Figures and figure supplements

Regulatory consequences of neuronal ELAV-like protein binding to coding and non-coding RNAs in human brain

Claudia Scheckel et al
Figure 1. Identification of nELAVL targets in human brain. (A) Illustration depicting the brain area analyzed by CLIP and RNAseq. The image was generated using BodyParts3D/Anatomography service by DBCLS, Japan. (B) SDS-PAGE separation of radiolabeled nELAVL-RNP complexes. nELAVL-RNP complexes from 40 mg of human brain were specifically immunoprecipitated with Hu-antiserum, compared to control serum (compare lane #4 to #1), which is dependent on UV irradiation (compare lane #4 to #2). Wide-range nELAVL-RNP complexes collapse to a single band in the presence of high RNAse concentration (lane #3). RNAse dilutions: + 19.23 Units/μl; +++ 3846 Units/μl. As in studies of mouse nELAVL (Ince-Dunn et al., 2012), higher molecular weight bands were present in nELAVL CLIP autoradiograms, which correspond at least in part to nELAVL multimers. (C) Shown is the most enriched motif in the top 500 nELAVL peaks, determined with MEME-ChiP. (D) Pie chart of the genomic peak distribution of 75,592 nELAVL peaks (p < 0.01; present in at least 5 individuals). (E) nELAVL binding correlates with...
Figure 1 continued

mRNA abundance. nELAVL binding (CLIP tags within binding sites per transcript) was compared to mRNA abundance (RNAseq tags per transcript). Only expressed genes with peaks are shown and the correlation coefficient is indicated. The top 1000 targets were identified as genes with highest normalized nELAVL binding (binding sites were normalized for mRNA abundance and summarized per gene). (F) Subnetwork of direct protein-protein interactions of top nELAVL targets. The 1000 top nELAVL target genes and six additional genes highly associated with AD (APP, BACE1, MAPT, PICALM, PSEN1 and PSEN2) were clustered using the organic layout algorithm in yEd. Genes with no direct interactions with other target genes were excluded, leaving 172 nodes from the top nELAVL target list (green) and 5 AD associated genes (blue) in this subnetwork. The size of the nodes is proportional to the connectivity degree. Six clusters (gray circles) containing at least 10 nodes were identified, and subjected to enrichment analysis (see Supplementary file 1F).

DOI: 10.7554/eLife.10421.003
Figure 1—figure supplement 1. Cross-correlation plot comparing nELAVL peak binding between eight individuals (n = 75,592). Shown are R values.
DOI: 10.7554/eLife.10421.004
Figure 1—figure supplement 2. Shown is the most enriched motif in the top 500 nELAVL peaks, determined with HOMER.

DOI: 10.7554/eLife.10421.005
Pie chart of the genomic peak distribution of 75,592 nELAVL peaks (p < 0.01; present in at least 5 individuals), normalized for region length.

**Figure 1—figure supplement 3.** Pie chart of the genomic peak distribution of 75,592 nELAVL peaks (p < 0.01; present in at least 5 individuals), normalized for region length.

DOI: 10.7554/eLife.10421.006
Figure 1—figure supplement 4. nELAVL peaks within 3'UTRs are higher than intronic binding sites. The genomic distribution of nELAVL binding sites is plotted as a function of nELAVL peak binding. The number of nELAVL binding sites (n) within each category is indicated.

DOI: 10.7554/eLife.10421.007
Figure 1—figure supplement 5. Cross-correlation plot comparing the mRNA abundance of all transcripts between eight individuals (n = 19,185). R values are depicted.
DOI: 10.7554/eLife.10421.008
Figure 1—figure supplement 6. Correlations between mRNA abundance and nELAVL binding. (A,B) mRNA abundance shows a higher correlation with nELAVL 3’UTR (A) than intronic binding (B). nELAVL binding was defined as CLIP tags within binding sites per transcript. Shown are genes with exclusively 3’UTR (A) or intronic (B) binding and R values are indicated.
DOI: 10.7554/eLife.10421.009
Figure 2. nELAVL mediated regulation is conserved in mouse and human. (A) Overlap of nELAVL targets in human and mouse. Human nELAVL targets (n = 8681) were intersected with mouse targets identified by RIP (Bolognani et al., 2010) or HITS-CLIP (Ince-Dunn et al., 2012). 538 genes were identified as nELAVL targets by RIP and were expressed in human brain. 1978 expressed genes had HITS-CLIP nELAVL clusters that were present in at least 3 samples (biological complexity (BC) ≥ 3). Both overlaps (n = 500 and n = 1835) were highly significant (p = 6.5e-74 and p = 2.3e-287; hypergeometric test), compared to expressed transcripts (n = 14,737). (B) Only few nELAVL binding sites are conserved between mice and human, which are predominantly present within 3'UTRs. The genomic distribution of all human nELAVL binding sites (total) and nELAVL binding sites conserved in mouse is shown. The number of nELAVL binding sites (n) within each category is indicated. (C) UCSC Genome Browser images illustrating the 3'UTRs of RAB6B, HCN3, and KCNMB2 and their normalized nELAVL binding profile in human brain. The maximum PeakHeight is indicated by numbers in the right corner. (D) The mRNA levels of transcripts with nELAVL 3'UTR binding decrease in Elavl3/4 knockout (KO) mice. Shown are the mRNA expression fold changes (knockout/wildtype) of RAB6B, HCN3, and KCNMB2. *p< 0.01 (two-tailed t test; Ince-Dunn et al., 2012). (E) UCSC Genome Browser images showing pink cassette exons in the DST, NRXN1, and CELF2 genes and Figure 2 continued on next page.
Figure 2 continued

