

## **SUPPLEMENTARY INFORMATION**

### **Lin28B and miR-142-3p regulate neuronal differentiation by modulating Staufen1 expression**

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Running title: STAU1 expression in neuronal differentiation

## SUPPLEMENTARY FIGURE LEGENDS

**Supplementary Figure S1.** Change in STAU1 expression during differentiation also affects neuronal differentiation. **(a)** Schematic representation of the experiments. Differentiation was induced in SH-SY5Y cells by retinoic acid. **(b)** At day 3 of differentiation, cells were transiently transfected with siRNAs (siControl or siSTAU1) and then kept under differentiation conditions for an additional 4 days. Western blot was performed to detect the indicated proteins. **(c)** Depletion of UPF1 reduced neuronal differentiation. SH-SY5Y cells were transfected with siControl or siUPF1 to decrease the expression of UPF1 and then differentiated. The expression of specified proteins on the indicated incubation day was detected by Western blot.

**Supplementary Figure S2.** Conserved sequences in the 3'UTR of STAU1 and the predicted RNA secondary structures. **(a)** miR-142-3p binding sites in the 3'UTR of STAU1 were conserved among mammals. **(b)** The secondary structures of WT or mutated pre-miR-142 (predicted using the mFold web server) and the changes in free energy by loss of "GG" and mutation of "A" to "G" in normal pre-miR-142.  $\Delta G$ , Gibbs free energy.

**Supplementary Figure S3.** Depletion or overexpression of STAU1 or Lin28B did not affect cell proliferation. SH-SY5Y cells that were transiently transfected with STAU1<sup>55</sup>-HA<sub>3</sub> **(a)** or FLAG-Lin28B **(b)** proliferated for 5 days. Protein expression was analyzed by Western blot (left panel), and the number of cells in a 35-mm dish were counted at the indicated day (right panel). Western blot and cell counting were also performed with STAU1- **(c)** and Lin28B-depleted cells **(d)**. Cell numbers were obtained by counting cells by using a hemocytometer on the indicated days. \*\*,  $p < 0.01$ .

**SUPPLEMENTARY TABLE**

**Supplementary Table S2. List of transcripts upregulated upon UPF1 and STAU1 depletion and differentiation (categorized to “anatomical structure formation involved in morphogenesis”).**

Gene name	NCBI reference	Description	Fold change		
			siUPF1 /siControl	siSTAU1 /siControl	Differentiation /proliferation
CD36	NM_001001547	CD36 molecule	59.20	11.40	2.83
PERP	NM_022121	PERP, TP53 apoptosis effector	3.40	15.20	2.40
RAB17	NM_022449	RAB17, member RAS oncogene family	4.60	7.60	68.00
TBX5	NM_080171	T-box 5	7.20	7.60	7.00
TBC1D30	XM_037557	TBC1 domain family, member 30	3.40	10.00	7.00
ANGPTL4	NM_139314	angiopoietin-like 4	3.14	2.31	148.40
ANGPTL6	NM_031917	angiopoietin-like 6	4.60	2.40	2.76
COL4A3	NM_000091	collagen, type IV, alpha 3	2.40	3.60	6.00
EPAS1	NM_001430	endothelial PAS domain protein 1	2.69	2.31	7.17
FAP	NM_001291807	fibroblast activation protein, alpha	2.40	4.80	26.80
HOXA1	NM_005522	homeobox A1	2.40	4.80	10.60
ITGA3	NM_005501	integrin, alpha 3	2.40	17.80	3.95
KRT8	NM_002273	keratin 8	3.35	32.92	5.45
MEGF11	NM_032445	multiple EGF-like -domains 11	2.40	6.00	2.80
NGFR	NM_002507	nerve growth factor receptor	2.40	6.00	8.83
PTK2B	NM_004103	PTK2B protein tyrosine kinase 2 beta	35.20	20.40	2.12
RS1	NM_000330	retinoschisin 1	2.48	4.57	2.33
THBS2	NM_003247	thrombospondin 2	8.00	4.00	4.22
TMEM100	NM_018286	transmembrane protein 100	7.20	2.40	2.09

