Supplemental Experimental Procedures

Nuclear/cytoplasmic RNA fractionation from hESCs

H9 or H14 cells were grown in 10 cm dishes plated with Matrigel. After reaching 70% confluence, the clones were individualized with Accutase (Innovative Cell Technologies, Inc., San Diego, CA) for 10 minutes at 37°C. Cells were centrifuged at 1,000 rpm for 5 min and rinsed with ice-cold PBS. Cell pellets were resuspended by gentle pipetting in 200 μL lysis buffer A [10 mM Tris (pH8.0), 140 mM NaCl, 1.5 mM $MgCl_2$, 0.1% Igepal, 2 mM vanadyl ribonucleoside complex (VRC; Invitrogen)], and incubated on ice for 5 min. 1/5 of the total RNAs were extracted by Trizol reagent (Invitrogen). The rest of the lysate was centrifuged at 1,000 g for 3 min at 4°C to isolate the cytoplasmic fractionation and pellet the nuclei. Cytoplasmic fractions were further centrifuged at maximum speed and nuclear pellets underwent two additional washes with 160 μL lysis buffer A before extraction with Trizol. In all analyses, equal amounts of cytoplasmic and RNA samples were used, representing comparable cell-equivalents.

Antibodies used in immunofluorescence microscopy and immunoblots

Antibodies used in immunofluorescence microscopy were Mouse anti-SSEA4 (1:50, Developmental Studies Hybridoma Bank (DSHB), Rat anti-SSEA3 (1:50, Santa Cruz Biotechnology), mouse anti-Troma-1 (1:50, DSHB), mouse anti-$p54^{nrb}$ (1:150, BD Transduction Laboratories, Palo Alto, CA), mouse anti-PSF (1:150, Sigma, St. Louis, MO), Rabbit anti-PSP1α (1:100, Dundee Cell Products, UK), mouse anti-SC35 (1:200, Sigma) and mouse anti-dsRNA, J2 antibody (1:200, English & Scientific Consulting, Hungary) were used as the primary antibodies. TRITC-labeled anti-mouse IgG (1:500, Molecular Probes), Alexa Fluor 488-labeled anti-rabbit

IgG (1:500, Molecular Probes) and Alexa Fluor 488-labeled anti-rat IgG (1:500, Invitrogen) were used to detect each specific primary antibody in cells. The nuclei were counterstained with DAPI.

Antibodies used in immunoblots were mouse anti-PSF (1:1,000), mouse anti-p54^{nrb} (1:2000), Rabbit anti-PSP1α (1:1,000), rabbit anti-Sox2 (1:1000, Millipore, Billerica, MA), goat anti-ADAR1 (C-16) (1:250, Santa Cruz Biotechnology).

Primers

Primers used for hNEAT1:

NEAT1-middle-S: GGGGAGAGGGTTGGTTAGAG;

NEAT1-middle-AS: GGTTCTCGGAAAACTGGTGA;

NEAT1-3‘end-S: TTGGTTCTGAGCTGCGTCTA;

NEAT1-3‘end-AS: TGTGCTGTAAAGGGGAAGAAA

Primers used to test hESCs' pluripotency (Takahashi et al., 2007):

Oct3/4-S: GACAGGGGGAGGGGAGGAGCTAGG;

Oct3/4-AS: CTTCCCTCCAACCAGTTGCCCCAAAC;

Sox2-S: GGGAAATGGGAGGGGTGCAAAA GAGG;

Sox2-AS: TTGCGTGAGTGTGGATGGGATTGGTG

Primers used for northern blotting anaylsis:

Lin28-upstream-S: GGGGAATCACCCTACAACCT;

Lin28-upstream-AS: CTTGGCTCCATGAATCTGGT;

Paics-upsteam-S: TGGCTGTTCAACCGTACTTTC;

Paics-upsteam-AS:CTTGCTTGATTTTC CTTCAGC

Primers used for editing analysis of the *AluSc* in *Lin28* mRNA:

*Lin28-AluSc-*S: TGATAACTTGGTGTATTCATGCCAAAC;

*Lin28-AluSc-A*S: TAAGGAGGTTTGATCCATCC

Primers used for mRNA nuclear and cytoplasmic distribution analysis:

actin-S: GCTCGTCGTCGACAACGGCTC;

actin-AS: CAAACATGATCTGGGTCATCTTCTC;

Flnb-S: CAGCAGACAGACCAGGAACA;

Flnb-AS: GATGAGGAGCGGTAAAGCAG;

Hyou1-S: GAATCCTTTGCCTGTGGGTA;

Hyou1-AS: CCTGGTAACTGAGGCTCTGC;

Lin28-upstream-S: GGGGAATCACCCTACAACCT;

Lin28-upstream-AS: CTTGGCTCCATGAATCTGGT;

Lin28-downstream-S: GGAGGCCAAGAAAGGGAATA;

Lin28-downstream-AS: CCGCCCCATAAATTCAAGAT;

Nup43-upstream-S: CACCAAGGAGGAAGAAGCAG;

Nup43-upstream-AS: TCGGTTCCACAAACAAGACA;

Nup43-downstream-S: TGTGTGAGTGGGATCCAAAA;

Nup43-downstream-AS: CTGGTGATTGGCTCATCTCA;

Paics-upsteam-S: TGGCTGTTCAACCGTACTTTC;

Paics-upsteam-AS:CTTGCTTGATTTTC CTTCAGC;

Paics-downstream-S: CGTAATTTTGGACTGCCACA;

Paics-downstream-AS: GCTAAGCATAGCACATCAGAGC;

Pccb-upstream-S: GAGTCTTTGGCTGGCTATGC;

Pccb-upstream-AS: CCTCCTGGGTAACATCCTCA;

Pccb-downstream-S: CGGTGTACAGCATCTGTTGG;

Pccb-downstream-AS: CATCTCTGCCAAAAACCACA;

Smc1a-S: CATGAAGGCAAGAAACAGCA;

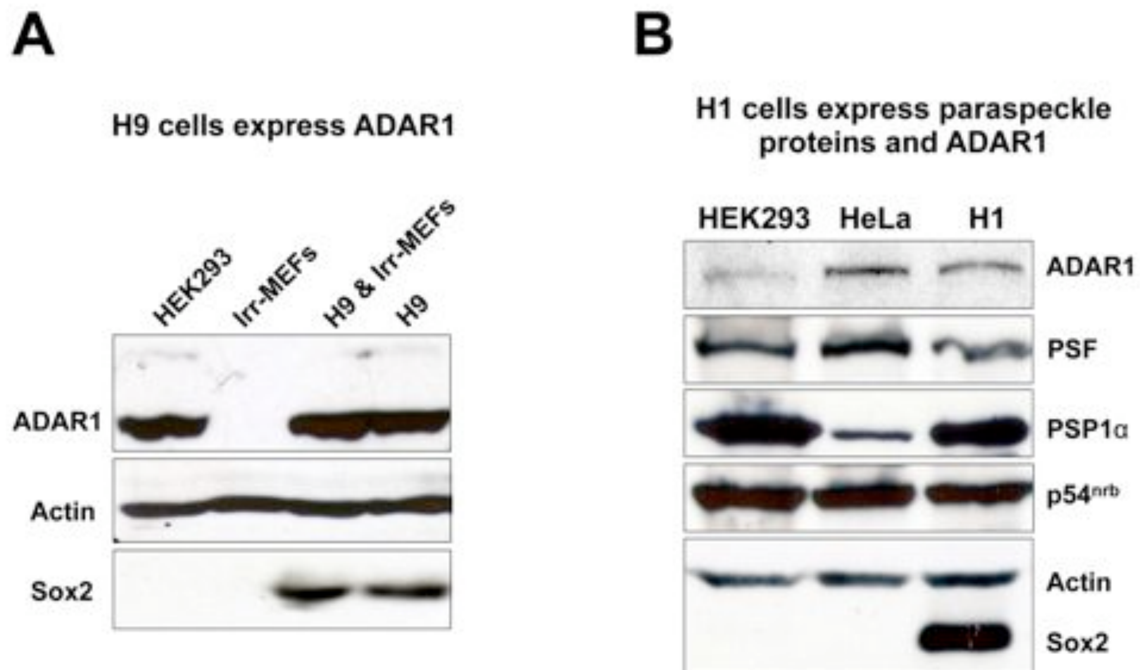
Smc1a-AS: TGCCGTACCTGTTTGTGGTA;

