Supporting Information

A Synthetic DNA-binding inhibitor of HES1 alters the Notch signaling pathway and induces neuronal differentiation

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KEYWORDS: Synthetic DNA-binding inhibitor, Artificial transcription modulators, Neurogenesis, Regenerative medicine, Therapeutics

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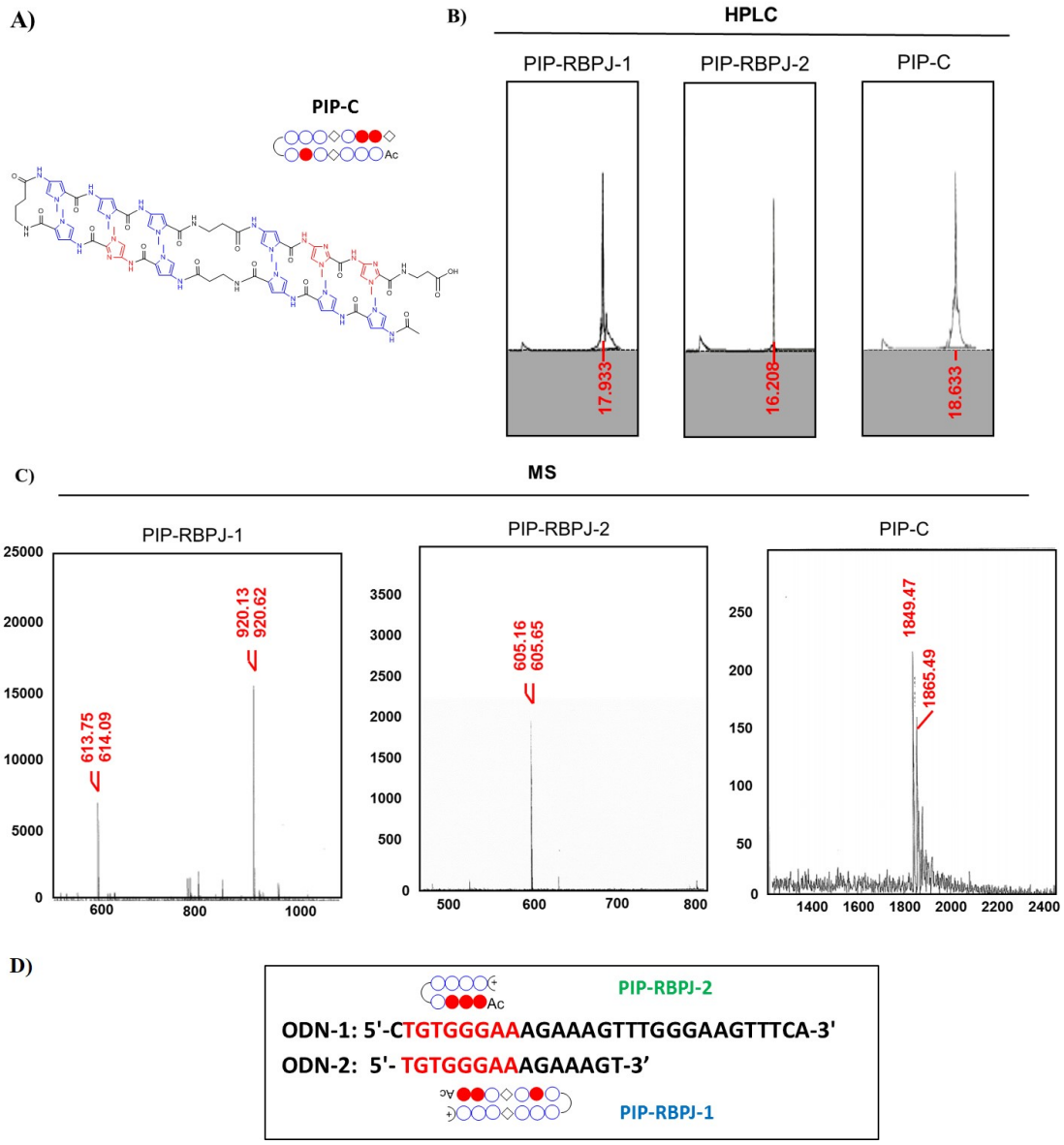


Figure S1. A) The chemical structure of the control PIP called PIP-C**. B)** HPLC and **C)** MS profile of the PIPs used in this study. **D)** The ODN sequence and binding site of PIPs used in the T_m assay. Red color stand for the core RBPJ protein binding site.

