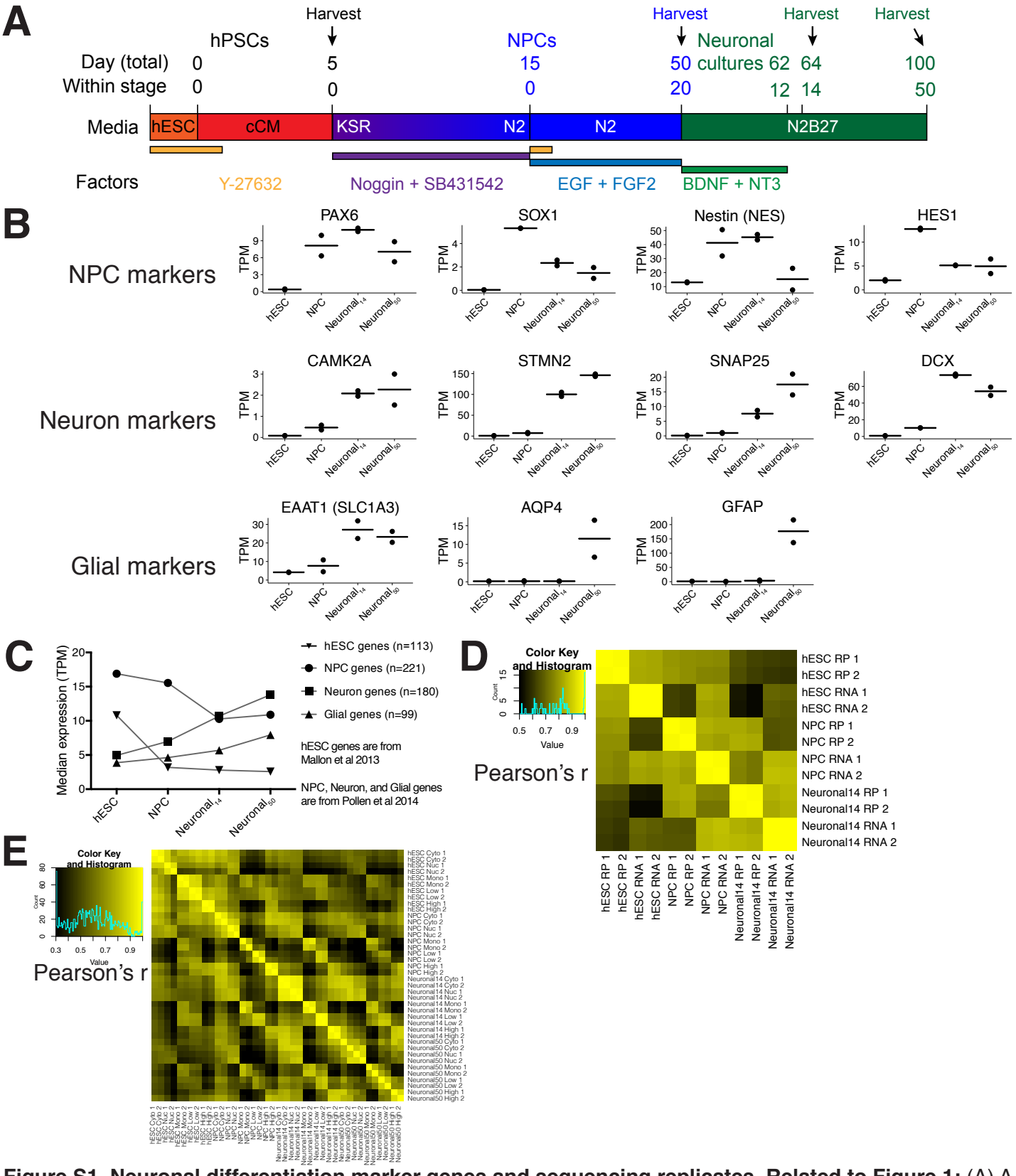

Figure S1. Neuronal differentiation marker genes and sequencing replicates, Related to Figure 1: (A) A schematic of the neuronal differentiation protocol. (B) Marker gene expression in cytoplasmic RNAseq. TPM: transcripts per million. Bar: mean; points: expression in each replicate. (C) Median expression for classes of genes representing different cell types. Gene classes from single cell sequencing of human fetal brains in Pollen et al. 2014 or analysis of hESCs in Mallon et al. 2013. Note that Pollen (2014) cannot separate NPC genes from hESC genes as they sequenced human fetal brains (which have no hESCs), leading to overlap. (D) Replicate correlations for ribosome profiling and matched RNAseq. (E) Replicate correlations for cytoplasmic, nuclear, and polysomal (TrIP-seq) RNAseq samples.