Son-S: GGAGCTGCCTAAGACCACAG;

Son-AS: CTCAGGCACTGGGGTAGAAA

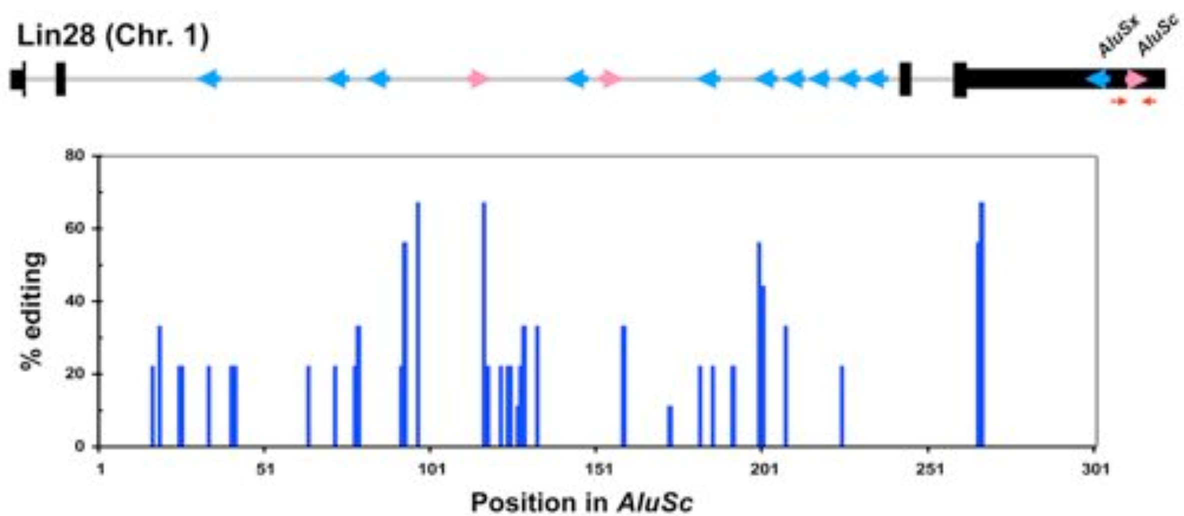
Supplemental Figures and legends

Supplemental Figure 1. **A**. H9 cells express ADAR1 under different culture conditions. Western blot analysis of ADAR1 in HEK293 cells, irradiated (Irr) mouse embryo fibroblasts (MEFs), H9 cells cultured on Irr-MEFs, and H9 cells cultured with Irr-MEFs conditioned medium. **B**. ADAR1 and paraspeckle-related proteins are expressed in H1 cells. Western blotting was used to monitor protein expression in HEK293 cells, HeLa cells and H1 cells.



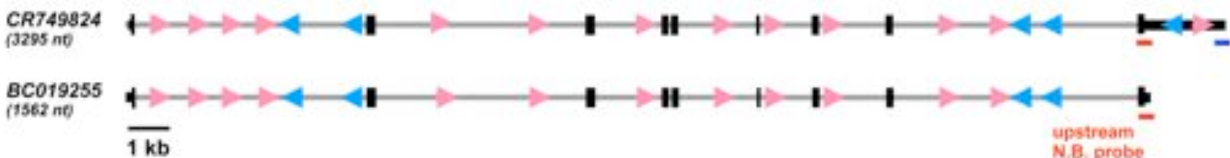
Supplemental Figure 2. Editing analysis of IR*Alu*s-RNA in hESCs. Total RNA from H9 cells was isolated and the indicated primers (red arrows) were used for RT-PCR, followed by TOPO cloning. 10 out of 20 clones showed evidence of editing (A residues converted to G residues in the clones). This figure shows the percentage of A-to-G changes at each A site within the *AluSc* element. The overall pattern of editing closely resembles the pattern seen in differentiated cells.

