HDAC1	ab7028	Abcam, UK
HDAC3	ab7030	Abcam, UK
PI3K p110α	4249	CST, USA
PI3K p110β	ab151549	Abcam, UK
H3K4me3	9751	CST, USA
H3K27me3	9733	CST, USA
H3K9me2	4658	CST, USA
H3K9Ac	ab10812	Abcam, UK

Table S3

Primer sequences used for quantitative RT-PCR analysis Related to Figure 3.

Genes	Forword	Reverse	
DNMT1	5′-GGTTCTGCGCGGGGACAGAC-3′	5′-CCGGCAACATGGCCTCAGGG-3′	183bp
DNMT3a	5′-GGTGTGTGTCGAGAAGCTCA-3′	5′-CCAAGGGCCCACTCAATCAT-3′	222bp
DNMT3b	5′-GGGCCGCTACCACGTTCAGG-3′	5′-AGGGCCGTCCTGGCTCAAGT-3′	178bp
DNMT3L	5′-GTATGCCCGGCCTCGCCAAG-3′	5′-CAGGTCCGCGTGCTTGCTCT-3′	208bp
Ngn1	5′-TGTAGCAGTTTGCTGGTCCT-3′	5′-GTAGCTCTGCACGACGATGT-3′	144bp
β-Actin	5′-GCGTCCACCCGCGAGTACAA-3′	5′-ACATGCCGGAGCCGTTGTCG-3′	118bp

Supplemental Experimental Procedures

5-Bromo-2′-deoxyuridine (BrdU) Labeling

Cells in the different treatment groups were incubated in 24-well plates in each group with 10 μg/L BrdU (Sigma-Aldrich) for 24 h. After incubation, the cells were seeded onto 100 μg/mL poly-L-lysine-coated coverslips. An immunocytochemical assay was used to determine the incorporation. Coverslips were mounted with 4′6-diamidino-2-phenylindole (DAPI) was used to counter stain nuclei. Immunoreactive cells were visualized by fluorescence microscopy. Experiments were done by triplicate.

CCK-8 Cell Assay

The CCK-8 assay was used to quantitatively assess cell survival. Briefly, NSCs were seeded in 96-well plates with six replicates in each group, and subjected to the different treatments described. 10 μL CCK-8 solutions were added to each well, and NSCs were incubated for 4 h. The absorbance at 450 nm was measured with a microplate reader (Thermo Fisher Scientific, Japan). Each experiment was repeated for four times.

DNA Methylation and Hydroxymethylation Assay

DNA was prepared using the GeneElute mammalian genomic DNA miniprep kit (Sigma-Aldrich) according to the manufacturer's instructions. DNA quality and concentration was measured by NanoDrop 1000 spectrophotometer (Thermo Scientific). Relative quantitation of *5-mC and 5-hmC* within a specific locus *was performed using* EpiMark™ 5-hmC and 5-mC Analysis Kit (Epigentek, NY) according to the manufacturer's instructions, followed by real-time PCR analysis. The forward and reverse primers for Ngn1 were 5′-TGTAGCAGTTTGCTGGTCCT-3′ and 5′-GTAGCTCTGCACGACGATGT-3′. The PCR cycle conditions were 50 ℃ for 2 min, 95 ℃ for 10 min, 95 ℃ for 15 s, and 60 ℃ for 1 min (40 cycles).

Western Blot

Western blotting was performed as described previously (Xuan et al., 2015). Briefly, cellular proteins were extracted with RIPA lysis buffer containing protease inhibitor cocktail. Protein samples were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Blotting membranes were incubated with 3 % bovine serum albumin in tris buffered saline with tween and probed with corresponding primary antibodies. After incubation with horseradish peroxidasecoupled secondary antibodies for 2 h at room temperature, bands were normalized to β-actin and quantified by densitometry (syngene, UK). The name, catalogue numbers and supplier for antibodies are listed in Supplementary Table 2.

Real Time RT-PCR

Total cellular RNA was extracted using TriZol reagent (Invitrogen) and quantified using the spectrophotometer. Reverse transcription was performed with an ExScript RT Reagent Kit (Takara Bio Inc., China). Real-time PCR analysis was undertaken using SYBR Premix Ex Taq (Takara Bio

Inc., China). The real-time PCR conditions were as follows: initial denaturation at 95 ℃ for 10 s followed by 39 cycles of 95 ℃ for 5 s and 60 ℃ for 20 s. Relative expression for the studied genes was normalized to the mean signals of ß-actin. Primers used are listed in Supplementary Table 3.