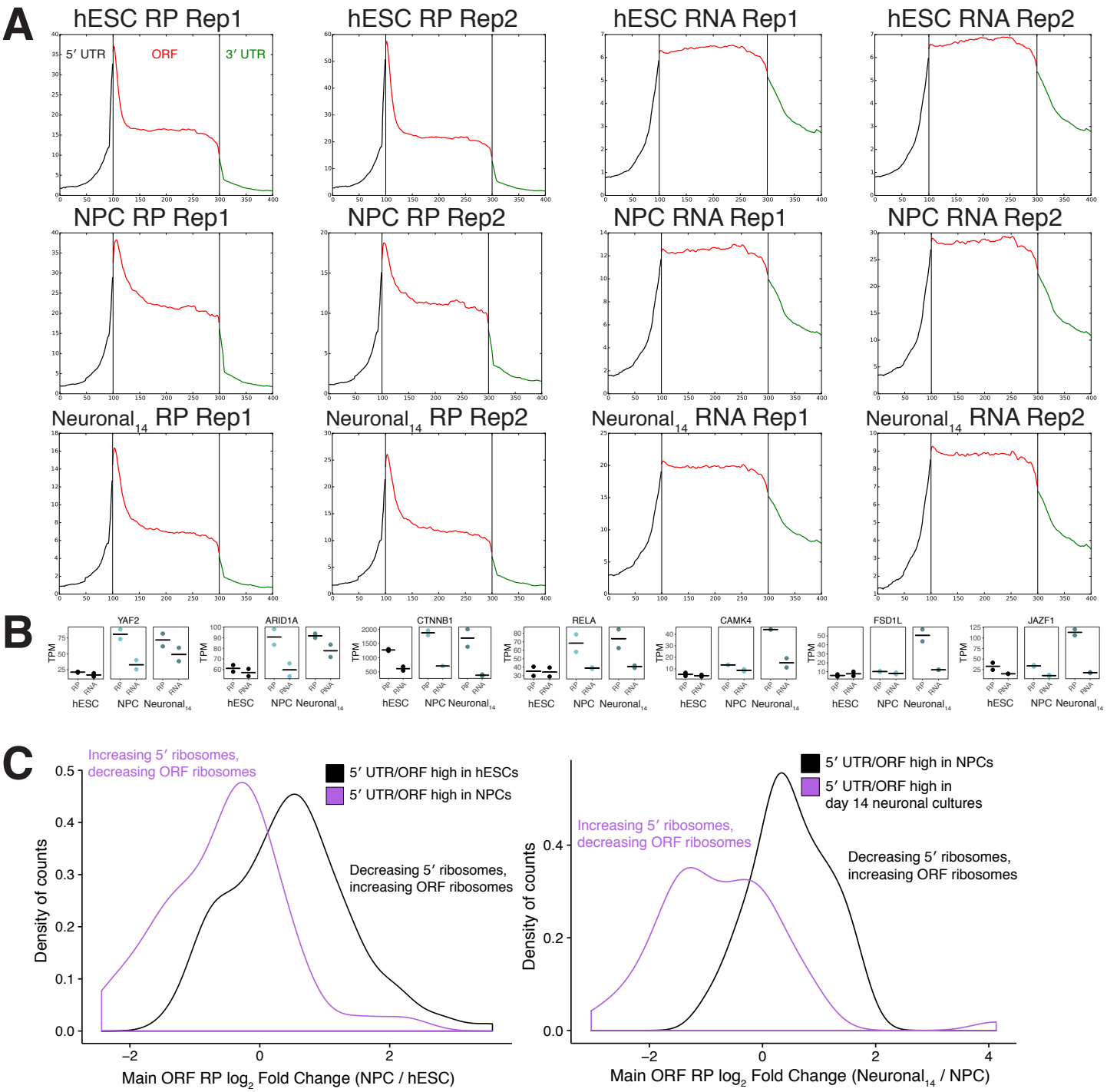

Figure S2. Metagene plots and 5′ UTR ribosome translation changes, Related to Figure 2: (A) Metagene plots for each replicate of ribosome profiling and RNAseq in hESCs, NPCs and 14 day neuronal cultures. Metagene plots are constructed by collapsing each ORF to 200 bins and averaging RP density across genes, while 5′ and 3′ UTR regions are the 100 nt before and after each ORF averaged across genes. (B) RP and RNAseq gene expression changes for seven genes related to transcription (left four) or translationally activated in day 14 neuronal cultures (right three). (C) Fold change distributions in ribosome profiling on the main ORF for classes of genes with changes in 5′ UTR / ORF occupancy during differentiation. Note the reciprocal relationship between 5′ UTR ribosomes and ORF ribosomes. The gene sets with high 5′ UTR / ORF ribosome are those in Figure 2B with a fold change > 2 (log2FC > 1).