Efficiency of editing of Lin28 mRNA in hESCs

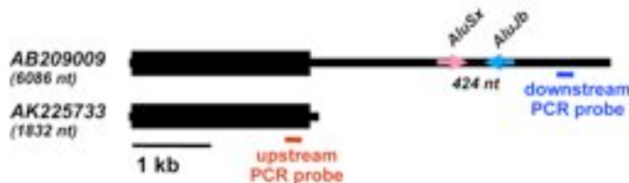


Supplemental Figure 3. Organization of several IR*Alu*s containing RNAs used in this study. The genomic sequence of Paics and the mature mRNAs of *Pccb* and *Nup43* are drawn roughly to scale. Exons and UTRs are shown as black bars, with coding regions being thicker. The *Alu* elements present in the gene are pink and blue arrows with the indicated orientations. There is a single pair of IR*Alu*s in the 3'-UTR in each gene. Evidence of editing in each 3'-UTR IR*Alu*s pair can be found in the available sequences in the UCSC genome browser. Blue bars indicate primers used to detect the full-length IR*Alu*s containing RNAs in the nuclear retention analysis of Fig. 5. Note that these primers allowed us to discriminate the IR*Alu*s-containing RNAs from their shorter isoforms which lack IR*Alu*s in their 3'-UTRs. Red bars indicate primers used to detect all transcripts.

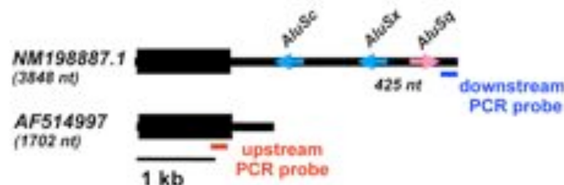
PAICS (phosphoribosylaminoimidazole carboxylase) pre-mRNA



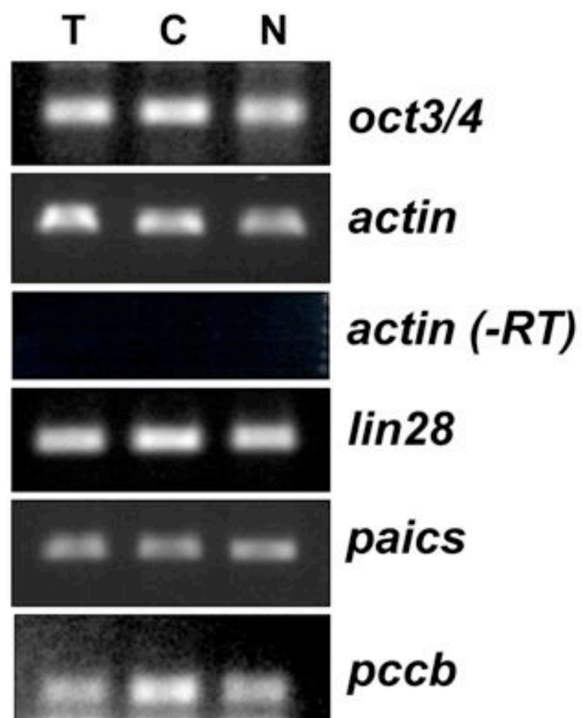
PCCB (propionyl Coenzyme A carboxylase, beta) mRNAs



