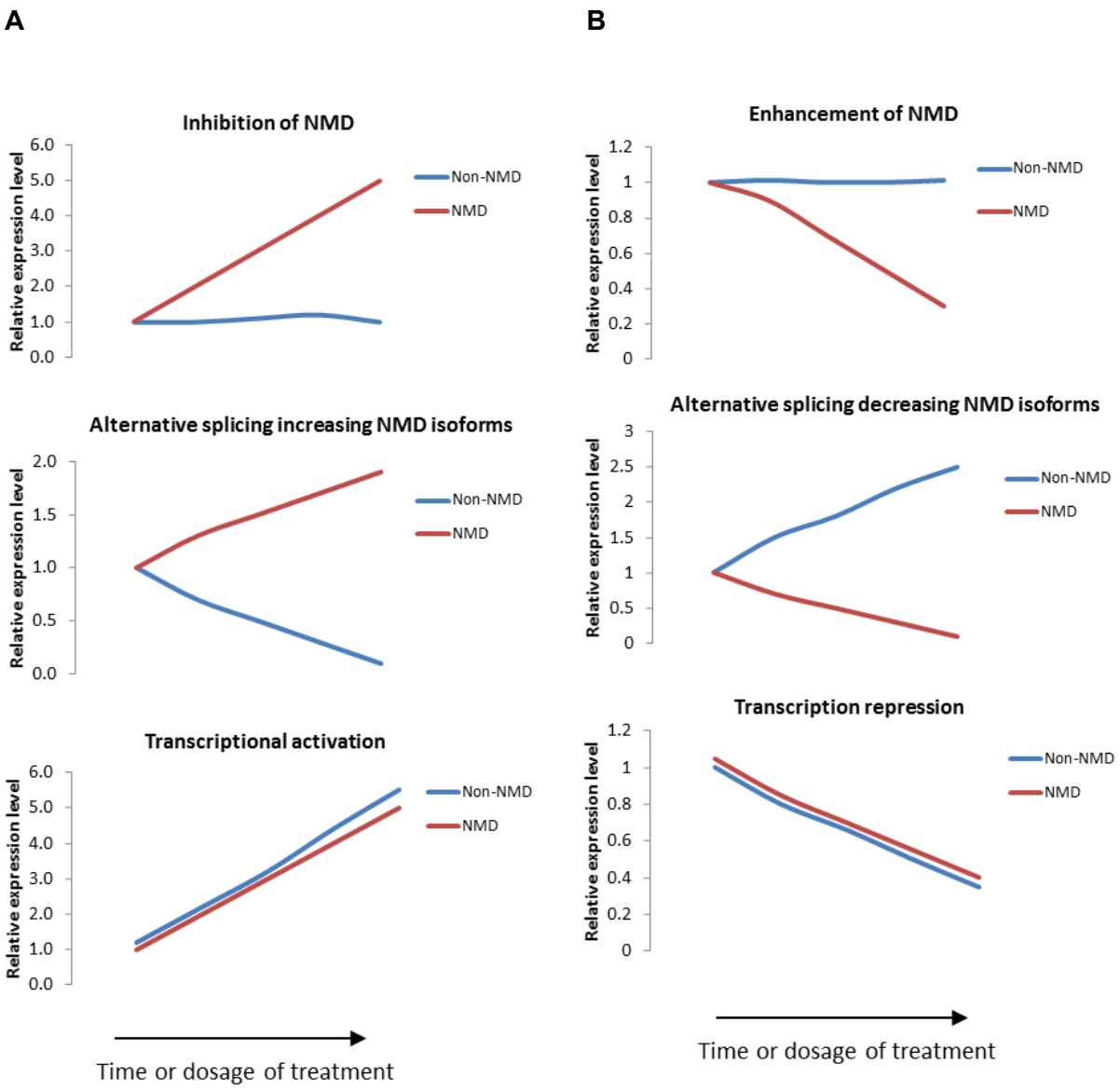
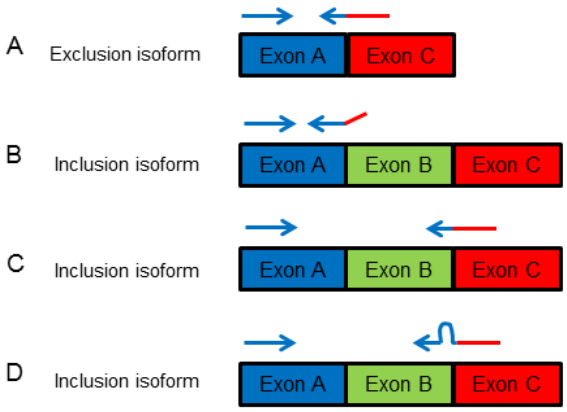
