

# Supplemental Materials

*Molecular Biology of the Cell*

Hirose et al.

## Supplemental Figure Legends

**Figure S1. Paraspeckle enlargement upon MG132 treatment.** Paraspeckles in HeLa cells treated with DMSO or 5  $\mu$ M MG132 for 6 h were visualised by RNA-FISH of NEAT1 (magenta) as well as immunofluorescence of PSPC1 (green). The nuclei were visualized by DAPI staining. The cell images by low magnification are shown on the left. The scale bars are shown.

**Figure S2. Proteasome inhibition-dependent aggresome formation in HeLa cells.** Efficiency of MG132 treatment was assessed by observation in the electron microscope of typical aggresomes. **A.** Ultrastructure of electron-dense cytoplasmic aggregates (arrows) formed around the centrioles (arrow-heads) after 3 h of 5  $\mu$ M MG132 treatment. **B.** Detection of ubiquitin conjugates in centrioles-associated aggresomes (arrow-heads) by immunoelectron microscopy. **C.** Aggregates of ubiquitinated proteins progressively invade the cytoplasm after long-term inhibition of the proteasome (MG132 5  $\mu$ M, 17h) as revealed by I-EM with an anti-ubiquitin antibody. Bars = 1, 0.2 and 0.5  $\mu$ m in A, B and C respectively.

**Figure S3. Proteasome inhibition does not affect the location of NEAT1 lncRNA isoforms within the paraspeckles.** Portions of the NEAT1 transcripts were detected by EM-ISH with biotinylated DNA probes (red bars). DNA-RNA

hybrids were detected with an anti-biotin antibody coupled to 10 nm gold particles. For both the control (DMSO) and MG132-treated (5  $\mu$ M, 17h) HeLa cells, peripheral labelling was observed with 5' and 3'-end probes and internal labelling with the D1 probe in. Bars = 0.2  $\mu$ m

**Figure S4. Confirmation of NEAT1 knockdown with ASO.** HeLa cells were treated with ASOs that target either GFP RNA (control), or NEAT1 (#12 or #17), and total RNAs were prepared after 6, 12 and 24h and used for microarray and qRT-PCR validation. NEAT1 levels in ASO-treated cells were quantified by RT-qPCR with four primer sets (the position of the primers are shown in Figure 5B) and normalized to the level of GAPDH mRNA.

**Figure S5. Paraspeckle disruption by NEAT1 knockdown does not affect subcellular localization of ADARB2 mRNA.** **A.** Subcellular localization of ADARB2 mRNA and NEAT1 lncRNA. HeLa cells were fractionated according to the procedure shown at the top of the panel. The relative amounts of ADARB2 mRNA and NEAT1 lncRNA in each fraction were monitored by RT-qPCR. **B, C.** Subcellular distribution of ADAR1, ADARB1 and ADARB2 mRNAs were unchanged upon NEAT1 knockdown. The ASO-treated HeLa cells were fractionated into nuclear and cytoplasmic fractions as indicated at the top of panel A. The level of each ADAR mRNA was quantified by RT-qPCR (B). The

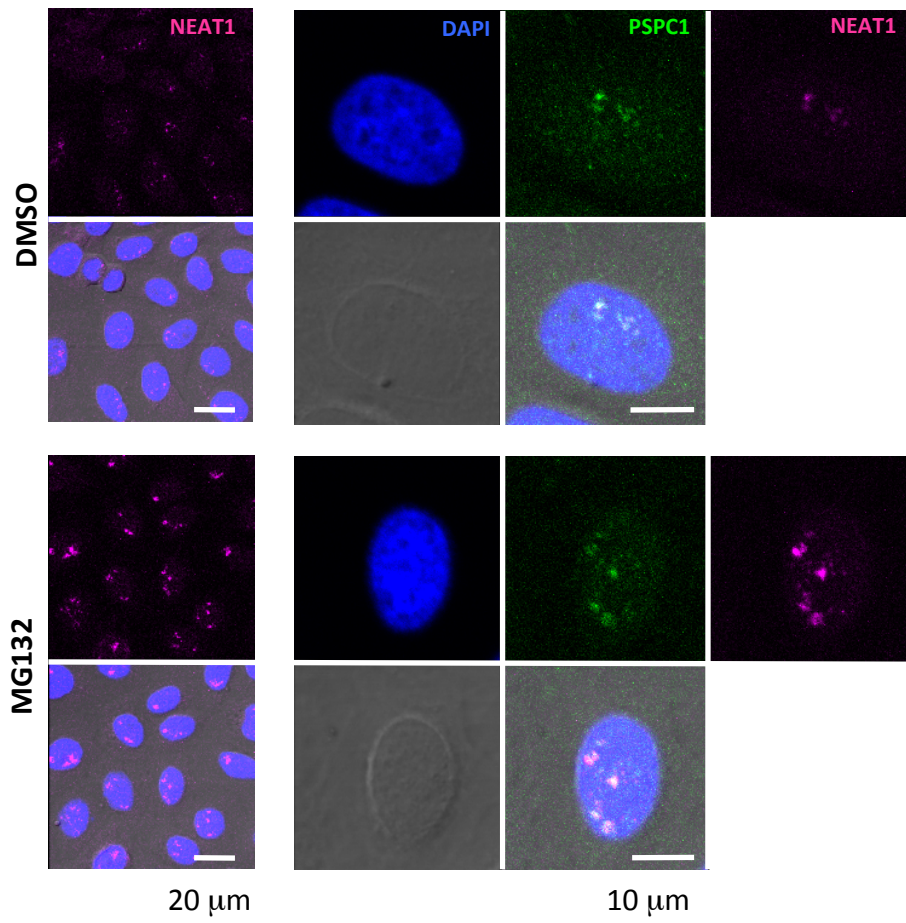
nucleocytoplasmic ratio of each ADAR mRNA in ASO-treated cells was shown in C. The ASO used (GFP, #12 and #17) are shown below.