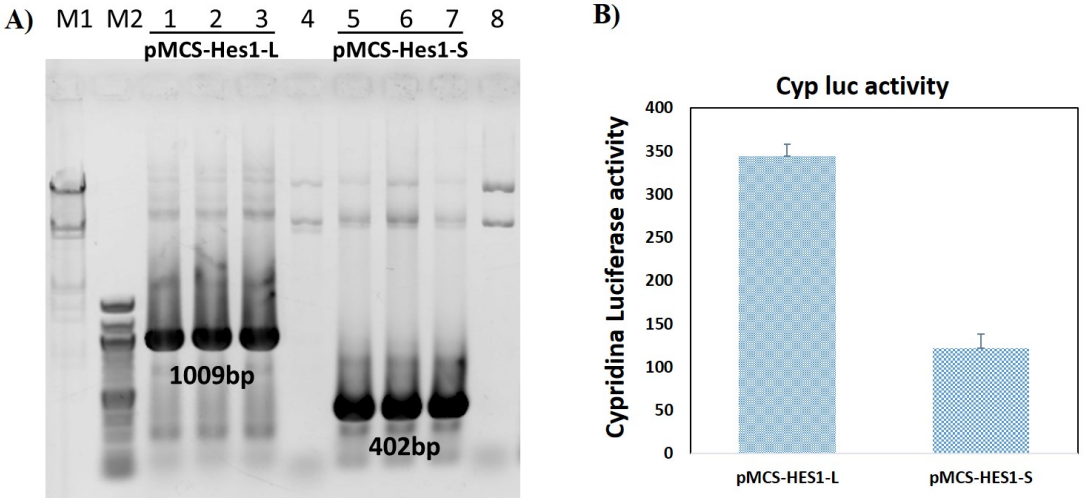


Figure S2 The design of plasmid for *HES1*. A) The cloning of the plasmid pMCS-HES1-L and pMCS-HES1-S were checked. The cloned *E.coli* were selected and the plasmid was checked using PCR. The 1009 bp and 402 bp promoter region was correctly inserted into the plasmid. Lanes 4 and 8 represents the negative control without the inserted sequence. B) Luciferase activity of the pMCS-HES1-L and pMCS-HES1-S plasmids show that the 1008bp inserted promoter exhibited a better luciferase activity.