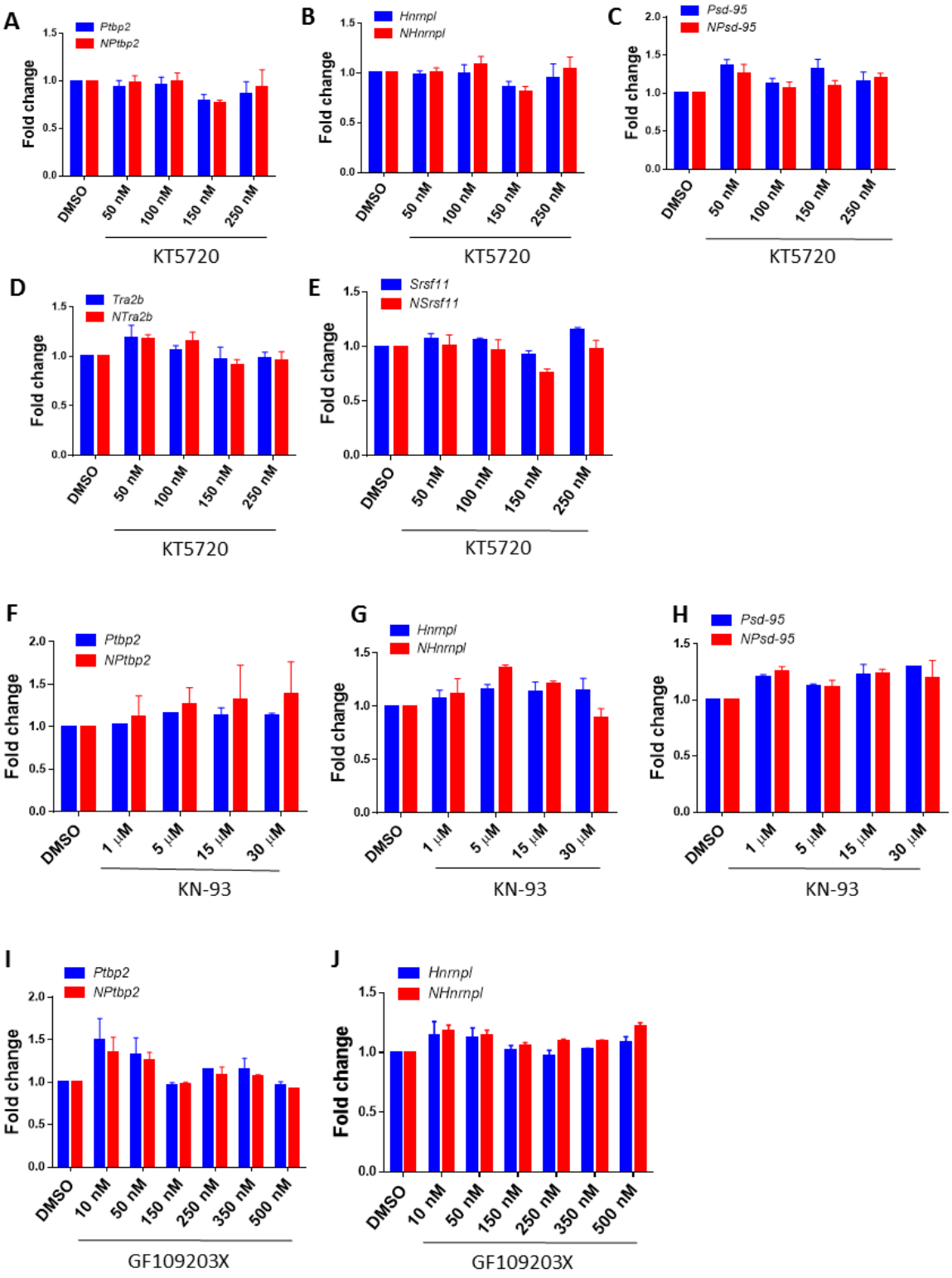


