

## **Supplementary data**

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Table S1. Primers used for qRT-PCR, MeRIP-qPCR and RIP-qPCR assay.

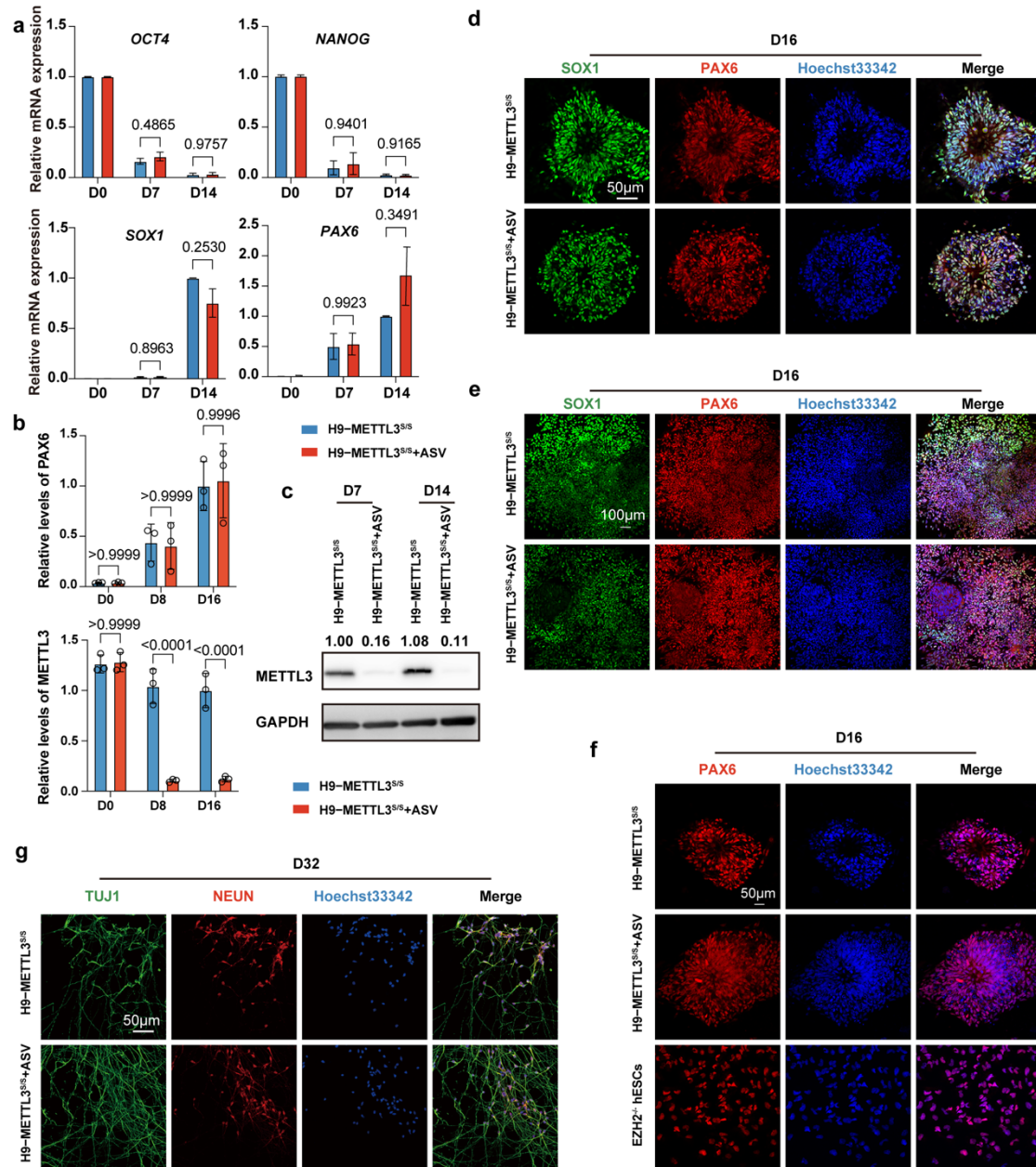
Table S2. Primers used for vector construction.

Table S3. Antibodies used in this study.

Table S4. GO of m<sup>6</sup>A downregulated peaks.