their normalized nELAVL binding profiles in human brain. The maximum PeakHeight is indicated by numbers in the right corner. (F) nELAVL binding adjacent to a cassette exon in the DST gene prevents exon inclusion. Downstream nELAVL binding promotes the inclusion of cassette exons in the NRXN1 and CELF2 genes. The change in alternative exon inclusion (delta inclusion (ΔI): wildtype - Elavl3/4 KO) is shown. * significantly changing (analyzed by Aspire2; Ince-Dunn et al., 2012).

DOI: 10.7554/eLife.10421.010
Figure 2—figure supplement 1. Comparison of nELAVL binding (CLIP tags within binding sites per transcript) between mice and human. The correlation coefficient is indicated.

DOI: 10.7554/eLife.10421.011
Figure 3. nELAVL proteins regulate mRNA abundance of human brain targets. (A) nELAVL depletion causes mRNA level changes in IMR-32 neuroblastoma cells. The mRNA abundance change was plotted against average mRNA abundance. Significantly changing transcripts (FDR < 0.05; n = 784) are colored in blue. Shown are only expressed genes (n = 12,743), and ELAVL1/2/3/4 transcripts are indicated. (B) nELAVL with exclusively 3'UTR binding decrease upon nELAVL RNAi depletion. Box plots represent the distribution of mRNA level differences between mock and nELAVL RNAi. We compared genes with exclusively 3'UTR (n = 2346) or intronic (n = 1693) binding that were expressed in IMR-32 cells. nELAVL binding was defined as CLIP tags within binding sites per transcript. Transcripts with exclusively 3'UTR binding were less abundant upon nELAVL RNAi compared to remaining transcripts (p = 3.8e^{-15}; two-tailed t-test). In contrast, mRNA levels of transcripts with exclusively intron binding were even slightly increased compared to remaining transcripts (p = 1.7e^{-4}; two-tailed t-test). (C) Transcripts with nELAVL 3'UTR binding decrease upon nELAVL RNAi. Cumulative fraction curves for genes with no 3'UTR nELAVL binding in human brain, 3'UTR binding, and top 3'UTR targets. Top targets were identified as 1000 genes with highest normalized nELAVL 3'UTR binding (binding sites were normalized for mRNA abundance before summarized per gene). 952 of the top 1000 Figure 3 continued on next page
targets were expressed in IMR-32 cells. A curve displacement to the left indicates a downregulation of mRNA abundance upon nELAVL RNAi. p values were calculated with a one-sided KS test, comparing (top) targets to non-targets. (D) Many transcripts that are decreasing upon nELAVL depletion are top nELAVL 3'UTR targets. The mRNA abundance change (nELAVL/mock RNAi) of transcripts expressed in IMR-32 cells and in human brain (n = 12,242) was plotted against average mRNA abundance. Significantly changing transcripts (FDR<0.05; n = 743) are colored in blue and additionally boxed if they are top nELAVL 3'UTR targets. Transcripts shown in E/F are indicated. (E) UCSC Genome Browser images illustrating the 3'UTRs of APPBP2, ATXN3, and SHANK2 and their normalized nELAVL binding profile in human brain. The maximum PeakHeight is indicated by numbers in the right corner. (F) The mRNA abundance of top nELAVL 3'UTR targets decreases upon nELAVL RNAi. Shown are the mRNA level changes (nELAVL/mock RNAi) of APPBP2, ATXN3, and SHANK2. * FDR<0.05 (derived from edgeR). DOI: 10.7554/eLife.10421.012
Figure 3—figure supplement 1. Western blot and its quantification showing protein levels of nELAVL and the housekeeping genes HSP90 and Histone H3 in mock and nELAVL RNAi-treated IMR-32 cells. Protein expression was normalized to the housekeeping gene Histone H3 and to mock RNAi treated cell. Error bars represent SEM. * p<0.01 (two-tailed t-test).
DOI: 10.7554/eLife.10421.013
Figure 4. nELAVL regulates splicing of human brain targets. (A) Analysis of splicing changes upon nELAVL RNAi. Shown is the exon inclusion fraction of cassette exons that are expressed in IMR-32 cells and in human brain (n = 7903). Significantly changing exons (FDR<0.05 and ΔI>0.1) are colored in light blue (n = 473), and additionally boxed in dark blue if adjacent (+/- 2.5 kb) to intronic nELAVL binding sites (n = 155). Significantly changing exons shown in (B/C) are boxed in pink. The two alternative events within PICALM correspond to the same alternative exon with two different 3’ splice sites. (B) UCSC Genome Browser images depicting cassette exons in pink in the BIN1, PICALM, and APP genes and their normalized nELAVL binding profiles in human brain. The maximum PeakHeight is indicated by numbers in the right corner. (C) nELAVL binding downstream of cassette exons in BIN1 and PICALM promotes exon inclusion, whereas intronic nELAVL binding of APP prevents exon inclusion downstream and upstream. The change in alternative exon inclusion (ΔI: mock – nELAVL RNAi) is shown. *FDR<0.0005; **FDR<1e-4; ***FDR<1e-16 (GLM likelihood ratio test). (D) Normalized nELAVL binding map of nELAVL regulated exons. Only exons that changed significantly upon nELAVL RNAi (FDR<0.05 and ΔI>0.1) and that are adjacent (+/- 2.5 kb) to intronic nELAVL binding sites (n = 155) were included. Red and blue peaks represent binding associated with nELAVL-dependent exon inclusion and exclusion, respectively.