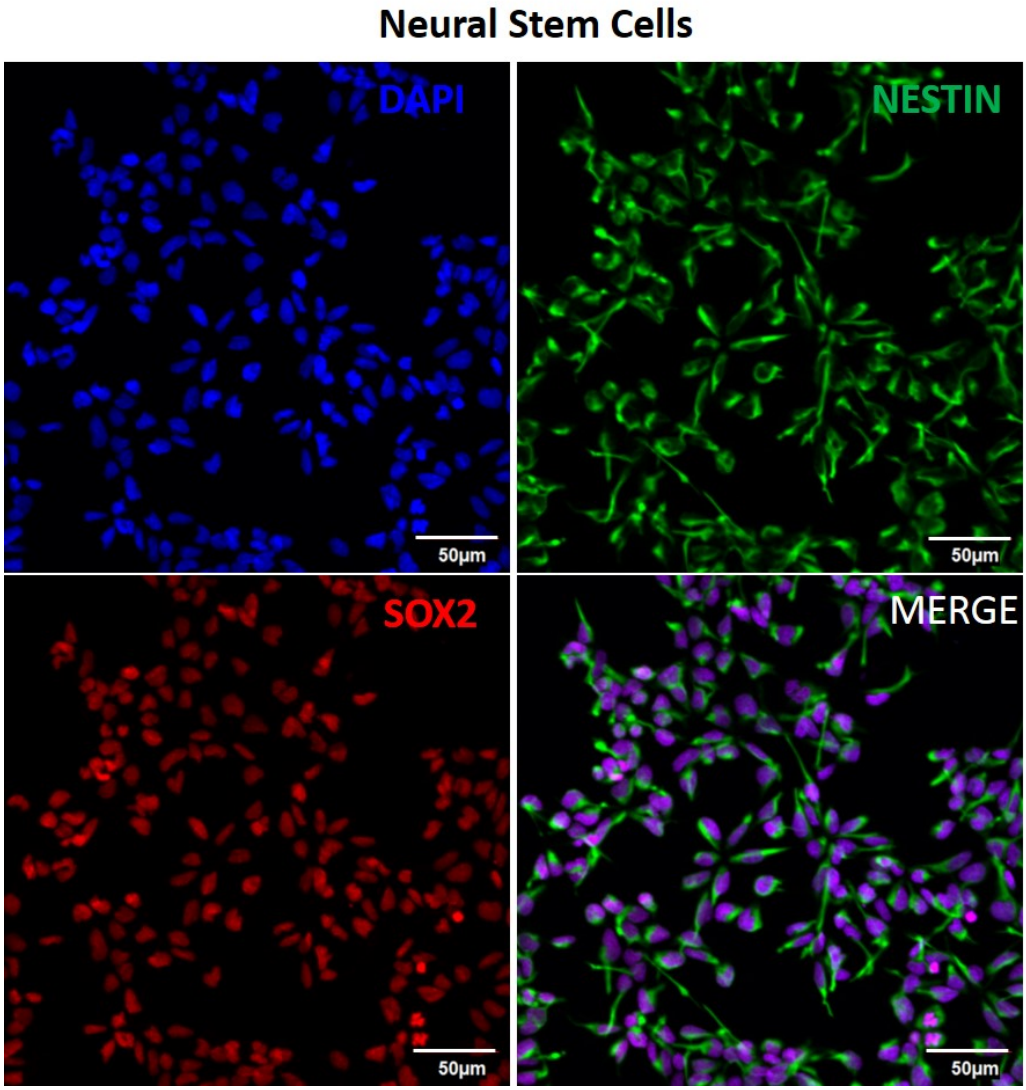


Figure S3. Immunostaining of hNSCs with the markers suggesting the presence of undifferentiated cells. Fluorescence image of the hNSCs at P5 with the neuron markers NESTIN (green) and proliferation marker Sox2 (red). Cell nuclei were stained with DAPI (blue).