**Supplementary Table S3. Sequences used in this study**

RT-qPCR primer sequences	
Primer name	Sequence
STAU1-F	5'- TCAATCCGATTAGCCGACTG -3'
STAU1-R	5'- TCTTTATGGGTGTCTTCTCCTCTG -3'
FLuc-F	5'- CCCCATCTTCGGCAACCAG -3'
FLuc-R	5'- GCGCAAGAATAGCTCCTCC -3'
RLuc-F	5'- GTGGGCCACGACTGGGGGGC -3'
RLuc-R	5'- ACCATTTTCTCGCCCTCTTCGC -3'
Synaptophysin-F	5'- CAACACCTCGGTGGTGTTCG -3'
Synaptophysin-R	5'- CCTGAGGCCCGTAGGAATC -3'
Nestin-F	5'- CGCACCTCAAGATGTCCCTC -3'
Nestin-R	5'- CAGCTTGGGGTCCTGAAAGC -3'
HMGA2-F	5'- CTGCTATACACAAGCAATGCAAG -3'
HMGA2-R	5'- GTAAGGAGATTGCTTCTTTAACTG -3'
U6-F	5'- TCGCTTCGGCAGCACATATAC -3'
U6-R	5'- TCGTGTGCATCCTTGCGCAG -3'
pri-miR-142-3p-F	5'- CGGACTTCTCATGTCCGG -3'
pri-miR-142-3p-R	5'- CGTCGTCACCGCACTA -3'
pri-let-7a-F	5'- CCTGGATGTTCTTCTTCACTG -3'
pri-let-7a-R	5'- GCCTGGATGCAGACTTTTCT -3'
pri-miR-16-F	5'- GTCAGCAGTGCCTTAGCAGC -3'
pri-miR-16-R	5'- GTCAACCTTACTTCAGCAGC -3'
pre-miR-142-F	5'-GACAGTGCAGTCA-3'
pre-miR-142-R	5'-CTCATCCATAAAG-3'
pre-let-7a-F	5'-TGGGATGAGGTAGTAGG-3'
pre-let-7a-R	5'-TAGGAAAGACAGTAGATTG-3'
mSTAU1-F	5'-TGCGCTGAAGCGGAATTTGC-3'
mSTAU1-R	5'-CCCTTGCCGCATTCTTCTTGGAG-3'
mLin28B-F	5'-GCACATGGTGGCCAACTG-3'

mLin28B-R	5'-GCTGCAAATGCTTTCGTGGC-3'
mLin28A-F	5'-CTTTGTGCACCAGAGCAAGC-3'
mLin28A-R	5'-TAAAGGTGAACTCCACCGCC-3'
mNestin-F	5'-GGAGTGTGCTTAGAGGT-3'
mNestin-R	5'-TCCAGAAAGCCAAGAGAA-3'
mGAPDH-F	5'-CTCATGACCACAGTCCATGC-3'
mGAPDH-R	5'-TTCAGCTCTGGGATGACCTT-3'
ARHGDIB-F	5'-CAGAAGTCCCTGAAAGAGCTG-3'
ARHGDIB-R	5'-GGCTTCCAGATCTCCAGTAAG-3'
DACT1-F	5'-TCTTGGAGGAGAACATCTTGC-3'
DACT1-R	5'-TCACTCAGCTCATAAAACCCTG-3'
HPSE-F	5'-TGGTTAGGAGAAACAAGCTCTG-3'
HPSE-R	5'-ACCACTTCTATTCCCATTTCGG-3'
KCNG1-F	5'-CGGGAACCTGAGGCCCGA-3'
KCNG1-R	5'-TAGAACGCGCCCTTGATGG-3'
DEPDC4-F	5'-GAACCGTAGAGATGGCTTCTG-3'
DEPDC4-R	5'-ACTTAAGACCACATCGACAGC-3'
COL5A1-F	5'-CAGATGGTGAATACTGGGTCG-3'
COL5A1-R	5'-CACATAGGAGAGCAGTTTCCC-3'
PPARG-F	5'-TTGAATGTCGTGTCTGTGGAG-3'
PPARG-R	5'-CAAGGCATTTCTGAAACCGAC-3'
B3GAT1-F	5'-AGAACTTGTCACCCTCAACG-3'
B3GAT1-R	5'-TTCTTGCCCTCATTACCAG-3'
FBXO4-F	5'-GGAAAGAGATAGAGCAAGGGAAG-3'
FBXO4-R	5'-GAAAATTCATCTTGCCATTCATGTC-3'
ERN1-F	5'-TCCTCATATCCATGCCCAATG-3'
ERN1-R	5'-AGAAAAGATGTCCACCGTGTAG-3'
PTHLH-F	5'-GTGTCCCCTAACTCCAAGCC-3'
PTHLH-R	5'-TTGAGCGGCTGCTCTTTGTA-3'
DLX2-F	5'-CCTTACTCCGCCAAGAGCAG-3'
DLX2-R	5'-CTCCTTCTCAGGCTCGTTGTT-3'