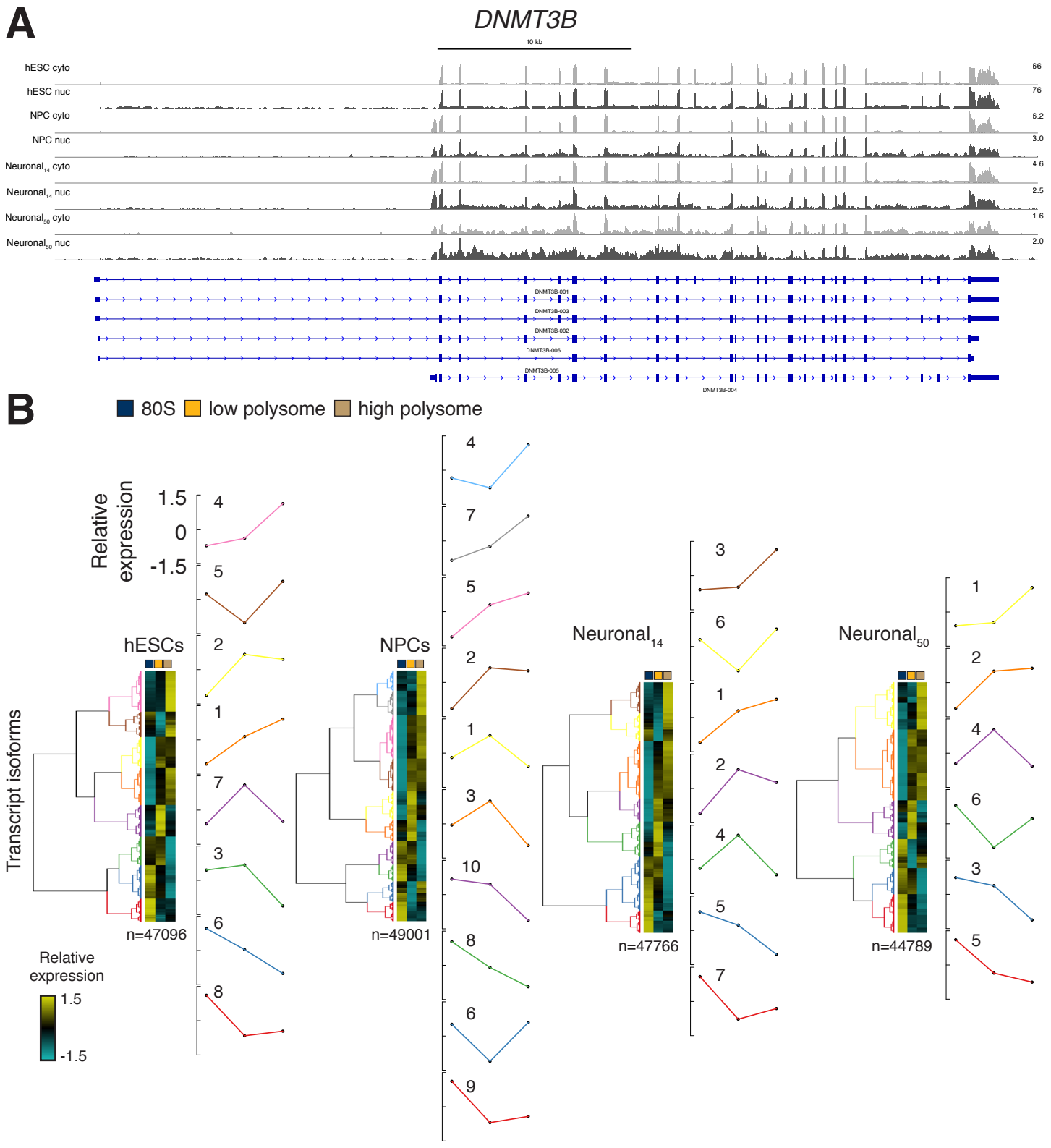

Figure S3. Intron retention and transcript clusters from TrIP-seq data, Related to Figure 5. (A) Unannotated intron retention increases in DNMT3B during neural induction. Gray: cytoplasmic RNAseq. Dark gray: nuclear RNAseq. Note also the cell type specific exon inclusion in, for example, the 2nd and 3rd to last exons, among others. (B) The clustering in Figure 5A is reproduced here with associated dendrograms and plots of average expression of all transcripts in each color. Colors of the expression profiles match the colors of the dendrogram. All average plots run from 1.5 to -1.5 in units of relative expression (rlog; see Methods). Cluster IDs are the numbers to the upper left of each cluster average plot.