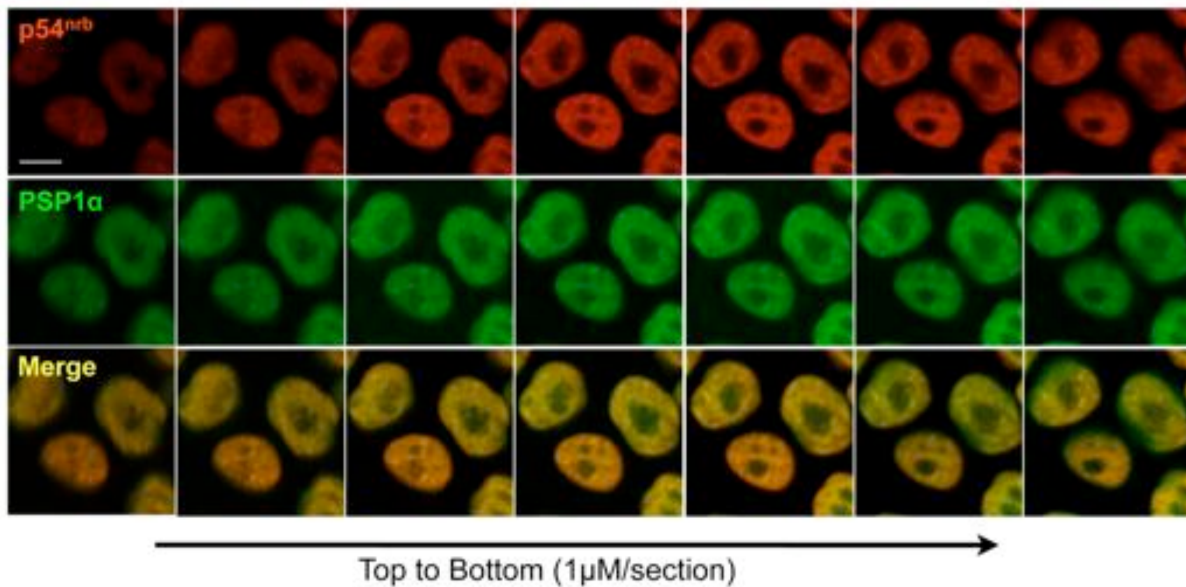
Nup43 (Nucleoporin 43 kDa) mRNAs



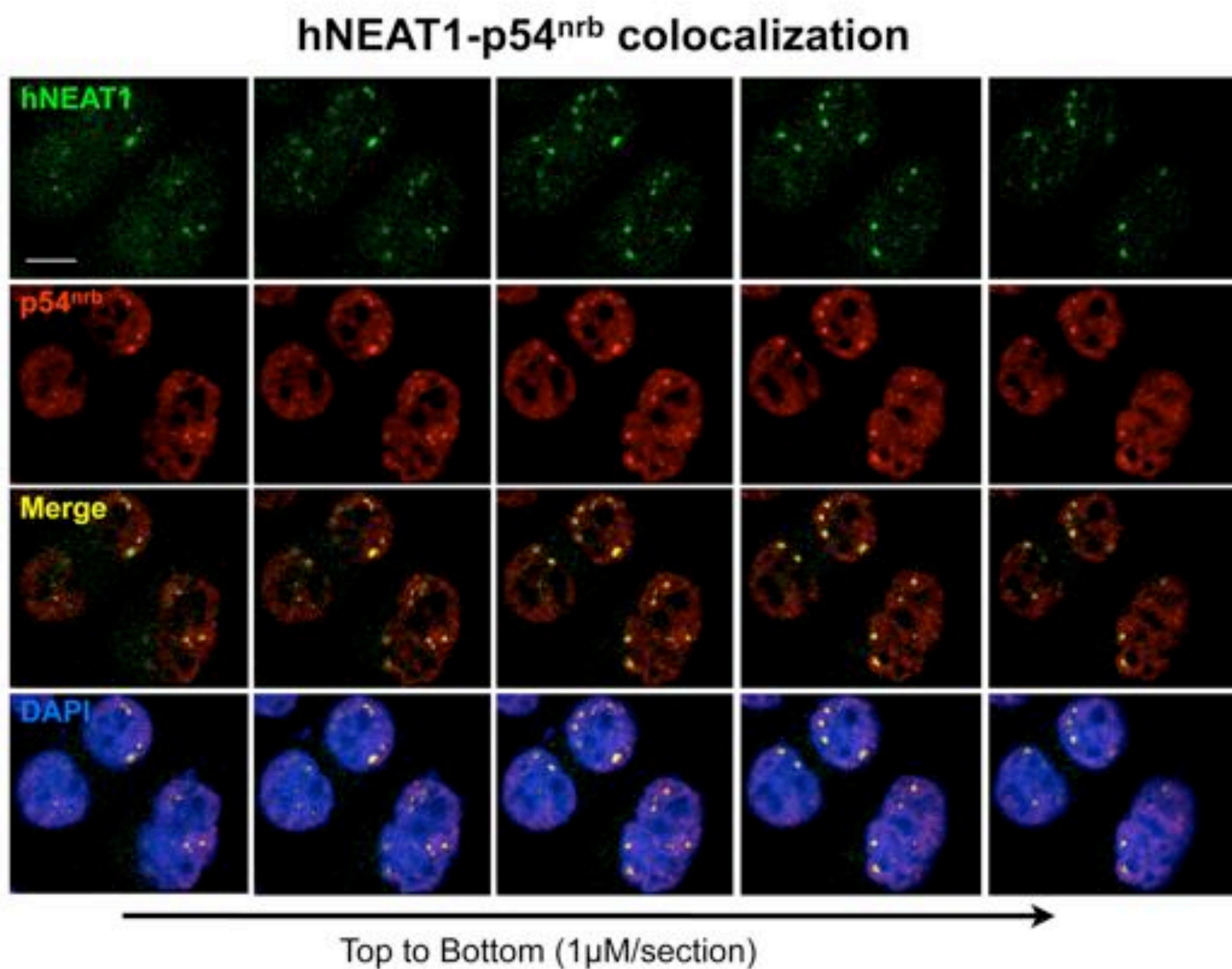
Supplemental Figure 4. IR*Alu*s containing mRNAs escape nuclear retention in H14 cells. Nuclear and cytoplasmic RNAs were isolated from H14 cells and then analyzed by RT-PCR. PCR primers are located in the downstream of IR*Alu*s in the 3'-UTRs to detect the full-length mRNAs (Fig. S3). In H14 cells, the full-length IR*Alu*s containing mRNAs *Lin28, Paics,* and *Pccb* show very similar nuclear and cytoplasmic distribution to *actin* and *oct3/4*.