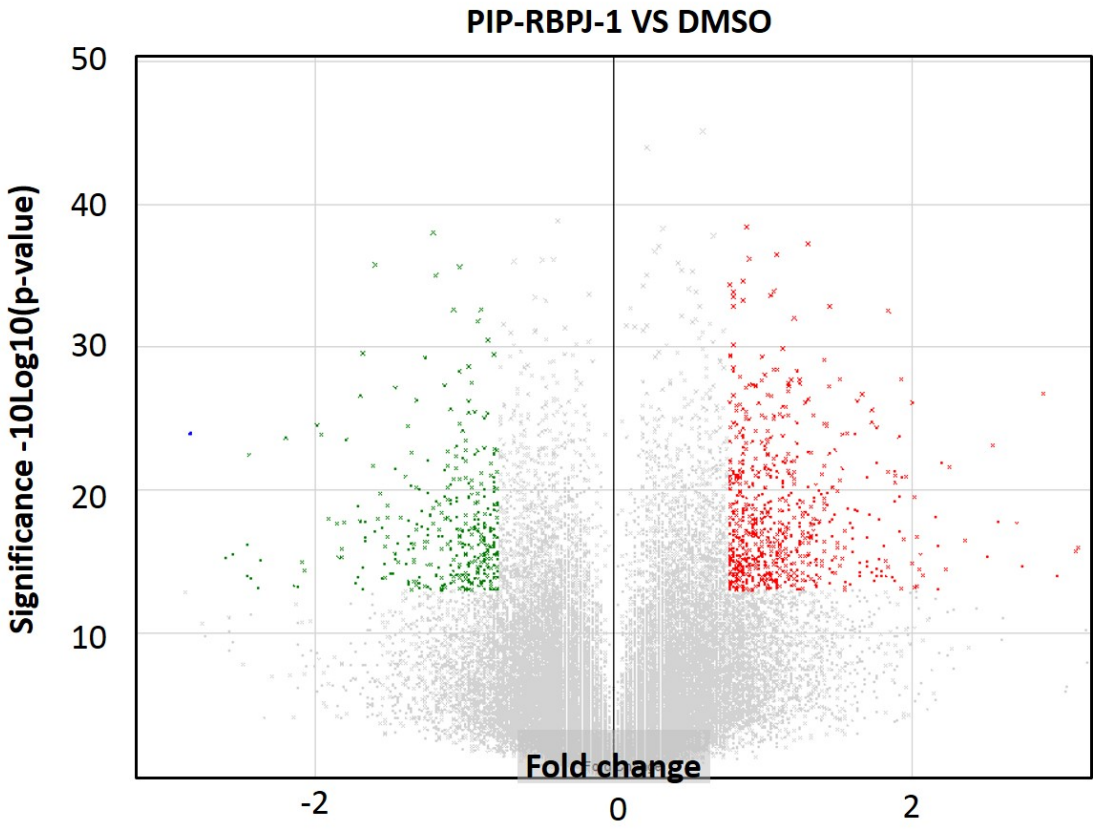
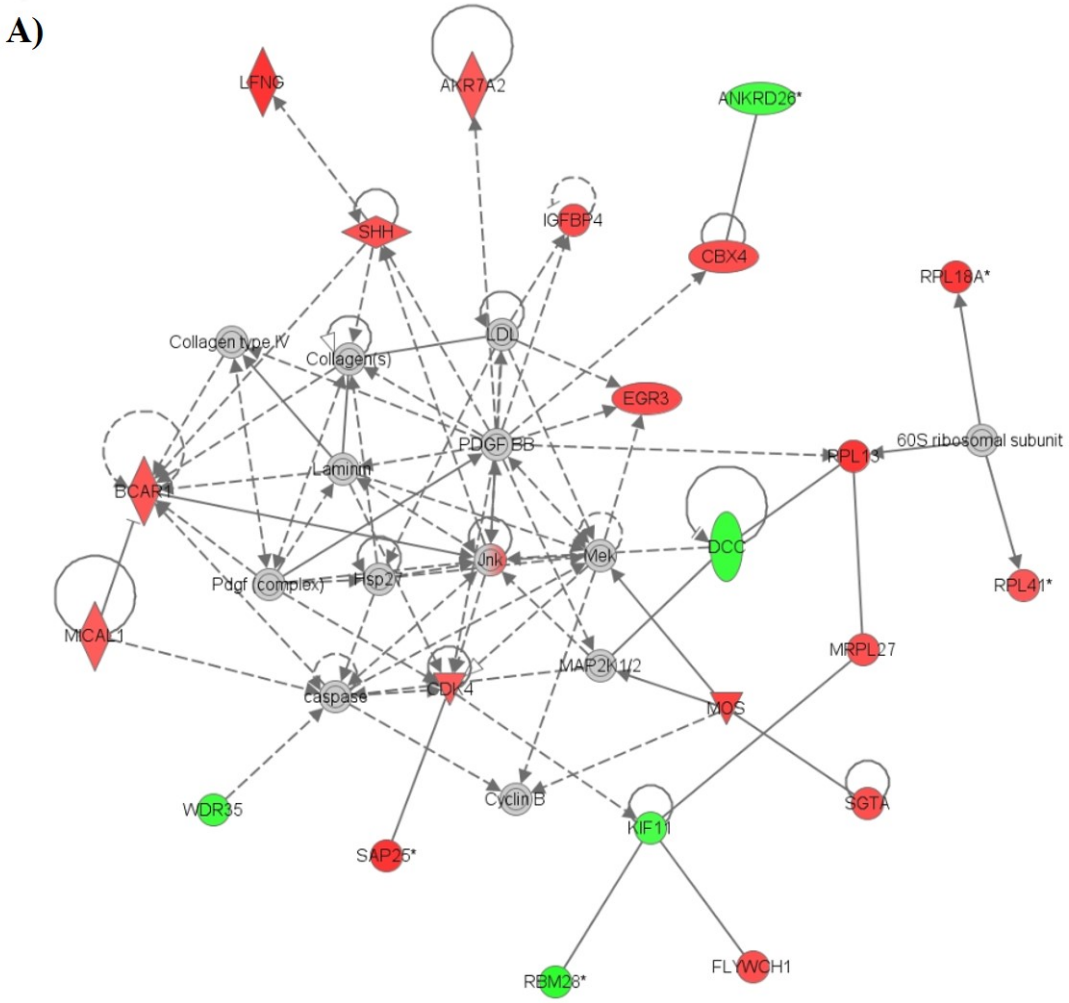