Supplemental Figure S1. (**A**) In scenarios where NMD isoform levels increase, assaying the non-NMD isoforms distinguishes NMD regulation from transcriptional activation and alternative splicing regulation. (**B**) In scenarios where NMD isoform levels decrease, assaying the non-NMD isoforms distinguishes NMD regulation from transcriptional repression and alternative splicing regulation.

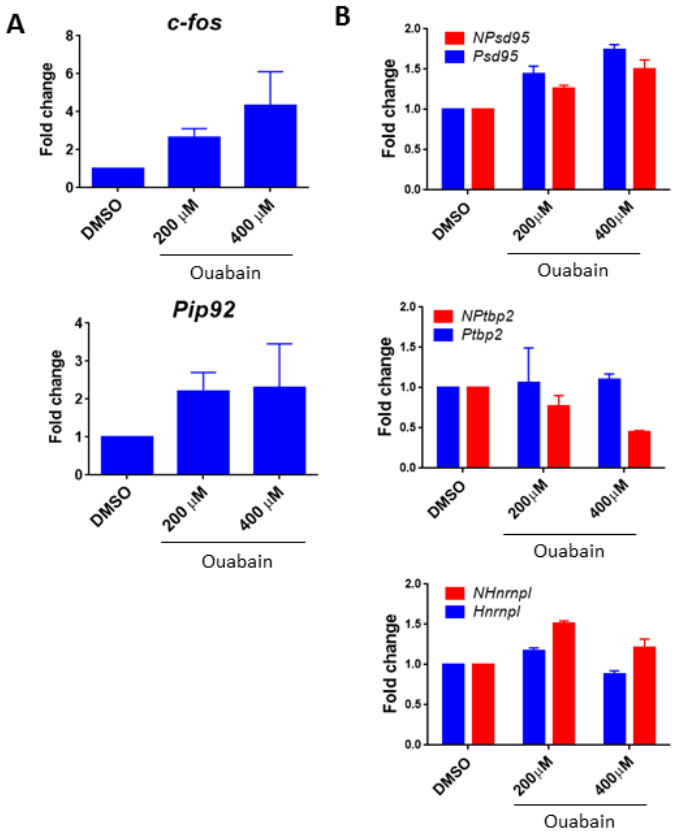


Supplemental Figure S2. Schematics of potential complementary binding of the skipping-isoform specific junction primers to (A) the exclusion isoform or (B-D) the inclusion isoform.



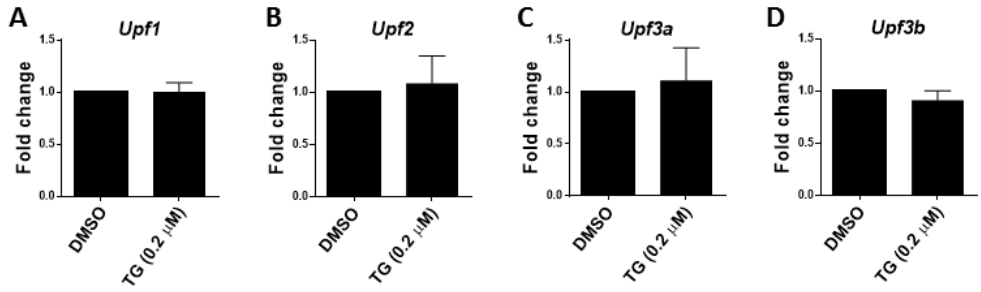
Supplemental Figure S3. Chemical inhibitors that did not have effects on NMD.

N2a cells were treated with each inhibitor at the indicated concentrations for 5 hours. Expression of NMD and non-NMD isoforms was examined by RT-qPCR. KT5720: PKA inhibitor (Tocris Bioscience, 1288100U). KN-93: CamKII inhibitor (Fisher Scientific, NC0362431). GF109203X: PKC inhibitor (R&D, A00061). N = 3. Error bars represent mean ± SEM.