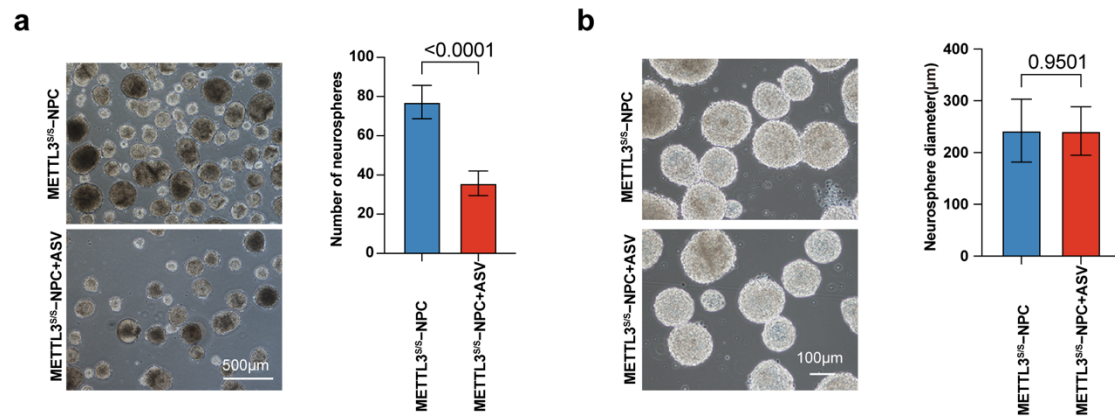
Table S5. GO of upregulated genes in RNA-seq.

Table S6. GO of downregulated genes in RNA-seq.

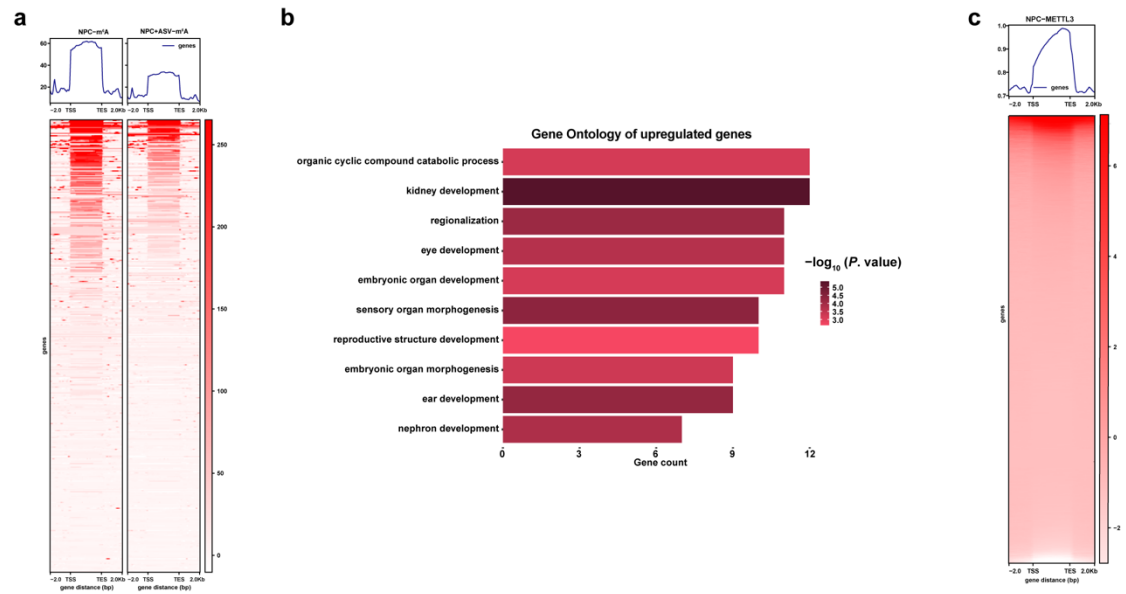


**Figure S1.** NPC differentiation and neuron differentiation of METTL3<sup>s/s</sup> hESCs using EB method. (a) qRT-PCR analysis on the expression level of the pluripotent genes (*NANOG* and *OCT4*) and the hNPC genes (*PAX6* and *SOX1*) in H9-METTL3<sup>s/s</sup> hESCs and H9-METTL3<sup>s/s</sup> hESCs after ASV treatment at day 0, day 7 and day 14 of neural differentiation. H9-METTL3<sup>s/s</sup> hESCs serve as control. Data represent mean ± SD (n = 3, two-way ANOVA). (b) Relative intensities analysis of western blot in Figure 2c. (c) Western blot of H9-METTL3<sup>s/s</sup> hESCs and H9-METTL3<sup>s/s</sup> hESCs after ASV treatment for METTL3 at day 7 and day 14 of neural differentiation. GAPDH was