Figure S4. Volcano plot of the PIP-RBPJ-1 treated NSCs compared with DMSO treated cell. The red mark indicate the up-regulated genes and the green mark indicate the down-regulated genes with fold change more than 1.3 and *p*-value <0.05.

A)



Neurological disease related Network

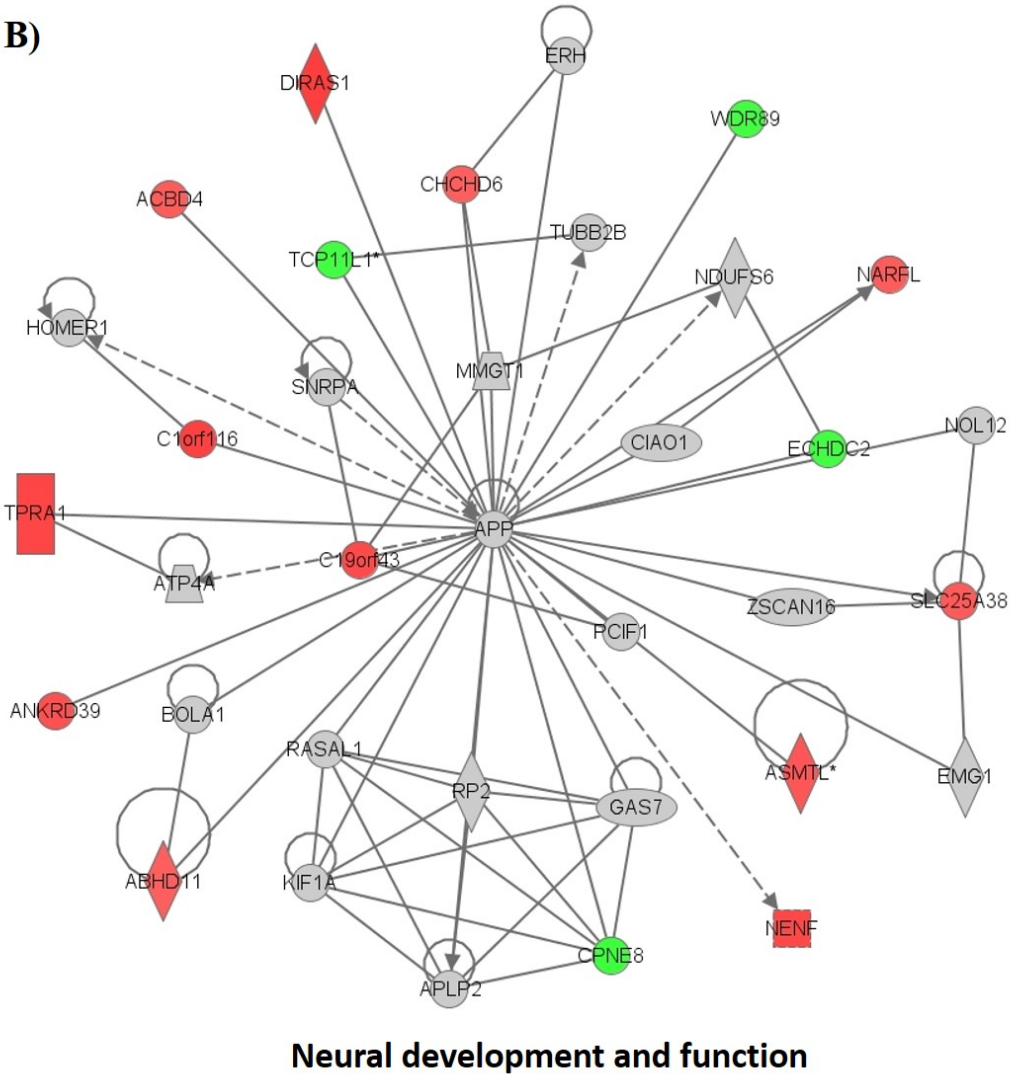


Figure S5. Network analysis of PIP-RBPJ-1 modulated genes showing the networks associated with A) neurological diseases, B) neural development and function. 1.3-fold induction was chosen as the effect to account for the down-stream effect. Each result is the summary of analysis of data derived from two individual culture plates. Data were analyzed through the use of IPA (Ingenuity® Systems, www.ingenuity.com).