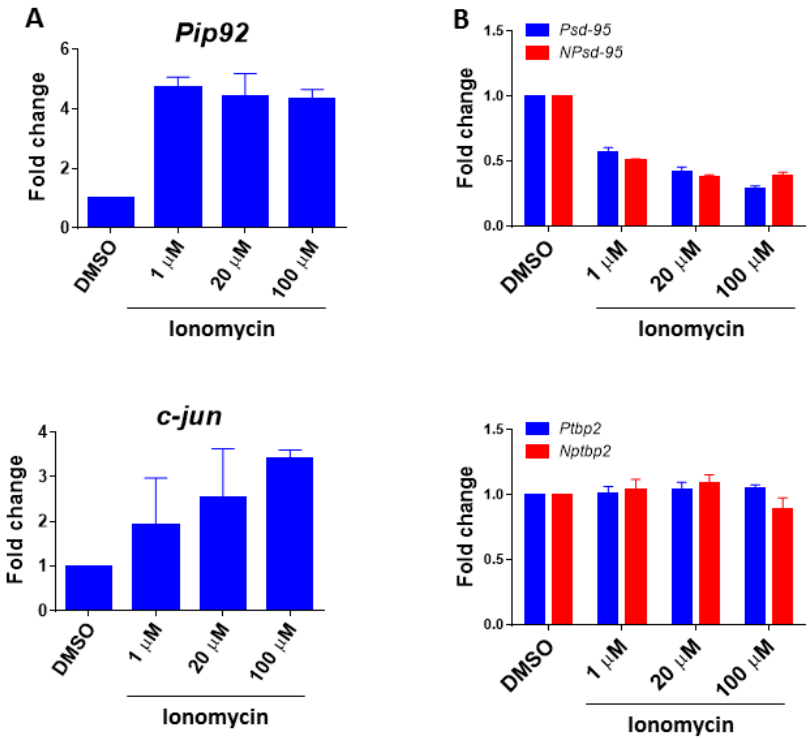
Supplemental Figure S4. Ouabain did not cause consistent changes in the NMD targets.

RT-qPCR assay of (**A**) immediate early genes *c-fos* and *Pip92* as well as (**B**) the NMD and non-NMD isoforms of *Psd-95*, *Ptbp2* and *Hnrnpl* in N2a cells treated with ouabain. Ouabain clearly induced *c-fos* and *Pip92*, but did not unequivocally affect NMD activity. N = 3. Error bars represent mean ± SEM.



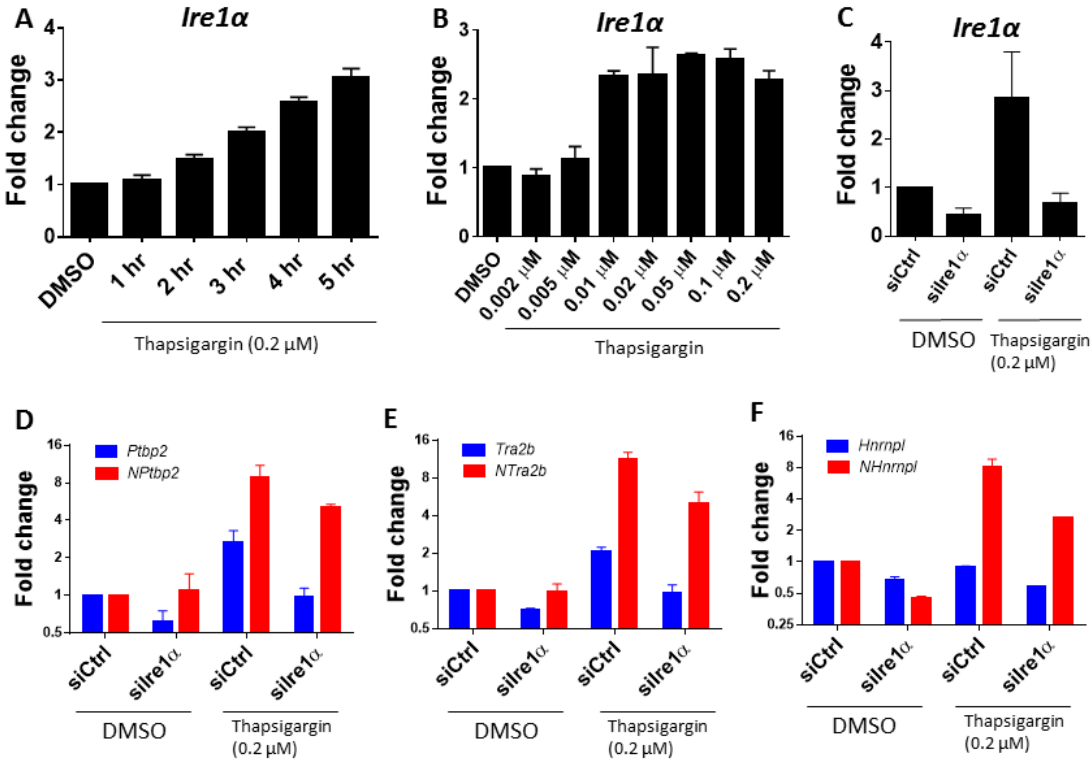
Supplemental Figure S5. *Upf1*, *Upf2*, *Upf3a* and *Upf3b* expression after thapsigargin treatment.