**Figure S6. The control experiment of Figure 5A.** Levels of GAPDH mRNA were determined by RT-qPCR analyses, as in Figure 2A.

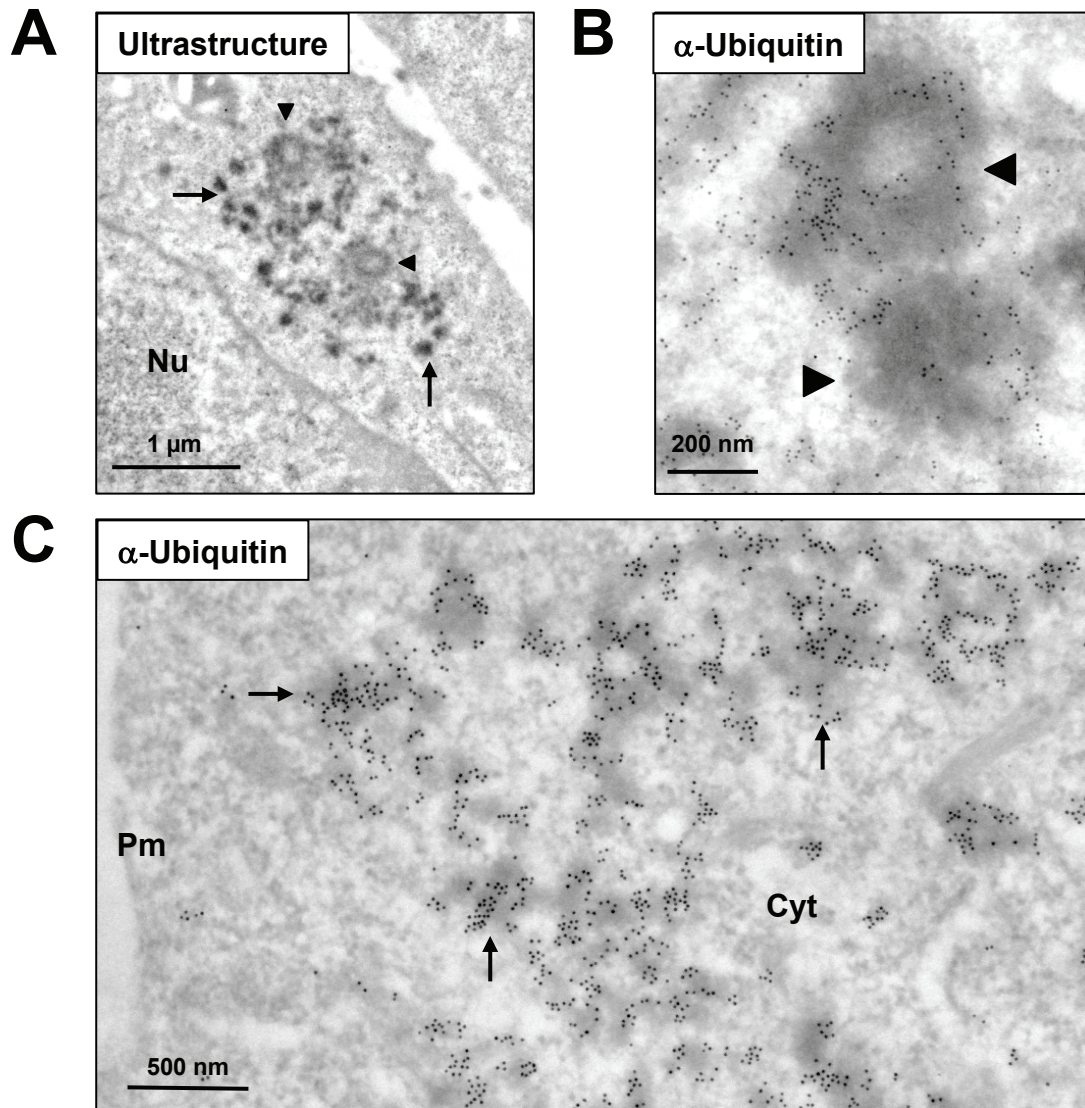
**Figure S7. NEAT1 lncRNA acts to attenuate MG132 induced apoptosis.**

Neat1<sup>-/-</sup> MEFs have an immediate sensitivity to MG132, compared to Neat1<sup>+/+</sup> MEFs, that are resistant for the first 2.5 h after MG132 addition. The calculated difference between the cell index of the control (DMSO) cells and the MG132 treated cells was graphed, using the first 4 h of data from three separate real time growth assays, each with different batches of MEFs. After MG132 addition, Neat1<sup>-/-</sup> MEFs begin to die more quickly than Neat1<sup>+/+</sup> MEFs, displaying an almost immediate difference in growth for the control compared to the MG132 treatment. In contrast, the Neat1<sup>+/+</sup> MEFs have indistinguishable growth between the two treatments over the first 2.5 h of the experiment. The error bars represent SD between the experiments.