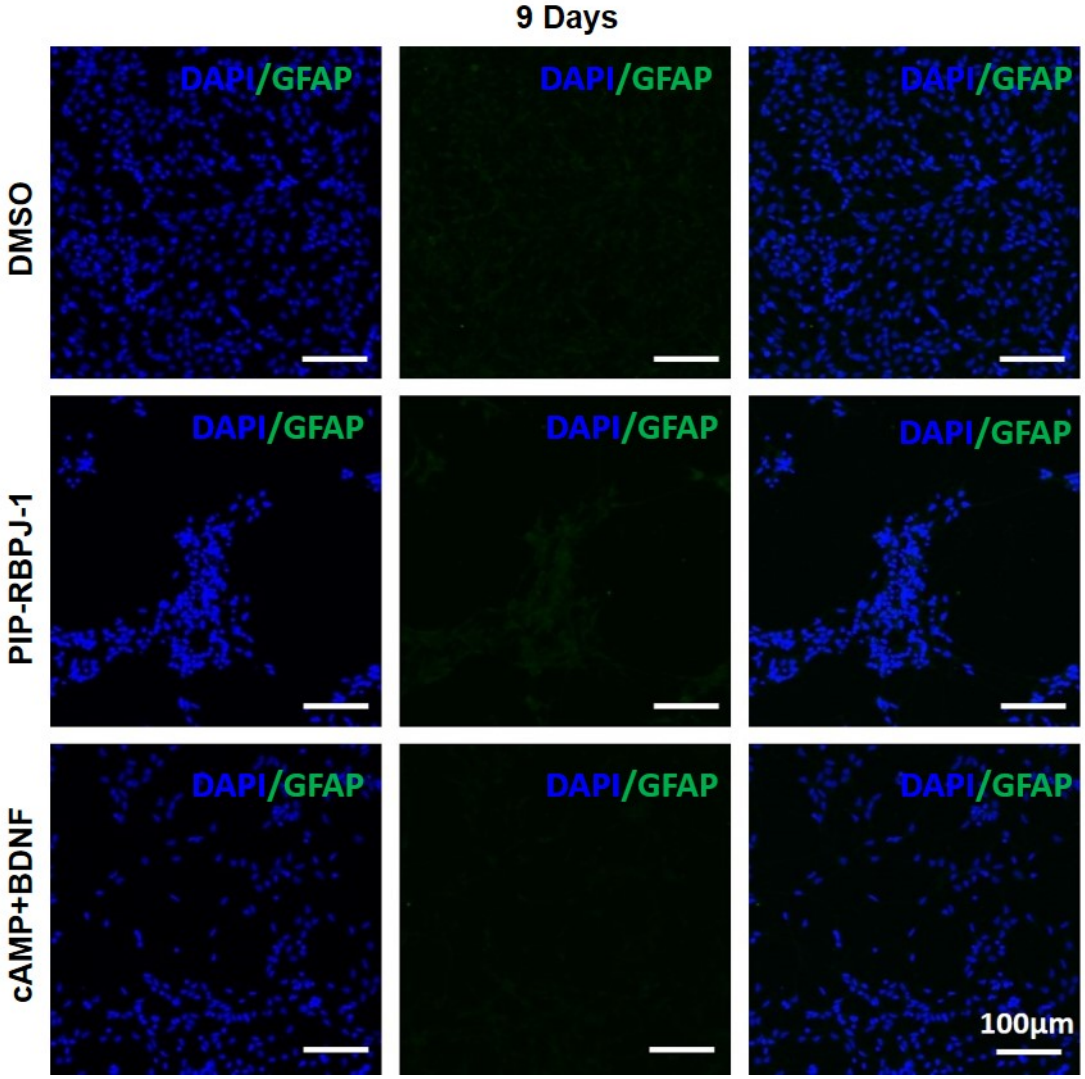


Figure S6. Glial fibrillary acidic protein (GFAP) immunostaining of 9 days treatment of hNSCs. The glial cell marker GFAP was stained with Alex488 (Green), cell nuclei were stained with DAPI (blue).