RT-qPCR assay targeting four major NMD factors (*Upf1*, *Upf2*, *Upf3a*, and *Upf3b*) in N2a cells showed no significant change in expression levels after 5 hours of DMSO or 0.2 μM thapsigargin (TG) treatments. N = 6. Error bars represent mean ± SEM.



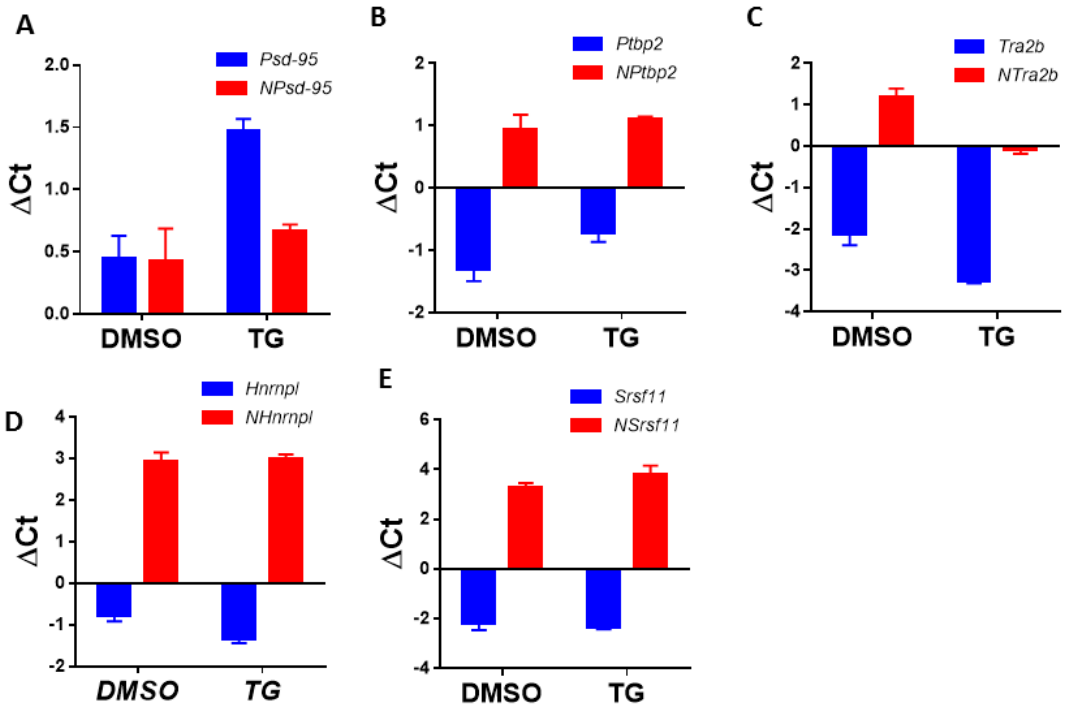
Supplemental Figure S6. Ionomycin did not have uniform effects on NMD targets.

RT-qPCR assay of (**A**) immediate early genes *c-fos* and *Pip92* as well as (**B**) the NMD and non-NMD isoforms of *Psd-95* and *Ptbp2* in ionomycin-treated N2a cells. Ionomycin induced *c-fos* and *Pip92* expression and exhibited gene specific effects on different AS-NMD targets. Ionomycin downregulated both isoforms of *Psd-95* but did not affect *Ptbp2*. N = 3. Error bars represent mean ± SEM.