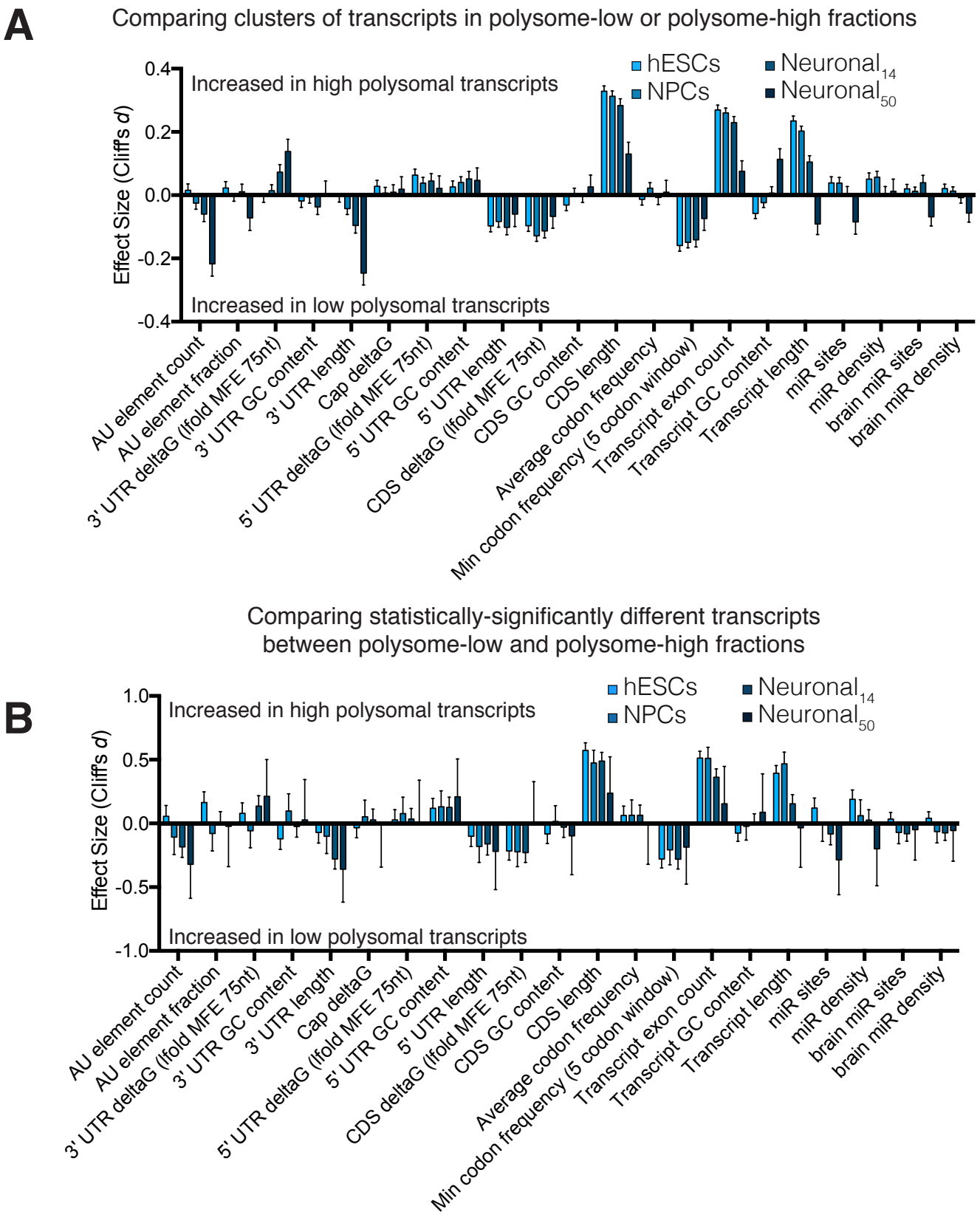

Figure S4. Transcript features that affect translation between transcript isoforms, Related to Figure 6: (A) The effect size of 21 different features between transcripts in high polysomal clusters versus those in low polysomal clusters. Different transcript isoforms of the same gene were contrasted. Error bars are 95% confidence intervals. MFE 75nt: minimum free energy across a 75 nucleotide sliding window in the region. Codon frequency is the usage of codons in the human transcriptome. Note: higher deltaG for a set of transcripts implies less stable structures. See Methods for more details. (B) as in (A) but for transcripts identified by DESeq2 as significantly different ($p < 0.01$) between low polysomal fractions and high polysomal fractions.

Table S3. Gene ontology terms, Related to Figure 2

Term (translationally up in hESC vs NPC)	ID	N	p (Benjamini-corrected)
rRNA processing	GO:0006364	85	1.07E-45
translational initiation	GO:0006413	66	2.42E-41
viral transcription	GO:0019083	60	6.76E-41
translation	GO:0006412	85	1.20E-39
SRP-dependent cotranslational protein targeting to membrane	GO:0006614	54	7.65E-39
nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	GO:0000184	58	9.49E-37
mitochondrial translational elongation	GO:0070125	37	1.96E-20
mitochondrial translational termination	GO:0070126	36	3.40E-19
mitochondrial translation	GO:0032543	16	9.60E-8
cytoplasmic translation	GO:0002181	11	0.000