Table S1 Functions Annotation of the nervous genes modulated in PIP-treated cells

Functions Annotation in nervous system	p-Value
Outfolding of myelin sheath	1.27E-03
Proliferation of cerebellar granule cell	2.51E-03
Quantity of lutenizing hormone-releasing hormone neurons	4.12E-03
Contact repulsion	5.74E-03
Proliferation of motor neurons	1.11E-02
Synaptic transmission of superior cervical ganglion neurons	1.11E-02
Coordination	1.45E-02
Proliferation of anterior pituitary cells	1.73E-02
Activation of oxytocin neurons	2.08E-02
Area of lateral cerebral ventricle	2.08E-02
Arrest in cell cycle progression of astrocytes	2.08E-02
Curvature of nerves	2.08E-02
Development of vestibulocochlear cranial nerve ganglion	2.08E-02
Differentiation of Bergmann glia	2.08E-02
Differentiation of excitatory synapses	2.08E-02
Differentiation of inhibitory synapse	2.08E-02
Exit from cell cycle progression of oligodendrocyte precursor cells	2.08E-02
Extension of climbing fiber	2.08E-02
Fission of synaptic vesicles	2.08E-02
Generation of cholinergic neurons	2.08E-02
Length of dorsal root ganglion cells	2.08E-02
Morphology of stratum lucidum	2.08E-02
Quantity of cortical precursor cells	2.08E-02
Quantity of oxytocin neurons	2.08E-02
Quantity of striatonigral neurons	2.08E-02
Regeneration of cavernous nerve	2.08E-02
Synaptic transmission of excitatory synapses	2.08E-02
Synaptic transmission of inhibitory synapse	2.08E-02
Vacuolation of axons	2.08E-02
Quantity of Purkinje cells	2.60E-02
Morphology of dorsal root ganglion	2.72E-02
Proliferation of nervous tissue cell lines	3.30E-02
Cell cycle progression of neurons	3.32E-02
Myelination of central nervous system	3.56E-02
Morphology of spinal cord	3.59E-02
Migration of nervous tissue cell lines	3.77E-02
Synaptic transmission of nervous tissue	4.05E-02

Table S2 The calculated neuron efficiency and neurite length difference of PIPs and the DMSO-treated hNSCs.

	Neuron Efficiency	Neurite Length
DMSO	12.4%	62.6
PIP-RBPJ-1	42.3%	117.5
PIP-RBPJ-2	14.4%	59.1

Table S3 Primers used in q-RT PCR and *HES1* plasmid construction

q-RT-PCR

	Forward sequence	Reward sequence
HES1	ACGACACCGGATAAACCAAA	CGGAGGTGCTTCACTGTCAT
ASCLI1	GTGCGTGTTAGAGGTGATGG	TGCACTCCAATCATTCACG
NGN2	GGGACAGGAAAGGGAACC	TCAGACATGGACTATTGGCAG
NOTCH1	GAACCAATACAACCCTCTGCG	TAGCTCATCATCTGGGACAGG
DLL1	ACAGCAAGCGTGACACCAAG	TGAAGTTGAACAGCCCGAGT
JAG1	GTCGGGATTTGGTTAATGGTTATCG	TCACTGGCACGGTTGTAGCAC
TUJ1	GCTCAGGGGCCTTTGGACATCTCTT	TTTTCACACTCCTTCCGCACCACATC
ACTIN-β	CAATGTGGCCGAGGACTTTG	CATTCTCCTTAGAGAGAAGTGG

***HES1* promoter cloning**

	Forward sequence	Reward sequence
***HES1*-L**	CGGAATTCGCAGAACCTAAAGCCTACGG	CCCAAGCTTCACAAAACTACTGAGCAAGTGC
***HES1*-S**	CGGAATTCCAAAGCCCAGAGGGAGAGT	CCCAAGCTTCACAAAACTACTGAGCAAGTGC