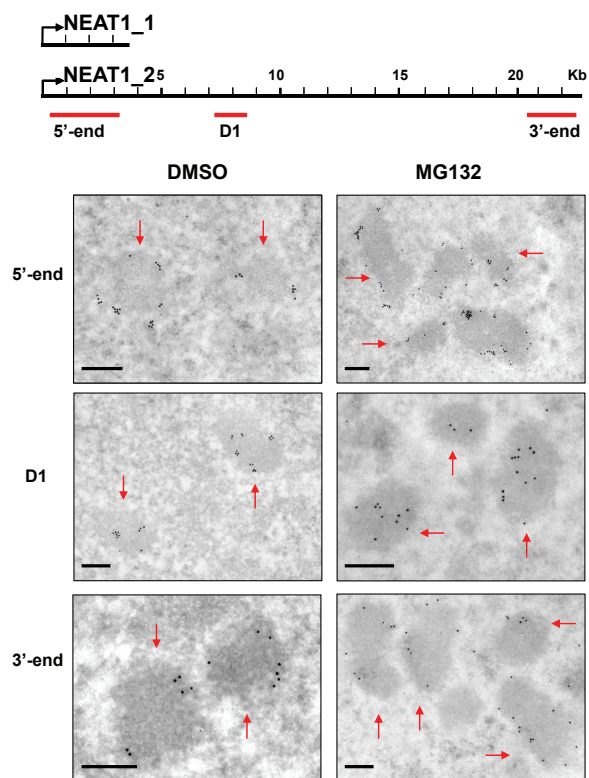
Supplemental Fig.S1



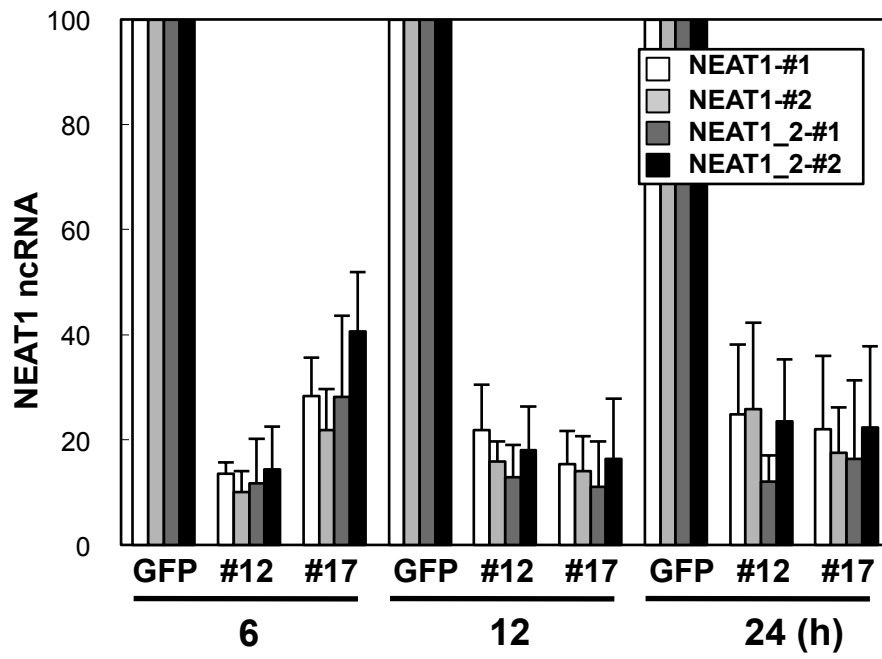
Supplemental Fig.S2



## Supplemental Fig.S3



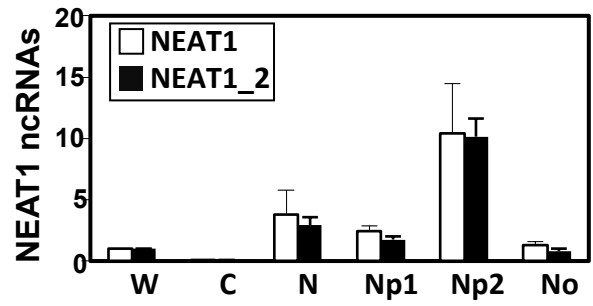
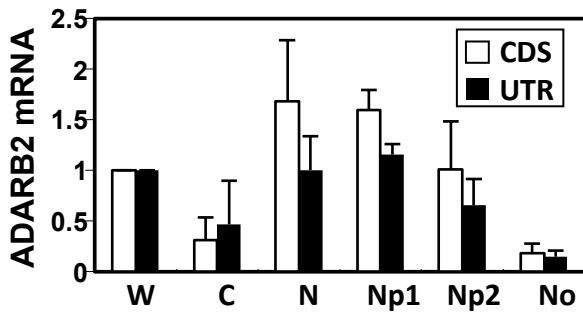
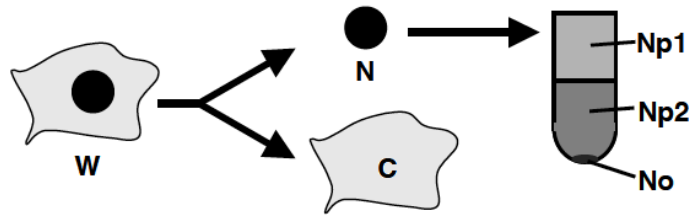
Supplemental Fig.S4