negative regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	GO:0051436	17	0.000
positive regulation of establishment of protein localization to telomere	GO:1904851	7	0.001
positive regulation of ubiquitin-protein ligase activity involved in regulation of mitotic cell cycle transition	GO:0051437	17	0.001
regulation of mRNA stability	GO:0043488	20	0.001
ribosomal small subunit assembly	GO:0000028	9	0.001
anaphase-promoting complex-dependent catabolic process	GO:0031145	17	0.001
positive regulation of telomerase RNA localization to Cajal body	GO:1904874	8	0.001
aerobic respiration	GO:0009060	11	0.001
protein folding	GO:0006457	27	0.001
ribosomal small subunit biogenesis	GO:0042274	8	0.002
mRNA splicing, via spliceosome	GO:0000398	30	0.003
cell-cell adhesion	GO:0098609	34	0.003
positive regulation of protein localization to Cajal body	GO:1904871	6	0.003
maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	GO:0000462	10	0.006
NIK/NF-kappaB signaling	GO:0038061	14	0.008
RNA processing	GO:0006396	17	0.011
mitochondrial electron transport, ubiquinol to cytochrome c	GO:0006122	7	0.011
oxidation-reduction process	GO:0055114	56	0.019
protein stabilization	GO:0050821	20	0.022
tRNA aminoacylation for protein translation	GO:0006418	10	0.029
mitochondrion organization	GO:0007005	14	0.031
ribosomal large subunit biogenesis	GO:0042273	8	0.030
regulation of cellular amino acid metabolic process	GO:0006521	11	0.040