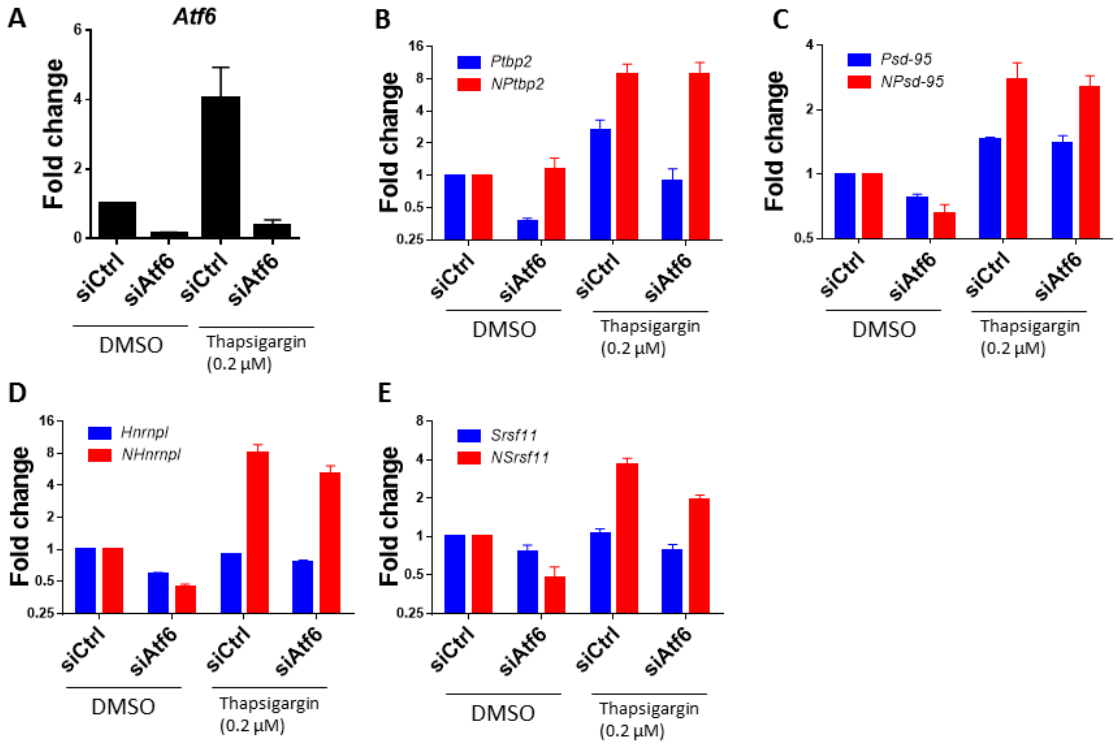
Supplemental Figure S7. *Ire1α* expression and its effect on thapsigargin-induced NMD inhibition

(**A-B**) *Ire1α* (*Ern1*) expressions upon thapsigargin treatment at various time points and dosages measured by RT-qPCR. (**C**) Expression level of *Ire1α* in cells treated with Silencer Select siRNA targeting *Ire1α*. (**D-H**) Expression (log2 scale) of AS-NMD targets in *Ire1α*-depleted N2a cells at 5 hours after treatment with 0.2 μM thapsigargin. N = 3. Error bars represent mean ± SEM.



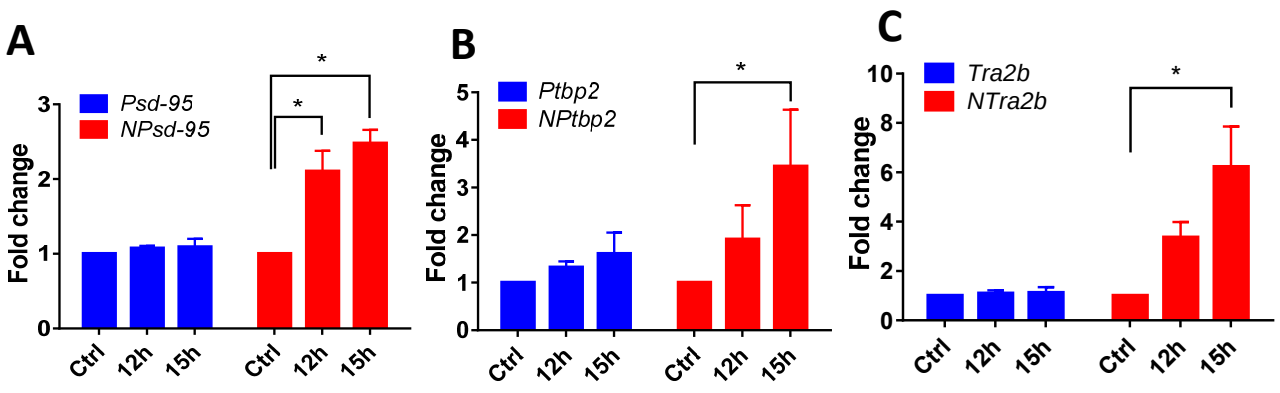
Supplemental Figure S8. Abundance of AS-NMD targets in the polysome fractions.