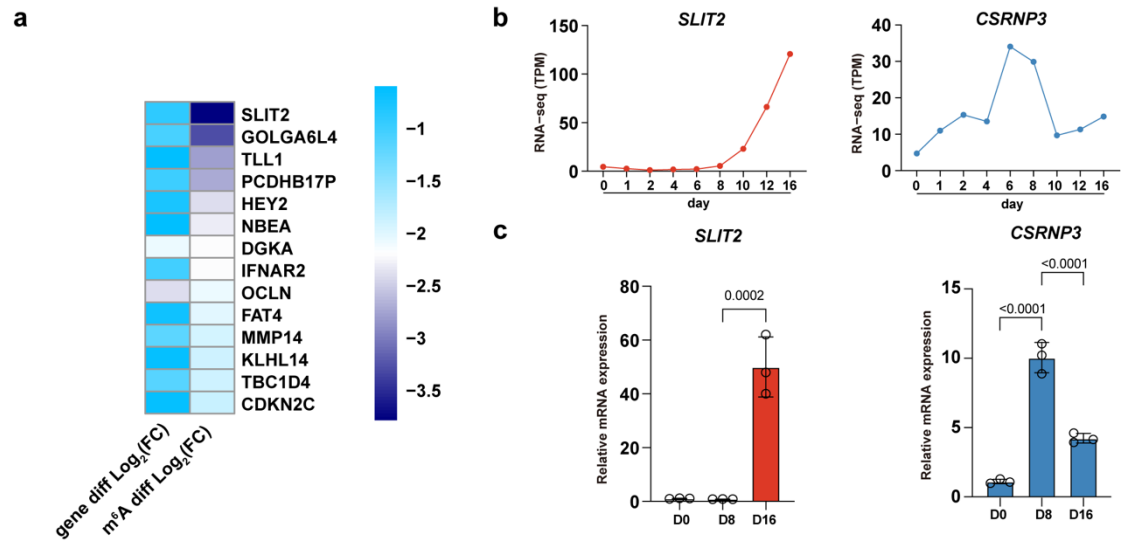
probed as an internal loading control. **(d)** Immunostaining images of H9–METTL3<sup>s/s</sup> hESCs and H9–METTL3<sup>s/s</sup> hESCs after ASV treatment for SOX1 (green) and PAX6 (red) at day 16 of neural differentiation. Scale bar, 50  $\mu$ m. **(e)** Immunostaining images show the rosette-like morphology of H9–METTL3<sup>s/s</sup> hESCs and H9–METTL3<sup>s/s</sup> hESCs after ASV treatment for SOX1 (green) and PAX6 (red) at day 16 of neural differentiation. Scale bar, 100  $\mu$ m. **(f)** Immunostaining images show the rosette-like morphology of H9–METTL3<sup>s/s</sup> hESCs, H9–METTL3<sup>s/s</sup> hESCs after ASV treatment and EZH2<sup>-/-</sup> hESCs (negative control) at day 16 of neural differentiation. Scale bar, 50  $\mu$ m. **(g)** Immunostaining images of H9–METTL3<sup>s/s</sup> hNPCs and H9–METTL3<sup>s/s</sup> hNPCs after ASV treatment for TUJ1 (green) and NEUN (red) at day 32 of neural differentiation. Scale bar, 50  $\mu$ m.



**Figure S2.** Characterization of METTL3<sup>s/s</sup> hNPCs using EB method. **(a)** The number of neurospheres in METTL3<sup>s/s</sup> hNPCs and METTL3<sup>s/s</sup> hNPCs after ASV treatment. **(b)** The diameter of neurospheres in METTL3<sup>s/s</sup> hNPCs and METTL3<sup>s/s</sup> hNPCs after ASV treatment.