Term (translationally up in NPC vs hESC)	ID	N	p (Benjamini-corrected)
positive regulation of transcription, DNA-templated	GO:0045893	46	0.0029
vascular endothelial growth factor receptor signaling pathway	GO:0048010	14	0.0077
cell migration	GO:0016477	21	0.020
axon guidance	GO:0007411	20	0.017

Table S6: List of antibodies used, Related to Figure 1

Antibody	Host	Company	Cat #	Western blot dilutio
4EBP1	Rb	Cell Signaling	9644	1:1000
phospho-4EBP1 (Ser65)	Rb	Cell Signaling	9451	1:1000
phospho-4EBP1 (Thr37/46)	Rb	Cell Signaling	2855	1:1000
B-Actin	M	Sigma	a1972	1:15000
MAP2	Ch	AbCam	ab5392	1:10000
MAP2	M	AbCam	ab11267	1:2000
OCT4	Rb	AbCam	ab19857	1:1000
S6	Rb	Cell Signaling	2317S	1:1000
phospho-S6 (Ser240/244)	Rb	Cell Signaling	5364S	1:2000
TSC2	Rb	Cell Signaling	4308	1:1000
eIF3D	Rb	Bethyl	A301-758A	1:1000
RPLP1	Rb	Abcam	Ab121190	1:250
eIF4B	M	Cell Signaling technology	13088S	1:1000
Pax6	Rb	Covance	PRB-278P-100	1:500
Syn1	M	SySy	106001	
CamKIIa	M	Cell Signaling technology	50049	1:500
GLURI	Rb	Millipore	Ab1504	1:500
phospho-TSC2 (Ser1387)	Rb	Cell Signaling technology	5584	1:1000
phospho-p70S6K (Thr389)	Rb	Cell Signaling technology	9234	1:500
p70S6K	Rb	Cell Signaling technology	2708	1:500

Supplemental experimental procedures:

RNA-seq library prep:
An Agilent Bioanalyzer was used to determine the RNA integrity (RIN) for each sample, which was typically > 6. Libraries were prepared using the TruSeq Stranded Total RNA Library Prep Kit (Illumina) starting with 100 ng of RNA from each sample or pool with 6 minutes fragmentation time and 13 PCR cycles.

Ribosome profiling library prep:
Ribosome profiling libraries were prepared using a standard protocol (McGlincy and Ingolia, 2017), with minor modifications. Sucrose gradient purification of 80S monosomes was used and RNA was extracted from the 80S peak as above. A larger fragment size was selected, from about 20nt to 40 nt, as reports have shown variability in the protected footprint size (Guydosh and Green, 2014; Lareau et al., 2014). Matched RNA-seq libraries were constructed with the ribosome profiling library prep using RNA that was fragmented by incubating for 20 minutes at 95 degrees in 1 mM EDTA, 6 mM Na2CO3, 44 mM NaHCO3, pH 9.3. PCR was performed for 8 or 10 PCR cycles, depending on the library. The RNase digestion repeatedly overdigested ribosomes derived from 50 day old neuronal cultures causing them to fall apart during purification, precluding collection of ribosome protected footprints from these cells. Metagene plots were made using metagene-maker (https://github.com/stephenfloor/metagene-maker).