ULK2-F	5'-CGGATGACTTTGTTTTGGTGC-3'
ULK2-R	5'-AGGAACTGGAATTGGTGCTG-3'
CELF2-F	5'-GCATGAAACGCTTGAAGGTG-3'
CELF2-R	5'-AGAAACAATGACGGGACGATG-3'
KLRG2-F	5'-TGTCATTGTGGTCCTGGC-3'
KLRG2-R	5'-GTCCTGGGTGTGGCTTAG-3'
THBS2-F	5'-GACAACTGCCCCTACATCTC-3'
THBS2-R	5'-ACAAATATCACCCCGTCCATC-3'
NGFR-F	5'-CCTGTCTATTGCTCCATCCTG-3'
NGFR-R	5'-CTGGTCATGCAGGCTCTG-3'
NRG2-F	5'-CACTCCTGTTCTCCTTCTCAC-3'
NRG2-R	5'-CAGAAGTCAGGCTCTCAGAAC-3'
PTK2B-F	5'-CTAGGAAAGATGGTGAGAAGCG-3'
PTK2B-R	5'-ATTCAGGACCACATCTTCACG-3'
STAT4-F	5'-GACATTCCCAAAGACAAAGCC-3'
STAT4-R	5'-CTCTCAACACCGCATAACA-3'
PPARG-F	5'-TTGAATGTCGTGTCTGTGGAG-3'
PPARG-R	5'-CAAGGCATTTCTGAAACCGAC-3'
NPNT-F	5'-CGGCAACCTTCAAATGACTTG-3'
NPNT-R	5'-TGTCAGATATTGTCCACCTGC-3'
ADIPOQ-F	5'-TGCAGTCTGTGGTTCTGATTC-3'
ADIPOQ-R	5'-TCGTGGTTTCCTGGTCATG-3'
CD36-F	5'-CAGCCTCATTTCACCTTTTG-3'
CD36-R	5'-TGTCTGGGTTTTCAACTGGAG-3'
PERP-F	5'-GGTGATCTGTTTCATCCTCTCC-3'
PERP-R	5'-CCGTAGGCCCAGTTATAGATG-3'
c-Jun-F	5'-CTTGAAAGCTCAGAACTCGG-3'
c-Jun-R	5'-TCAGCCCCCGACGGTCTCTC-3'
IL7R-F	5'-GCTCAAAATGGAGACTTGGAAG-3'
IL7R-R	5'-CGAGGGCCCCACATATTC-3'
ZNF75D-F	5'-GACCCACTACCATCTTCCAAG-3'