Polysomal RNA expression for both non-NMD and NMD isoforms of (**A**) *Psd-95*, (**B**) *Ptbp2*, (**C**) *Tra2b*, (**D**) *Hnrnpl* and (**E**) *Srsf11* was assessed by RT-qPCR to derive the ΔCt values in comparison to housekeeping gene *Sdha*. Both NMD and non-NMD isoforms were readily detectable on the polysomes. N = 3. Error bars represent mean ± SEM.



Supplemental Figure S9. *Atf6α* expression and its effect on thapsigargin-induced NMD inhibition

(**A**) Expression level of *Atf6α* in cells treated with Silencer Select siRNA targeting *Atf6α* and 0.2 μM thapsigargin. (**B-E**) Expression (log2 scale) of AS-NMD targets in *Atf6α*-depleted N2a cells at 5 hours after treatment with 0.2 μM thapsigargin. N = 3. Error bars represent mean ± SEM.



Supplementary Figure S10. Deprivation of L-glutamine inhibits NMD.

Expression levels of the NMD and non-NMD isoforms of *Psd-95* (**A**), *Ptbp2* (**B**) and *Tra2b* (**C**) in N2a cells cultured in L-glutamine-free media for 12 and 15 hours. The NMD isoforms but not the non-NMD isoforms were significantly upregulated by L-glutamine deprivation. A two-way ANOVA followed by Dunnett's multiple comparison tests was used to determine significant changes in gene expression. *, $P < 0.05$. N=3. Error bars represent mean ± SEM.

Supplementary Table S1. RT-qPCR primers.

Targets	Forward	Reverse	Size	Slope	Y-inter	R^2	Eff%	Confirmed linear range (Cq)	Cq variation at lower limit
AS-NMD qPCR primers									
Psd95_qPCR	TCCAGTCTGTGCGAGAGGTA	ACGGATGAAGATGGCGATAG	116	-3.275	32.001	0.990	100%	22.007 - 32.139	0.2
NPsd95_qPCR	CGAGAGGTAGCAGAGCAGAGA	AAGCACTCCGTGAACTCCTG	105	-3.296	31.473	0.990	100%	21.050 - 27.916	0.1
Ptbp2_qPCR	TTACGCCCCAAAGTCTGTTT	CCCATCAGCCATCTGTATCA	107	-3.417	30.309	0.988	96%	20.029 - 26.873	0.389
Ptbp2_NMD_qPCR	GAGTCTCAGCTGGTGGCAAT	TGCACATCTCCATAAACACCTC	72	-3.346	34.659	0.990	99%	24.604 - 31.296	0.586
Srsf11_qPCR	TCCAGACTCAGCAGTTGTGG	TCTCATCAGGAATAACTCTTCAGC	100	-3.418	29.948	0.998	96%	19.755 - 30.042	0.07
Srsf11_NMD_qPCR	TCCAGACTCAGCAGTTGTGG	GGCTGAACCAGGGAAAAGA	102	-3.397	35.431	0.994	97%	25.036 - 31.648	0.234
Tra2b_qPCR	GGAGCTTGACAGCTTCAGGA	AAGCAGAACGGGATTCCC	105	-3.340	29.231	0.998	100%	19.217 - 25.841	0.043
Tra2b_NMD_qPCR	TGGAATCAGAAAGCACTACGC	GAGTCTTCCTTGGAGCGAGA	116	-3.458	35.032	0.998	95%	24.690 - 31.606	0.248
Hnrnpl_qPCR	CAACCTCAGTGGACAAGGTG	CCTCATATTCTGCGGGATGA	92	-3.211	28.059	0.999	104%	18.394 - 24.832	0.117
Hnrnpl_NMD_qPCR	GGTCGCAGTGTATGTTTGATG	GGCGTTTGTTGGGGTTACT	100	-3.394	34.321	0.996	97%	24.209 - 30.801	0.313
ER stress reporter									
Xbp1s_qPCR	CTGAGTCCGCAGCAGGTG	GGCAACAGTGTCAGAGTCCA	74	-3.226	34.042	0.975	104%	24.113 - 30.566	0.447
Bip_qPCR	CTGAGGCGTATTTGGGAAAG	CAGCATCTTTGGTTGCTTGT	93	-3.647	30.698	0.996	88%	19.634 - 26.928	0.051
Ern1_qPCR	TCCTAACAACCTGCCCAAAC	AGATACGGTGGTCGGTGTGT	121	-3.766	32.98	0.997	84%	21.711 - 29.303	0.244
Atf6a_qPCR	GCAGGAGGGGAGATACGTT	GTGGTCTTGTTGTGGGTGGT	82	-3.853	34.269	0.986	82%	22.918 - 30.624	0.48
Immediate early genes									
Pip92	CCGACAATATGCTCAACGTG	CTCGAAAGAAGCCACCAGAG	70	-3.271	33.069	0.995	102%	23.076 - 33.004	0.012
c-fos	GCAGAAGGGGCAAAGTAGAG	GCAGCCATCTTATTCCGTTC	82	-3.260	35.667	0.994	102%	26.013 - 35.993	0.003
c-jun	GAAAAGTAGCCCCCAACCTC	GGGACACAGCTTTCACCCTA	102	-3.377	33.839	0.994	98%	23.605 - 30.308	0.02
Others									
Tardbp (Tdp43)	GGGGCAATCTGGTATATGTTG	TTCACTGCAGAGGAAGCATCT	82	-3.144	27.647	0.998	108%	18.262 - 24.55	0.102
eIF2ak3 (Perk)	GGTCTGGTTCCTTGGTTTCA	GGTCCCACTGGAAGAGGTC	99	-3.169	32.367	1.000	106%	22.886 - 29.224	0.033
Upf1	CCAGCGCTCTTACTTGGTG	ACGCAGGACAGAATGATGAAG	132	-3.405	30.229	0.998	97%	19.99 - 30.229	0.27
Upf2	TGTCCGCTTTATTGGAGAGC	TGCATGCCATTTCAATATGAT	116	-3.431	32.039	0.997	96%	21.756 - 28.617	0.264
Upf3a	GGAGACGAGAAGCAGGAAGA	CGAGATCTCTTGTCCCTTGG	104	-3.366	33.887	0.999	98%	23.75 - 30.556	0.01
Upf3b	GATAGGCAGGATCGCAACAG	TCCTGAAGCTGTTCCTTGGT	95	-3.329	33.615	0.995	100%	23.451 - 30.464	0.306
U6	CGCTTCGGCAGCACATATAC	ACGAATTTGCGTGTCATCCT	84	-3.415	29.4	0.999	96%	19.124 - 29.378	0.233
Gapdh	TGCGACTTCAACAGCAACTC	CTTGCTCAGTGTCCTTGCTG	200	-3.32	24.06	0.999	100%	13.54 - 21.84	0.08
Sdha	GCTTGCGAGCTGCATTTGG	CATCTCCAGTTGTCCTCTTCCA	145	-3.3	28.62	0.99	100%	18.32 - 25.26	0.15