Quantitative Chromatin Immunoprecipitation

Chromatin immunoprecipitation (ChIP) experiments were performed with the MAGnify ChIP kit (Life Technologies) according to manufacturer's protocol with the following adjustments. Antibodies against H3K4me3, H3K27me3, H3K9me2, H3K9Ac (all four from Diagenode) and H3 (Abcam) were used. A rabbit IgG (Diagenode) was used as a negative control. Cells were grown in 10-cm dishes, washed with Hank's Balanced Salt Solution (HBSS) and then crosslinked by adding 5 mL of HBSS with 1% formaldehyde for 8 min at room temperature. The crosslinking reaction was stopped by adding 500 μL of 1.25 M glycine and incubating 5 min. Dishes were washed 2 × with 5 mL HBSS and then the cells were scraped from the dishes in two steps using 2.5 mL of HBSS with 2% FBS, 0.1% EDTA and protease inhibitors for each scraping. Chromatin was sheared by sonication in a chilled Bioruptor set to high for 42 cycles of 30 sec on/off. Approximately 200,000 cells were used for each ChIP. Input DNA was purified from a sample aliquot equal to 10% of the total cells used for each ChIP. Equal volumes of ChIP and input DNA were quantified by QRT-PCR using the ABI 7500 Real-Time Detection System. The forward and reverse primers were 5′-TGTAGCAGTTTGCTGGTCCT-3′ and 5′-GTAGCTCTGCACGACGATGT-3′.

5-hydroxymethylated DNA Immunoprecipitation (5hmC DIP)-qPCR and 5-methylated DNA Immunoprecipitation (5mC DIP)-qPCR

Genomic DNA was extracted from cultured cells using the TIANamp Genomic DNA Kit (Tiangen Biotech) according to the manufacturer's instructions and sheared into an average 200–500 bp in length by sonication and immunoprecipitated with a rabbit anti-5hmC polyclonal antibody (Active Motif) and a mouse anti-5mC monoclonal antibody (Gene Tex). 5mC DIP-qPCR and 5hmC DIP-qPCR were performed using the EpiQuik methylated DNA Immunoprecipitation and

EpiQuik methylated DNA Immunoprecipitation Kits (Epigentek) according to the manufacturer's instructions. Immunoprecipitated DNA fragments were collected by magnetic beads, purified, and subjected to real-time qPCR using primers specific to Ngn1 promoter loci (5′-TTACGGGCACGCTCCAGG-3′ and 5′-CCTCAGGACCCCTTAAGTACGG-3′). Data were normalized to values of the input DNA.

DNA Extraction and Bisulfite Sequencing

Genomic DNA was isolated from cultured cells using the DNeasy tissue kit (QIAGEN, Valencia, CA). The extracted DNA was subjected to bisulfite conversion. The bisulfite conversion was carried out using 750 ng of DNA and EZ DNA Methylation Gold Kits (Zymo Research Corp., Orange, CA) following the manufacturer's instructions. The converted DNA was amplified by PCR using Platinum PCR SuperMix (Invitrogen, Carlsbad, CA) with one specific set of primers. One fragment of Ngn1 gene was amplified: fragment included the CpG island from −171 to +140, the forward primer was 5′-GYGAGTATAAATTATGTAAATAGTAGG-3′ and the reverse primer was 5′-CTATACCTACTACAAACRCRAA-3′, PCR amplification conditions were: 94 ℃ 3 min; 94 ℃ 30 s, 60 ℃ 45 s, 72 ℃ 45 s, 35 cycles. The PCR products were cloned into pTG19-T vector using a Generay TA Cloning Kit (Generay Biotech, Shanghai, China). 10 clones from each sample were randomly selected for DNA sequencing using QIAprep Spin Miniprep Kit (QIAGEN, Valencia, CA).

Dot Blot

Total DNA was extracted from cultured cells using the TIANamp Genomic DNA Kit (Tiangen Biotech). DNA was denatured at 99 ℃ and spotted on nylon membrane (Millipore). The membrane was incubated at 80 ℃ for 1 hour for crosslinking and then was blocked with 5% nonfat dry milk (BD Bioscience) dissolved in Phosphate Buffered Saline (PBS) containing 0.05% Tween-20 for 1 hour at RT. Membranes were incubated with primary antibodies against 5-hydroxymethylcytosine (5hmC) (1:1000, Active Motif) or 5-methylcytosine (5mC) (1:1000, Epigentek) at 4 ℃ overnight, followed by incubation with secondary antibodies for 1 hour at RT. Secondary antibodies were donkey anti-rabbit IgG or donkey anti-mouse IgG (1:10000, ABCOM).

Supplemental References

Xuan, A.G., Pan, X.B., Wei, P., Ji, W.D., Zhang, W.J., Liu, J.H., Hong, L.P., Chen, W.L., Long, D.H. (2015). Valproic acid alleviates memory deficits and attenuates amyloid-β deposition in transgenic mouse model of Alzheimer's disease. Mol. Neurobiol. 51, 300-312.