High-throughput sequencing:
All libraries were sequenced on an Illumina HiSeq 4000. Index bleed issues were corrected for in ribosome profiling libraries using read-internal indexes. Index bleed appeared to be a minor issue in polysomal TrIP-

seq data, as, for example, OCT4 expression was tightly restricted to hESC libraries (Figure 1C). Total RNA libraries were sequenced in 100bp paired-end format, while ribosome profiling libraries were sequenced in 50bp single-read format. Sequencing was performed at the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley.

Polysome profiling:
Sucrose gradients from 10-50% were made in 100 mM KCl, 20 mM HEPES pH 7.6 and 5 mM MgCl2 with 100 µg/ml cycloheximide and 0.66 U/µl Superase:In (Thermo-Fisher) and pre-chilled in centrifuge buckets for at least 30 minutes before use. 100 µl of lysate was then applied to the top of a 12 ml 10-50% sucrose gradient. Tubes were spun for 2 hours at 36,000 RPM (221,632 g) in a SW-41 rotor. The bottom of the tube was punctured and 2M sucrose pumped in using a peristaltic pump. Absorbance at 260 nm was monitored using a Brandel (Gaithersburg, MD) gradient fractionator and ISCO (Lincoln, NE) UA-6 detector. Peaks were fractionated into separate tubes, which were ethanol precipitated by adding 2 µl glycoblue (Thermo), 1:10 volumes 3M NaOAc pH 5.2 and 2 volumes 100% ethanol. Samples were resuspended, DNase treated using RQ1 RNase-free DNase (Promega), acid phenol:chloroform extracted, and ethanol precipitated as above. Samples were then resuspended in 20 µl DEPC-treated water and RNA concentration was measured using a Qubit (Life Technologies).

Western blotting:
Cells were harvested in lysis buffer containing 2 mM EDTA, 2 mM EGTA, 1% Triton-X, and 0.5% SDS in PBS with Halt phosphatase inhibitor cocktail and Complete mini EDTA-free protease inhibitor cocktail. Total protein was determined by BCA assay, and 10 µg of protein in Laemmli sample buffer were loaded onto 4–15% Criterion TGX gels. Proteins were transferred to PVDF membranes, blocked in 5% milk in TBS-Tween for one hour at room temperature (RT), and incubated with primary antibodies diluted in 5% milk in TBS-Tween overnight at 4°C. The following day, membranes were incubated with HRP-conjugated secondary antibodies for one hour at RT, washed, incubated with chemiluminesence substrate and developed on GE Amersham Hyperfilm ECL. Membranes were stripped with 6M guanidine hydrochloride to re-blot on subsequent days.

Electrophysiology in day 50 neuronal cultures:
Whole cell patch clamp recordings were made at room temperature from visually identified neurons grown on coverslips for 54-56 days after differentiation from NPCs. Cells were superfused with ACSF containing (in mM): 123 NaCl, 25 D-Glucose, 10 HEPES, 25 NaHCO3, 5 KCl, 1 Na2H2PO4, 2 CaCl2, and 1 MgCl2. Internal solution contained (in mM): 135 KMeSO4, 10 HEPES, 4 MgCl2, 4 Na-ATP, 0.4 Na-GTP, 10 phosphocreatine-Na2, and 1 EGTA. For current clamp recordings, the membrane potential was adjusted to -70mV and one second steps of depolarizing current (from +5-50pA) were injected to elicit action potentials. To measure excitatory synaptic activity, voltage clamp recordings were obtained at -70mV. Spontaneous excitatory synaptic currents were recorded before and five minutes after wash-in of the AMPA receptor blocker NBQX (10uM).

Guydosh, N.R., and Green, R. (2014). Dom34 rescues ribosomes in 3' untranslated regions. Cell *156*, 950-962.
Lareau, L.F., Hite, D.H., Hogan, G.J., and Brown, P.O. (2014). Distinct stages of the translation elongation cycle revealed by sequencing ribosome-protected mRNA fragments. Elife *3*, e01257.
McGlincy, N.J., and Ingolia, N.T. (2017). Transcriptome-wide measurement of translation by ribosome profiling. Methods.