Supplemental Table S2. Different sensitivity of NMD targets and their PTC positions

Gene/isoform	3'UTR length	Distance from PTC to the first downstream splice junction (>50bp)	Sensitivity rank to *Upf2* knockout	Sensitivity rank to TG treatment
NHnrnpl	1244	83	1	1
NTra2b	3333	195	2	1
Nptbp2	2077	66	2	3
NSrsf11	2854	86	4	4
NPsd95 (Dlg4)	1024	80	4	5

Supplementary Table S3. RT-PCR primers for splicing assay and genotyping primers.

Splicing Primers	Forward	Reverse	Tm (°C)	Size (inclusion)	Size (exclusion)
Psd-95	TCTGTGCGAGAGGTAGCAGA	AAGCACTCCGTGAACTCCTG	60	211	111
Xbp1	TGGCCGGGTCTGCTGAGTCCG	GTCCATGGGAAGATGTTCTGG	56	98	72
ALS-associated cryptic exons				Cryptic Inclusion	Cryptic exclusion
Ups15	CCAGGTGCATCCAATTTTTC	GCCTGGCTGTTCATTGTTTC	60	338	174
A23004 6K03Rik	CAGCAGCTGCCAAACTTCTA	CATTGCATCTGTTGGTGAGG	60	408	210
Mib1	CAGCAATGCAAGCTGCTAGT	TGGGATGACAACCAAAATCC	60	332	273
Genotyping primers				Size	
Ptbp2 Wt	TCTACTTCATTGTGTTGTTTTG	GATACAGCAGGCTCCCCTCA	60	184	
Ptbp2 Null	TCTACTTCATTGTGTTGTTTTG	AAATAAGCATTTTCTAGCACCAA	60	455	