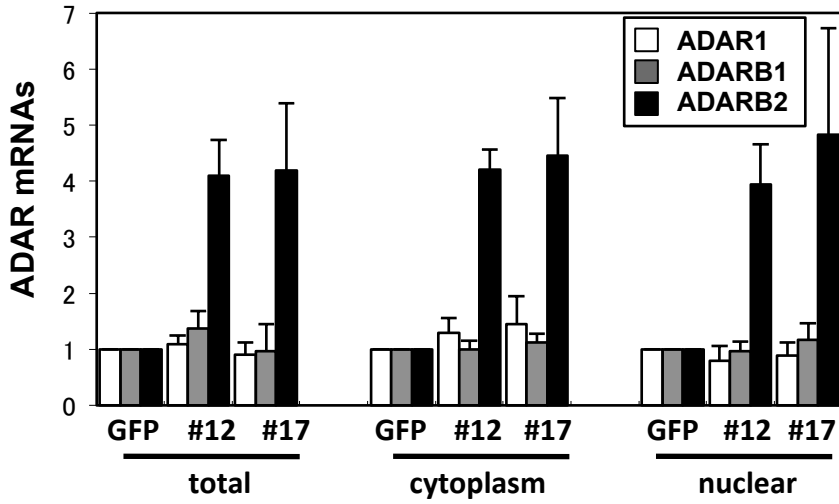


Supplemental Fig.S5

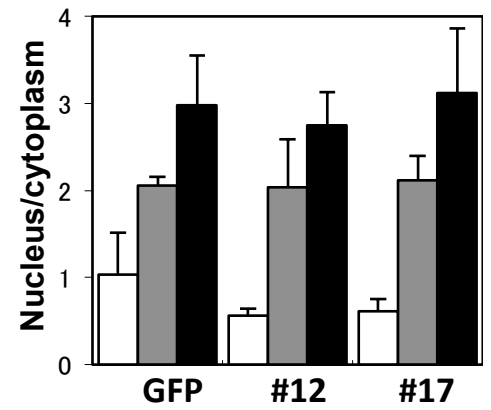
**A**



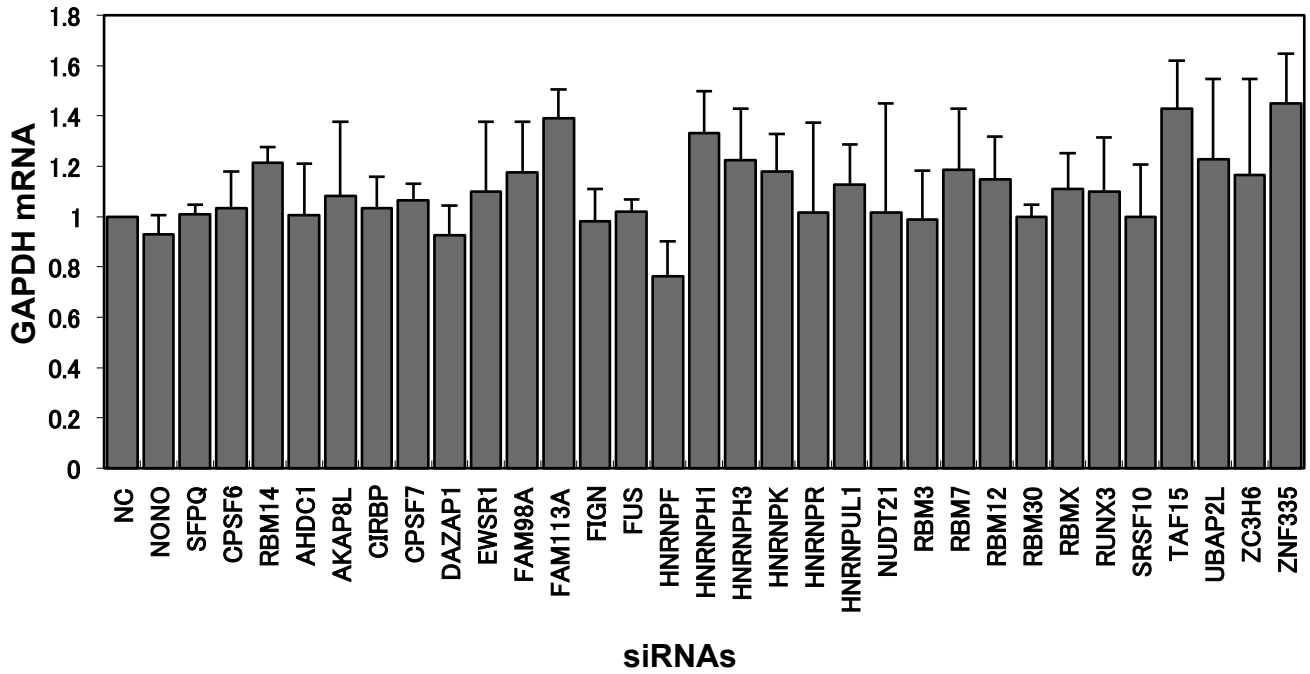
**B**



**C**



Supplemental Fig.S6



Supplemental Fig.S7

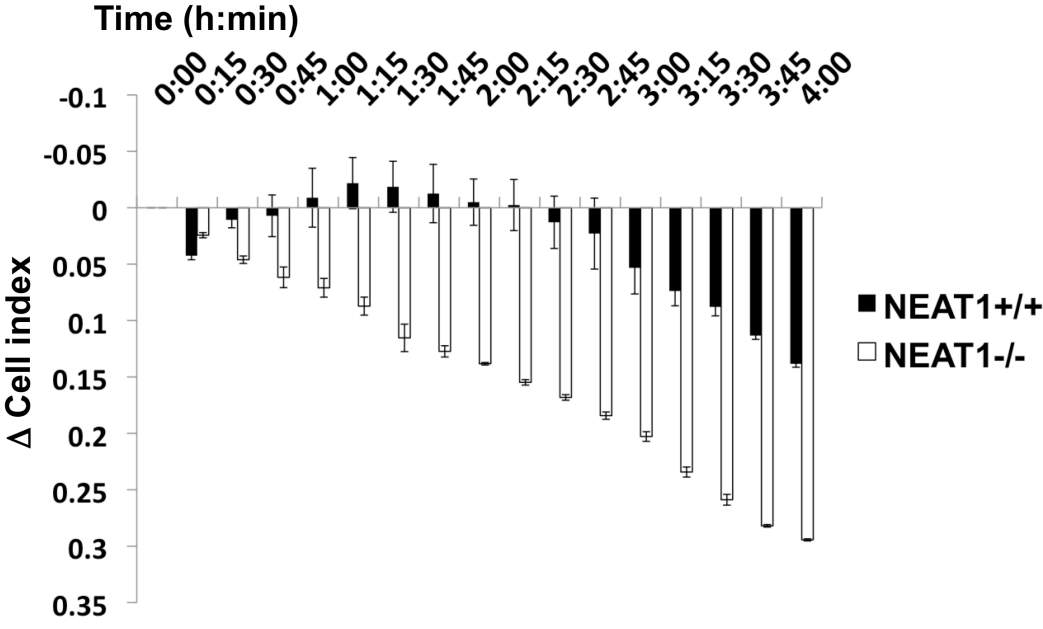


Table S1. List of genes with altered expression by NEAT1 knockdown

Samples (h)	Genes	Accession	fold changes
6	CDC40	NM_015891	3.76
6	EIF4G3	NM_003760	2.32
6	SH3MD1	NM_014631	2.24
6	ADARB2	NM_018702	2.12
6	FP15737	AF495725	2.09
6	CFI	NM_000204	2.07
6	DKFZp761B039	AL512711	0.50
6	PSEN1	AJ008005	0.48
6	EMD	NM_000117	0.47
6	CYP2U1	NM_183075	0.46
6	PFKFB3	NM_004566	0.45
6	CGI-301	AF531436	0.40
6	CHMP4B	NM_176812	0.39
6	DLST	NM_001933	0.35
6	FLJ11267	NM_019607	0.33
6	LMNB1	NM_005573	0.31
12	SSH1	NM_018984	3.67
12	STARD4	NM_139164	3.66
12	RPL10 (isoform)	AY927536	2.24
12	FLJ32797	AK057359	2.01
12	FLJ35102	AK092421	2.01
12	LFNG (isoform)	ENST00000359574	0.50
12	GPR157	BC018691	0.46
12	PTPRF	NM_002840	0.45
12	BC031940	BC031940	0.36
24	ADARB2	NM_018702	3.56
24	KATNAL1	NM_032116	2.76
24	SOCS2	NM_003877	2.73
24	PLAC4	NM_182832	2.62

24	OVC10-2	AF230201	2.24