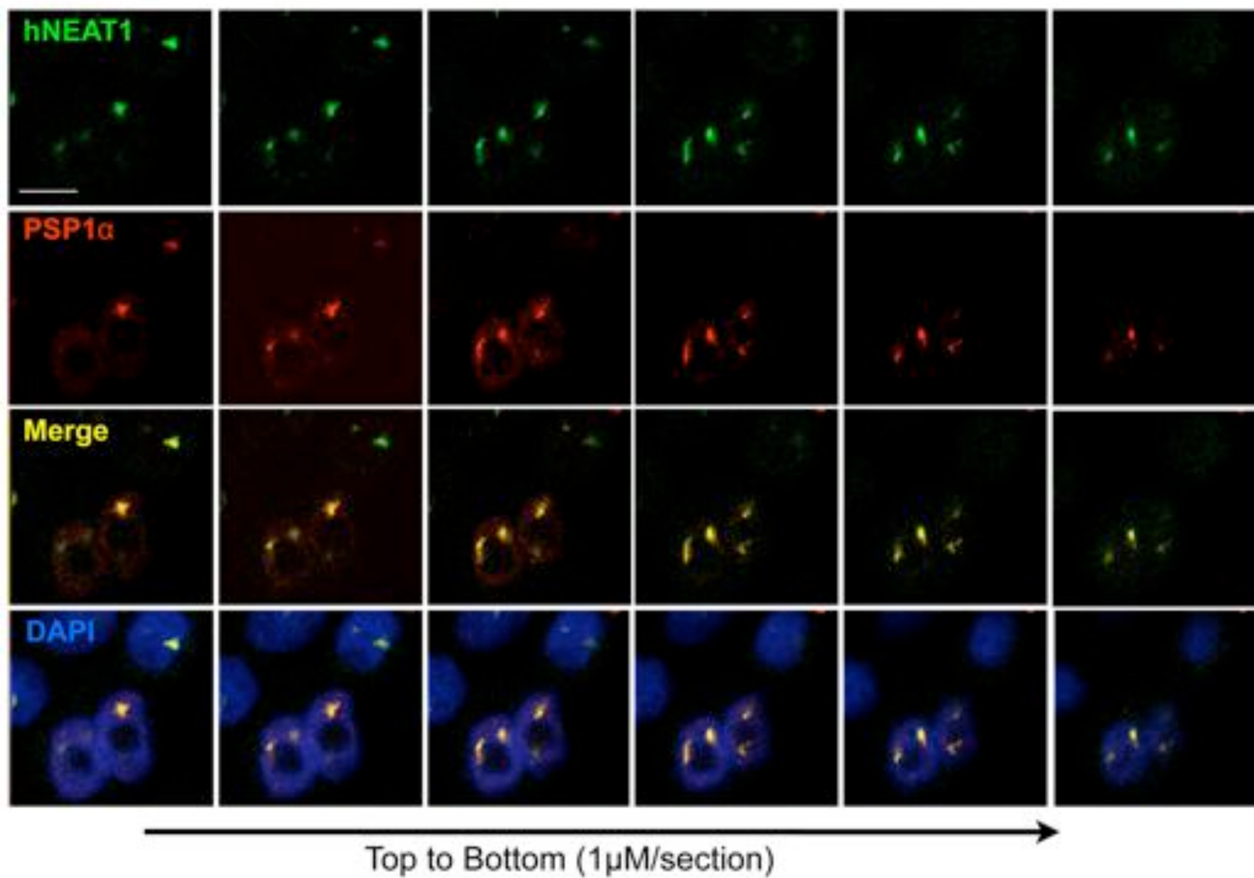
Supplemental Figure 5. H14 cells lack paraspeckles. $p54^{nrb}$ and PSP1α colocalize throughout the nucleoplasm in H9 cells, but show no apparent nuclear paraspeckles. $p54^{nrb}$ is shown in red, and PSP1α is shown in green. Representative z-section images (1 μM/section from top to bottom of cells) are shown.



Supplemental Figure 6. hNEAT1 RNA colocalizes with $p54^{nrb}$. RNA *in situ* hybridization was performed with green-dUTP-labeled antisense hNEAT1 probe in HeLa cells, and representative z-section images (1 μM/section from top to bottom of cells) are shown here. $p54^{nrb}$ is shown in red. hNEAT1 exclusively localizes in paraspeckles and forms 5-10 prominent accumulations per nucleus and always colocalizes with accumulations of $p54^{nrb}$.

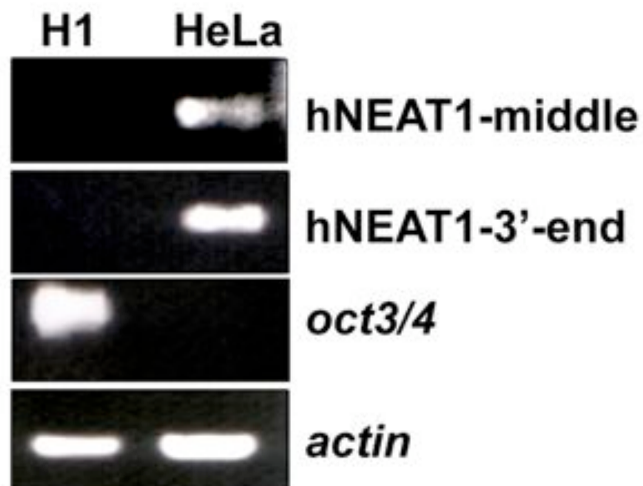


Supplemental Figure 7. hNEAT1 RNA colocalizes with PSP1α. RNA *in situ* hybridization was performed with green-dUTP-labeled antisense hNEAT1 probe in HeLa cells, and representative z-section images (1 μM/section from top to bottom of cells) are shown here. PSP1α is shown in red. hNEAT1 always colocalizes with accumulations of PSP1α.