**Figure S3.** Bioinformatic outcomes of METTL3<sup>s/s</sup> hNPCs with or without ASV. **(a)** The heatmap of m<sup>6</sup>A peaks in METTL3<sup>s/s</sup> hNPCs and METTL3<sup>s/s</sup> hNPCs after ASV treatment. **(b)** Gene Ontology (GO) analysis for upregulated genes in H9-METTL3<sup>s/s</sup> hNPCs after ASV treatment. **(c)** The heatmap of METTL3 peaks in H9-METTL3<sup>s/s</sup> hNPCs.



**Figure S4.** *SLIT2* is the key gene associated with hNPCs proliferation. **(a)** The heatmap of top genes in m<sup>6</sup>A differential genes and differential expression genes in METTL3<sup>s/s</sup> hNPCs and METTL3<sup>s/s</sup> hNPCs after ASV treatment. **(b)** The expression of *CSRNP3* and *SLIT2* in time-course RNA-seq. **(c)** qRT-PCR analysis on the expression level of *CSRNP3* and *SLIT2* in H9-METTL3<sup>s/s</sup> hESCs at day 0, day 8 and day 16 of neural differentiation. H9-METTL3<sup>s/s</sup> hESCs serve as control. Data represent mean  $\pm$  SD ( $n = 3$ , two-way ANOVA).

## Supplementary Tables

**Table S1.** Primers used for RT-qPCR, MeRIP-qPCR and RIP-qPCR assay.

hGAPDH-qF	ATGACATCAAGAAGGTGGTG	RT-qPCR
hGAPDH-qR	CATACCAGGAAATGAGCTTG	RT-qPCR
hOCT4-qF	ACATCAAAGCTCTGCAGAAA GAACT	RT-qPCR
hOCT4-qR	CTGAATACCTTCCCAAATAGA ACCC	RT-qPCR
hNANOG-qF	ATTCTTCCACCAGTCCCAA	RT-qPCR
hNANOG-qR	ATCTGCTGGAGGCTGAGGTA	RT-qPCR
hSOX1-qF	AATTTTATTTTCGGCGTTGC	RT-qPCR
hSOX1-qR	TGGGCTCTGTCTCTTAAATTT GT	RT-qPCR
hPAX6-qF	TCTTTGCTTGGGAAATCCG	RT-qPCR
hPAX6-qR	CTGCCCCGTTCAACATCCTTAG	RT-qPCR
qh-SLIT2-F2	AGCTTAGACGAATTGACCTG AGC	RT-qPCR
qh-SLIT2-R2	CCGAAGGCAGTTTATCTTGTT GG	RT-qPCR
qh-CSRNP3-F1	CTGTGACTGCCGAGTGTCT	RT-qPCR
qh-CSRNP3-R1	ACATACGATCCACCTGGCAC	RT-qPCR
MeRIP-qPCR-SLI T2-F	TGGGAACCATTGCAACTCGG G	MeRIP-qPCR&RIP-q PCR
MeRIP-qPCR-SLI T2-R	AGCTCCTCTGTCCCTCGTGC	MeRIP-qPCR&RIP-q PCR

**Table S2.** Primers used for vector construction.

METTL3-sg RNA-3-Olig o1	CACCGTATGGATTCTTAGCTCTGTA
METTL3-sg RNA-3-Olig o2	AAACTACAGAGCTAAGAATCCATAC
METTL3-H L-F2	GATCTTCCGGATGGCGTGAGCAGGTTGGTGTCAAAG
METTL3-H L-R	TATGGGTATAAATTCTTAGGTTTAGAGATGATACCATCTGG
METTL3-H R-F1	ATAACTTCGTATAATGTATGCTATACGAAGTTATAAGCACTTCC TTACAGAGCTAAG
METTL3-H R-R2	ATTGTAGGAGATCTTGTGGTGGGGTTGAAGAACTTGTTG
PURO-F1	GTACTAAATAACTTCGTATAATGTATGCTATACGAAGTTATTAC CGGGTAGGGGAGGCGC
PGK-R1	ACATTATACGAAGTTATCAGGCGGGGAGGCGGCCCAAAGGGA G
SMASh-F1	GAATTTATACCCATACGATGTTCCAGATTACGCTTATCCCTACG ACGTGCCTGATTATGCATACCCATATGATG
SMASh-F2	GATTATGCATACCCATATGATGTCCCCGACTATGCCGACGAGA TGGAGGAGTGTTCCCAG