24	FLJ33940	AK091259	0.50
24	MATN4	NM_003833	0.49
24	FRMD4A	NM_018027	0.49
24	LGR6	NM_001017403	0.47
24	MOP-1	AB014771	0.47
24	EMILIN3	NM_052846	0.44
24	PCSK1N	NM_013271	0.42
24	FLJ12650	NM_024522	0.37
24	KLHL5	NM_015990	0.37
24	LOC90353	NM_145232	0.35
24	SP5	NM_001003845	0.35
24	LCN7	NM_022164	0.30

Table S2. Antibodies used in this study

Antigen	Host	Supplier	Application	Dilution used
ADARB2	mouse	Santa Cruz	WB	1:400
SFPQ	mouse	Sigma	WB,IF,EM, ChIP/IP	1:2000, 1:100, 1:50, 5 $\mu$ g
PSPC1	rabbit	Naganuma et al.	IF	1:1000
RBM14	rabbit	Bethyl Laboratories	WB, IF	1:10000, 1:100
HNRNPK	mouse	Abcam	WB, IF	1:10000, 1:1000
FUS	mouse	Santa Cruz	WB, IF	1:1000, 1:1,000
DAZAP1	rabbit	Abcam	WB, IF	1:500, 1:100
HNRNPH3	rabbit	Abcam	WB, IF	1:1000, 1:100
NONO	Mouse	Transduction laboratories	EM	1:25
CPSF6 (CFIm68)	rabbit	Bethyl Laboratories	EM	1:25
Ubiquitin	rabbit	Dakocytomation	EM	1:25
SUMO-2/3	rabbit	Abcam	EM	1:50
SUMO-1	mouse	Zymed laboratories	EM	1:25
PML	rabbit	Kind gift of H. de Thé	EM	1:50
RNA polymerase II	mouse	Kind gift of H. Kimura	ChIP	0.5 $\mu$ g
$\alpha$ -tublin	rabbit	Abcam	WB	1:10000