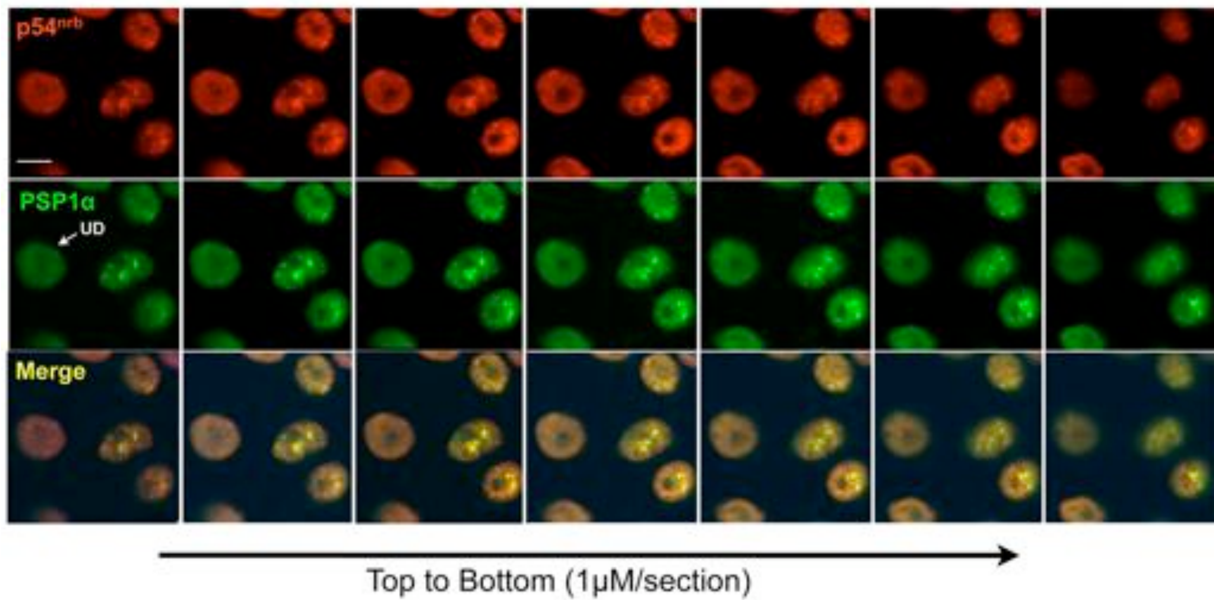


Supplemental Figure 8. RT-PCR shows that hNEAT1 is absent from undifferentiated H1 cells. RT-PCR was carried out with probes for either the middle or the 3'-end of hNEAT1 RNA.

H1 cells do not express hNEAT1



Supplemental Figure 9. Day 4 differentiated H14 cells show p54^{nrb} and PSP1α accumulations in the nuclei, indicating paraspeckles. UD, an H14 cell which has not yet differentiated and which shows no apparent nuclear paraspeckles. p54^{nrb} is shown in red, and PSP1α is shown in green. Representative z-section images (1 μM/section from top to bottom of cells) are shown.



Supplemental Figure 10. hNEAT1 knockdown in HeLa cells. hNEAT1 RNA *in situ* hybridization showed significant knockdown of hNEAT1 expression after AS treatment. Left panel, untreated HeLa cells. Right panel, HeLa cells were treated with hNEAT1 AS oligos.

hNEAT1 knockdown in HeLa cells