DOI: 10.7554/eLife.10421
Figure 5. RNA regulation changes in AD. (A) nELAVL binding changes in AD. The nELAVL peak binding change (AD/Control) was plotted against average nELAVL peak binding. Significantly changing peaks (FDR<0.05; n = 52) are colored in blue, and peaks within AD genes are colored in pink (1811 peaks within 69 genes). Shown are only peaks that are bound in control or AD brain (n = 115,393). (B) mRNA abundance changes in AD. The mRNA abundance change (AD/Control) was plotted against average mRNA abundance. Significantly changing transcripts (FDR<0.05; n = 3) are colored in blue, and AD transcripts are colored in pink (n = 89). Shown are only transcripts that are expressed in control or AD brain (n = 14,875). (C) Analysis of splicing changes in AD. Shown is the inclusion fraction of expressed cassette exons in control and AD subjects (n = 8163). Exons within AD genes are colored in pink (n = 79). Significantly changing exons (FDR<0.05 and Δ>0.1) are colored in light blue (n = 170), and additionally boxed in pink if within AD genes (n = 2). (D) BIN1 is alternatively spliced in AD. UCSC Genome Browser image illustrating a cassette exon in the BIN1 gene and normalized nELAVL binding profiles in control and AD brain. The maximum PeakHeight is indicated by numbers in the right corner. Bar graphs depict the difference in alternative exon inclusion (Δ: Control – AD) and nELAVL peak binding (AD/Control) in control and AD brain. Corresponding FDR values derived from edgeR are shown. The inclusion of the exon is promoted by nELAVL (see Figure 4), and exon inclusion as well as nELAVL peak binding are reduced in AD subjects.

DOI: 10.7554/eLife.10421.015
Figure 5—figure supplement 1. Examples of disease associated SNPs with corresponding nELAVL binding sites. UCSC Genome Browser images depicting the last exon of GABRB1, KCNJ10, KCNK2, LIPA, and USP24 and the normalized nELAVL binding profile in human brain. Pink bars illustrate SNPs overlapping with nELAVL binding sites, and the maximum PeakHeight is indicated by numbers in the right corner.
DOI: 10.7554/eLife.10421.016
Figure 5—figure supplement 2. Correlations between control and AD samples. (A) Cross-correlation plot comparing nELAVL peak binding between eight controls and nine AD subjects (n = 247,547). Shown are R values.

(B) Cross-correlation plot comparing the mRNA abundance of all transcripts between eight controls and nine AD subjects (n = 19,185). R values are depicted.