Table S3. siRNAs used in this study

Target proteins	siRNAs	Sense (5'-3')	Antisense (5'-3')
AHDC1	PSP3#1	CCGAUGGCACCUUUGGCCAAGGCUU	AAGCCUUGGCCAAAGGUGCCAUCGG
	PSP3#2	GCUCCCGAGCUUGAUGGCAAGCAUU	AAUGCUUGCCAUCAAGCUCGGGAGC
AKAP8L	PSP4#1	CCAGAAUUAACCAGCGCUUAGAUAU	AUAUCUAAGCGCUGGUUAAUUCUGG
	PSP4#2	GCCGCAUUUGGAGACAGACAUGAUG	CAUCAUGUCUGUCUCCAAUUGCGGC
CIRBP	PSP6#2	GGAGGCUCCAGAGACUACUUAUAGCA	UGCUAUAGUAGUCUCUGGAGCCUCC
	PSP6#3	UCCUACAGAGACAGUUACGACAGUU	AACUGUCGUAACUGUCUCUGUAGGA
CPSF7	PSP7#1	UCAGAAGGAGUGGACUUGAUUGAUA	UAUCAAUCAAGUCCACUCCUUCUGA
	PSP7#3	GGCCCAUUCCCAGAUUCUAGUGAU	AUCACUAGAUAUCUCGGAAUUGGGCC
DAZAP1	PSP8#1	CCAAGAGACUCUGCGCAGCUACUUU	AAAGUAGCUGCGCAGAGUCUCUUGG
	PSP8#3	UGUGGUGAGACAGAGCUCAGGGAU	AUUCUUGAGCUCUGUCUCACCACA
EWSR1	PSP10#1	AUAGGUGUUCUGCUGAGAGUAACUG	CAGUUAUCUCAGCAGAACACCUAU
	PSP10#2	AAAGAAGUCUGCCAGAUCAUCUAGA	UCUAGAUGAUCUGGCAGACUUCUUU
FAM98A	PSP11#1	CCCAUUGUUGGAAGAUGGAGCGCUU	AAGCGCUCCAUCUCCAACAUGGG
	PSP11#2	GGGAGAUGAACUGCCCGUUAUCUUUC	GAAAGAUACGGGCAGUUAUCUCCC
FAM113A	PSP12#2	GCCACCUCCAUAUCCUGGCCCUAAU	AUUAGGGCCAGGAUUGGAGGUGGC
	PSP12#3	CGCUGCGAAGCGACAUGGUCCACUU	AAGUGGACCAUGUCGCUUCGCGCG
FIGN	PSP13#1	CCCUCUUCUACAGUACUGCUAAA	UUUAGCAGUACUGUAGGAAGGAGGG
	PSP13#3	CCCGUUAUUAUUAAGACUUUGAAA	UUUCAAGUCUUGAUUAGUAACGGG
FUS	PSP14#2	CGGGACAGCCCAUGAUUAAUUUGUA	UACAAUUAAUCAUGGGCUGUCCCG
	PSP14#3	GGUAAAGAAUUCUCCGAAAUCCUA	UAGGAUUUCCGGAGAAUUCUUUACC
HNRNPA1	PSP15#1	AUGAAGAGCUUCCUCAGCUGUUCGG	CCGAACAGCUGAGGAAGCUCUUCAU
	PSP15#2	UUCAGUGUCUUCUUUAAUUGCCACCA	UGGUGGCAUUAAGAAGACACUGAA
HNRNPA1L2	PSP16#1	GGCAGUUAUUAAGAAGCAGUGAA	UUCACGUGCUUCUUUAGUAACUGCC
	PSP16#2	CACCUUCACUUUGGGAUUGGAAUU	AAUCCAUAUCCCAAAGUGAAGGUG
HNRNPF	PSP17#2	GCUAUGUCCAAGACAGGGCCAUA	UAUUGGCCUGUCUUUGGACAUAGC
	PSP17#3	GAACUUGGAUCAGAAGAUGAUGUAA	UUACAUCAUCUUCUGAUCCAAGUUC
HNRNPH1	PSP18#1	GAACUUGAAUCAGAAGAUGAAGUCA	UGACUUAUCUUCUGAUUCAAGUUC
	PSP18#3	GGGUGUUGAAGCAUACUGGUCCAAA	UUUGGACCAGUAGCUUCAACACCC
HNRNPH3	PSP19#2	CCACUAAUCCAUAACGAGUUAUA	UAUGAACUCGUUUGGAUUUAGUGG
	PSP19#3	GGCUACGGAAGAGAUUGGAAUGGAUA	UAUCCAUAUCUUCUUCUCCGUGGCC
HNRNPK	PSP20#2	CCGCUCGAAUCUGAUGCUGUGGAU	AUCCACAGCAUCAGAUUCGAGCGG
	PSP20#3	UCAUCAGAGUCUAGCAGGAGGAUU	AAUUCUCCUGCUAGACUCUGAUGA
HNRNPR	PSP21#1	GAUCCAGAAGUCAUGGCUAAGGUAA	UUACCUUAGCCAUGACUUCUGGAUC
	PSP21#3	AGGAUACUUUUGGGCAACAGUGGAA	UUCACUGUUGCCAUAGUAUCCU
HNRNPUL1	PSP22#2	GCCCAGCAGUGGAACCAGUACUAUC	GAUAGUACUGGUUCCACUGCUGGGC
	PSP22#3	GGGAACUACGACUACGGGAGCUACU	AGUAGCUCUCCGUAUGCUAGUUC
NUDT21	PSP24#2	UGAACCUCCUCAGUAUCCAUAUAUU	AAUUAUUGGAUACUGAGGAGGUUCA
	PSP24#3	GCACCAGGAUUGGACCAUCAUUU	AAUUGAUGGGUCCAUAUCCUGGUGC
RBM3	PSP25#2	UCAGGAGGAAUUAACAGAGACAAUU	AAUUGUCUCUGUAAUUUCCUCCUGA
	PSP25#3	GCUCUUCGUGGGAGGGCUCACUUU	AAAGUUGAGCCUCCACGAAGAGC
RBM4B	PSP26#1	GGUUUUGGGCCAGAGUGAAUUUAU	AUAAUUCACUCUCUGGCCAUAAACC
	PSP26#3	GCAUAUGGAGCACUCGACUACUAUA	UAUAGUAGUCGAGUGCUCCAUAUGC
RBM7	PSP27#2	CAAACAUGAAGUGUCUGUCCUUAU	AUAAGGAACAGACACUUAUGUUUG