ZNF75D-R	5'-GTTCCCTCATTTCGATCCCTC-3'
LACZ-F	5'-GTACGTCTTCCCGAGCGAAA-3'
LACZ-R	5'-CTGTTGACTGTAGCGGCTGA-3'
GAPDH	5'-CTGTGGTCATGAGTCCTTCC-3'
GAPDH	5'-CAAGATCATCAGCAATGCC-3'

#### RT-sqPCR primer sequences

Primer name	Sequence
miR-142-3p-F	5'- CGCTGTAGTGTTTCCTACTTTATGGA -3'
let-7a-F	5'- GCCGATGAGGTAGTAGGTTG -3'
miR-16-F	5'- CGCTAGCAGCACGTAAATATTGGCG -3'

#### siRNA sequences

siSTAU1	5'- CAGAGAGGCUGGAGGUGAA(dTdT) -3'
siLin28B-1	5'- CTGGGATAACATAACTCCAGA(dTdT) -3'
siLin28B-2	5'- AACATGATAAGCGTTGCTCAA(dTdT) -3'
siUPF1-1	5'- GAUGCAGUUCGCUCCAUU(dTdT) -3'
siUPF1-2	5'- GCTTAGTCCATCAGCATCTTATTCT(dTdT) -3'

#### mimic miRNA sequences

miR-142-3p	5'- UGUAGUGUUUCCUACUUUAUGGA -3'
miR-142-5p	5'- CAUAAAGUAGAAAGCACUACUUU -3'

#### Cloning primer sequences

Primer name	Sequence	Reference
STAU1_XhoI-F	5'- <u>CGCCTCGAGATGAAACTT</u> GGAAAAAACCA -3'	underlined sequence represents the <i>XhoI</i> enzyme site
STAU1_XhoI-R	5'- GCGCTCGAGTACTATAG AATAGGGCCCTC -3'	

STAU1_3'UTR-XbaI-R	5'- GCGTCTAGATTAACAGTA TCGAGCA -3'	underlined sequence represents the <i>XbaI</i> enzyme site
STAU1_3'UTR-XbaI-F	5'- GCGTCTAGATATTAATAAAA ACATTGTGTAT -3'	
STAU1_3'UTR_ middle-WT	5'- GACTTAGCTTTCACT <u>ACACTA</u> <u>CAGGATATGATCTCCATGTAG</u> TCCATATA -3'	underlined sequence represents the 142-3p target site
STAU1_3'UTR_ middle-Mutant	5'- GACTTAGCTTTCACT <u>AGT</u> <u>GAACAGGATATGATCTCCATG</u> TAGTCCATATA -3'	underlined sequence represents the 142-3p target site
STAU1_3'UTR-5'	5'- TAGTGAAAGCTAAGTCCT CAAGAGCCATATGTATAGATA CACAATGTTTTTTAATA -3'	
STAU1_3'UTR-3'	5'- TTAACAGTATCGAGCACT CTGGAAAATCACTCTGCAGGT TTATATGGACTACATGGAGAT -3'	
Pre-miR-142_BamHI-F-WT	5'- GCGGGATCCGCAGTCACC CATAAAGTAGAAAGCACTACT AACAGCACTGGAGGGTGT -3'	underlined sequence represents the <i>BamHI</i> enzyme site
Pre-miR-142_XhoI-R-WT	5'-GCGCTCGAGGTACACTCA TCCATAAAGTAGGAAACACTA CACCTCCAGTGCTGTTA -3'	underlined sequence represents the <i>XhoI</i> enzyme site
Pre-miR-142_BamHI-F-Mut	5'-GCGGGATCCGCAGTCACC CATAAAGTAGGAAGCACTACTA ACAGCACTAGGGTGT-3'	underlined sequence represents the <i>BamHI</i> enzyme site
Pre-miR-142_XhoI-R-Mut	5'-GCGCTCGAGGTACACTC ATCCATAAAGTAGGAAACACTA CACCTAGTGCTGTTA-3'	underlined sequence represents the <i>XhoI</i> enzyme site