**Table S3.** Antibodies used in this study.

Antibodies	Manufacture	Catalog
METTTL3 antibody	Abcam (Cambridge, MA, USA)	ab195352
SOX1 antibody	R&D system (Minneapolis, MN, USA)	AF3369
PAX6 antibody	Biolegend (San Diego, CA, USA)	901301
MAP2 antibody	Abcam (Cambridge, MA, USA)	ab32454
SOX2 antibody	R&D system (Minneapolis, MN, USA)	AF2018
NEUN antibody	Milipore (Boston, MA, USA)	ABN78
TUJ-1 antibody	Biolegend (San Diego, CA, USA)	801201
m <sup>6</sup> A antibody	Abcam (Cambridge, MA, USA) /Synaptic Systems (German)	ab286164 /202203
Rabbit IgG	CST (Boston, CA, USA)	2729
Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488	Invitrogen (Carlsbad, CA, USA)	A11055
Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594	Invitrogen (Carlsbad, CA, USA)	A11058
Donkey anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488	Invitrogen (Carlsbad, CA, USA)	A11008
Donkey anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594	Invitrogen (Carlsbad, CA, USA)	A11012
Anti-rabbit IgG, HRP-linked Antibody	CST (Boston, CA, USA)	7074s

**Table S4.** GO of m<sup>6</sup>A downregulated peaks.

ID	Description	pvalue	Count
GO:0008380	RNA splicing	9.11E-16	68
GO:0022613	ribonucleoprotein complex biogenesis	6.96E-12	63
GO:0016570	histone modification	2.20E-08	55
GO:0140694	non-membrane-bounded organelle assembly	1.12E-10	52
GO:0048285	organelle fission	7.63E-06	50
GO:0000280	nuclear division	1.96E-06	48
GO:0006325	chromatin organization	6.15E-07	47
GO:0044772	mitotic cell cycle phase transition	1.71E-06	47
GO:0006260	DNA replication	5.66E-13	46
GO:0007346	regulation of mitotic cell cycle	5.67E-05	45
GO:0006417	regulation of translation	0.00128771	41
GO:0050808	synapse organization	0.00038446	40
GO:0010975	regulation of neuron projection development	0.00513974	37
GO:0061564	axon development	0.01736094	36
GO:0030900	forebrain development	0.00218787	34

**Table S5.** GO of upregulated genes in RNA-seq.

ID	Description	pvalue	Count
GO:0001822	kidney development	4.35E-06	12
GO:1901361	organic cyclic compound catabolic process	0.00065042	12
GO:0003002	regionalization	7.49E-05	11
GO:0001654	eye development	0.00020362	11
GO:0048568	embryonic organ development	0.00066608	11
GO:0090596	sensory organ morphogenesis	4.19E-05	10
GO:0048608	reproductive structure development	0.00223968	10
GO:0043583	ear development	5.60E-05	9
GO:0048562	embryonic organ morphogenesis	0.00049445	9
GO:0072006	nephron development	0.00014847	7

**Table S6.** GO of downregulated genes in RNA-seq.

ID	Description	pvalue	Count
GO:0007389	pattern specification process	7.24E-10	27
GO:0061564	axon development	5.32E-07	23
GO:0034329	cell junction assembly	8.50E-07	22
GO:0030900	forebrain development	6.08E-06	19
GO:0050808	synapse organization	3.11E-05	19
GO:0016055	Wnt signaling pathway	0.00016201	18
GO:0050673	epithelial cell proliferation	0.00016753	17
GO:0030111	regulation of Wnt signaling pathway	0.00022437	15
GO:0050678	regulation of epithelial cell proliferation	0.00040783	15
GO:0030198	extracellular matrix organization	0.00078352	14
GO:0031589	cell-substrate adhesion	0.00102055	14
GO:0010631	epithelial cell migration	0.00141276	13
GO:0043410	positive regulation of MAPK cascade	0.00339217	13
GO:0098742	cell-cell adhesion via plasma-membrane adhesion molecules	0.01021831	12
GO:0050767	regulation of neurogenesis	0.03291139	12