	PSP27#3	UCACAGCGUAAAGUCAGAAUGAAUU	AAUUCAUUCUGACUUUACGCUGUGA
RBM12	PSP28#1	GCUUUGAAACGAAACAGAAUGCUGA	UCAGCAUUCUGUUUCGUUUCAAAGC
	PSP28#2	UGCCACUGAUGAAGAUGCAAGGCUU	AAGCCUUGCAUCUUAUCAGUGGCA
RBMX	PSP29#2	GCAGAUCCGCCAGGAAAGCUCUUCA	UGAAGAGCUUUCUGGGCGAUCUGC
	PSP29#3	CCAAGUUCUCGUGAUACUAGAGAUU	AAUCUCUAGUAUCACGAGAACUUGG
RUNX3	PSP30#2	CCCUGACCAUCACUGUGUUCACCAA	UUGGUGAACACAGUGAUGGUCAGGG
	PSP30#3	UCACUCAGCACCCACAAGCCACUUCA	UGAAGUGGCUUGUGGUGCUGAGUGA
SRSF10	PSP31#1	CCGACGACACCAGGUCUGAAGACUU	AAGUCUUCAGACCUGGUGUCGUCGG
	PSP31#3	CAACUAUAGAAGAUUCGUUAGUCCU	AGGACUAUACGAUCUUCUAUAGUUG
SS18L1	PSP32#1	CCAUCCAGAAGAUGCUGGACGAGAA	UUCUCGUCCAGCAUCUUCUGGAUGG
	PSP32#2	GCCACGAUCGCAGACUCCAACCAGA	UCUGGUUGGAGUCUGCGAUCUGGCG
TAF15	PSP33#1	CCUUUGCCACUAGAAGACCUGAAUU	AAUUCAGGUCUUCUAGUGGCAAAGG
	PSP33#2	GCUCAUUAAGCCAGCAACCAUUAUA	UUUAUUGGUUGCUGGCUUAUUGAGC
UBAP2L	PSP34#2	CCCGCCACAAGUAUUGGUUAUGAU	AUCAUAACCAUUAUCUUGUGGCGGG
	PSP34#3	CAACACUGGCCACUUUGAACCCAGAU	AUCUGGUUCAAGUGGCCAGUGUUG
ZC3H6	PSP35#1	GGGAUCCAGGAAAUUGAGAGGGAA	UUCCUCUCAAUUUCUGGGGAUCC
	PSP35#2	GCAUCAUGCAAUUGCUGGCACUAAU	AUUAGUGCCAGCAUUUGCAUGAUGC
ZNF335	PSP36#1	AGACAGCCUUGGAUCUUCUGCUGAA	UUCAGCAGAAGAUCCAAGGCUGUCU
	PSP36#2	AGGAAGCCGCCUACAUCCAAGAGAU	AUCUCUUGGAUGUAGGCGGCUUCCU
TARDBP	TARDBP#2	UGAGCCCAUUGAAAUACCAUCGGAA	UUCCGAUGGUUUUCAAUGGGCUCA
	TARDBP#3	GACAGAUGCUCUACUACGAGUGAAA	UUUCACUGCUGAUGAAGCAUCUGUC
CPSF6	CPSF6#1	UCCGCAUGUGAACCCAGCUUUCUUU	AAAGAAAGCUGGGUUCACAUCGGGA
	CPSF6#2	ACUJCAUGGUCAGAAUCCUGUUGUA	UACAACAGGAUUCUGACCAUGAAGU
NONO	NONO#1	GGGAACAGGGUUACUGUAUACUGAA	UUCAGUAUACAGUAACCCUGUCCCC
	NONO#2	CAGUAGCUCUJAGACUCGCCUAUCU	AGAUAGGCGAGUCUAAGAGCUACUG
PSPC1	PSPC1#2	GCAGGUUGAUAGAAACAUCTT	GAUGUUUCUAUCAACCCUGCTC
	PSPC1#3	GCUAGGCAUGAACACCAAUTT	AUUGGUUGUUCUGCCUAGCTG
RBM14	RBM14#1	AGUCUGCAGCCUCCUCACUAGCUUA	UAAGCUAGUGAGGAGGCUGCAGACU
	RBM14#3	CCAAGGCCUCUUAUACUUGGAAGA	UCUCCAAGUAUUAAGAGGCCUUGG
SFPQ	SFPQ#14	CAGUCAUUGUGGAACCAUUGAACA	UGUUCAAGUGGUUCCACAAGACUG
	SFPQ#19	CCCUAUGGUUCAGGAGGCCAGAAAU	AUUUCUGGCCUCCUGAACCAUAGGG



Table S4. ASOs used in this study

ASO for knockdown	Sequence (5'-3')
GFP	mU*mC*mA*mC*mC*T*T*C*A*C*C*T*T*mC*mC*mA*mC*mU
NEAT1#12	mC*mC*mC*mU*mC*T*A*G*T*C*T*T* G*G*C*mU*mC*mA*mU*mU
NEAT1#17	mC*mC*mC*mU*mU*C*T*C*C*T*A*G* T*A*A*mU*mC*mU*mG*mC

\*Phosphorothioate backbone; N and mN represent deoxynucleotide and 2'-O-methylribonucleotide, respectively.

Table S5. PCR primers used in this study

Primers	Direction	Sequence (5'-3')
<b>qRT-PCR</b>		
ADARB2-CDS	forward	ATATTCGTGCGGTTAAAAGAAGGTG
	reverse	ATCTCGTAGGGAGAGTGGAGTCTTG
ADARB2-UTR	forward	GCCATCCCACATACCTCCAA
	reverse	GGGGTGCTACTGCCTCTCCT
ADAR1	forward	CTTCTGAGATTCTTTCCTTGTGATCTG
	reverse	AACTCCTACCCTTACAAATGTTTCACC
ADARB1	forward	CCTTTGTTTGTGCATGTCTGTGGAG
	reverse	AGAGGCATTAGGAAACTGAACGAAAG
SLC7A2-CDS	forward	ACAAGTCTTCTTGGATCCATTTTCC
	reverse	GTGTCTTCGTTTTGGAATTGATTG
SLC7A2-UTR	forward	TGTTCCGAGATTACAGAACATCACA
	reverse	CAGTTGCTTGAAGCGTATTAGGAGA
mNeat1	forward	TTGGGACAGTGGACGTGTGG
	reverse	TCAAGTGCCAGCAGACAGCA
mNeat1_2	forward	TCCTGCCAGTGATGAAGATG
	reverse	AACTGCTACCCACCGAGATG
GAPDH	forward	ATGAGAAGTATGACAACAGCCTCAAGAT
	reverse	ATGAGTCCTTCCACGATACCAAAGTT
<b>Primers for ChIP assay</b>		
-5962	forward	ATGCCAGTTCAAGCATCAAAACC
	reverse	CTGTGAGACTCCAATTCTTCATTTTCC
-5096	forward	AATAAATGGGCTGGGAGTC
	reverse	ATGATCCTGAAATGCCAAGG
-4116	forward	AAGAGCAACGGCTGAAGATG
	reverse	ATATCCCCTTCCCTCCATCA
-2924	forward	TGATCTATGGAGAGACAGACATTGAAG
	reverse	AGTTTTCTCAATCAAGGCCAGAAAG
-2049	forward	AGTTTTCAGTTTTGTTTCCCCTC
	reverse	ATACATGAACCTTGTTCCTCCTCAAG
-1788	forward	CAGATACAGAAATAGGGCAACTCTGG
	reverse	AGCTCTCTCACTTCTCTCTGCAATAC
-1183	forward	ATCAGCTCAGCACATAACAACACAG
	reverse	TATTCATTGCCTCACATGCAACC
-127	forward	AGAGCCAGAAAGCAGTTGTGATTG
	reverse	TACTTTTGCGCCTCTGCTAGTTGTT
+284	forward	GCAAGTCATAGAGAACAATTCGAGAGAC
	reverse	CACCTGCACCTGCCTCCTTC
+4973	forward	CTCAAGAGGAAGGGCTGGATTTACT
	reverse	GTCACTAGAAACCAGAGGCACTGATAC
+50705	forward	CCTCTGTCTCTCCATCAGCAA
	reverse	TCCCTGAAGCACCTTTTCTTTTATC

+55059	forward	GCCATCCCACATACCTCCAA
	reverse	GGGGTGCTACTGCCTCTCCT
<b>Subcloning of ADARB2 probe</b>		
ADARB2-northern	forward	TCGTGCGGTTAAAAGAAGGT
	reverse	AGTACATGGAGGGCGTGTCT