DOI: 10.7554/eLife.10421.017
Figure 6. Non-coding Y RNAs are bound by nELAVL in AD. (A) Secondary structures of Y1 and Y3. Binding sites of nELAVL and Ro are indicated. Modified from (Chen and Wolin, 2004). (B) The nELAVL binding motif (UUUUUU, allowing a G at any position) is enriched in nELAVL-bound Y RNAs compared to non-bound Y RNAs ($p = 1.1 \times 10^{-7}$; Fisher’s exact test). Y RNAs were scanned for (T)$_6$, allowing a G at any position. nELAVL-bound Y RNAs: nELAVL CLIP tags in at least two samples, $n = 320$. (C) nELAVL binding of Y RNAs increases in AD compared to control samples ($p = 4.47 \times 10^{-51}$; paired one-sided Wilcoxon rank sum test). The axes depict nELAVL Y RNA binding (nELAVL CLIP tags per Y RNA) in control and AD subjects. Y RNAs with nELAVL binding motif are colored in green. (D) Y RNA levels do not change in AD. Y RNA abundance (RNAseq tags per Y RNA) in AD subjects was plotted against Y RNA abundance in control subjects. 

DOI: 10.7554/eLife.10421.018
Figure 6—figure supplement 1. Y RNAs with a motif that are not bound are not expressed. Box plots represent the distribution of Y RNA abundance in human brain. Non-bound Y RNAs with an nELAVL binding motif show a lower expression than nELAVL-bound Y RNAs with motif (p = 0.002; two-tailed t test). DOI: 10.7554/eLife.10421.019
Figure 6—figure supplement 2. nELAVL:Y RNA binding increases in AD. (A) A subset of AD subjects shows increased nELAVL binding to Y RNAs. Box plots represent the distribution of nELAVL Y RNA binding (tags per Y RNA) for each individual. AD subjects were grouped into AD_Y and AD_nY subjects based on nELAVL binding. Only nELAVL bound Y RNAs are included: nELAVL CLIP tags in at least two samples; n = 320. (B,C) nELAVL Y RNA binding but not Y RNA expression changes in AD_Y subjects. Comparison of Y RNA abundance and nELAVL Y RNA binding changes (AD/Control) in AD_Y (B) and AD_nY (C) subjects.
DOI: 10.7554/eLife.10421.020
Figure 7. Y RNPs are remodeled during UV stress. (A) The nELAVL binding motif (UUUUUU, allowing a G at any position) is enriched in nELAVL-bound Y RNAs compared to non-bound Y RNAs (p = 6.2e-6; Fisher’s exact test). Y RNAs were scanned for (T)₆, allowing a G at any position. nELAVL-bound Y RNAs: nELAVL CLIP tags in at least two samples; n = 132. (B) nELAVL binding of Y RNAs increases during UV stress compared to non-stressed cells (p = 8.23e-29; paired one-sided Wilcoxon rank sum test). The axes depict nELAVL Y RNA binding (nELAVL CLIP tags per Y RNA) in control and UV stressed cells. Y RNAs with nELAVL binding motif are colored in green. (C) Y RNA levels do not change upon UV stress. Y RNA abundance (RNAseq tags per Y RNA) in UV stressed cells was plotted against Y RNA abundance in non-stressed control cells. (D) nELAVL binding is not required for Y RNA stability. Comparison of Y RNA abundance between mock and nELAVL RNAi treated UV stressed cells.

DOI: 10.7554/eLife.10421.021
Figure 7—figure supplement 1. Y RNAs with a motif that are not bound are not expressed. Box plots represent the distribution of Y RNA abundance in IMR-32 cells. Non-bound Y RNAs with an nELAVL binding motif show a lower expression than nELAVL-bound Y RNAs with motif (p = 0.08; two-tailed t-test). DOI: 10.7554/eLife.10421.022
Figure 7—figure supplement 2. UV-stressed cells show increased nELAVL binding to Y RNAs. Box plots represent the distribution of nELAVL binding (tags per Y RNA) for each individual. Only nELAVL bound Y RNAs are included: nELAVL CLIP tags in at least two samples; n = 132.
DOI: 10.7554/eLife.10421.023
Figure 7—figure supplement 3. UV does not induce changes in the nucleocytoplasmic localization of Y RNP components. (A) Validation of UV stress induction. Bar graphs depict the fold change in RNA expression in UV stressed cells compared to non-stressed control cells. CDKN1A (the most upregulated transcript in the RNAseq dataset) but not control mRNAs (ACTB, GAPDH, ELAVL4) nor Y RNAs increase upon UV stress. RNA expression was normalized to non-UV treated cell. Error bars represent SEM. p values were calculated with a two-tailed t test (ns: not significant; * p = 6.9e-5; two-tailed t-test). (B) UV stress does not induce changes in Y RNA distribution. Bar graphs depict the percentage of cytoplasmic RNA levels (cytoplasmic RNA levels divided by the sum of cytoplasmic and nuclear RNA levels) of mRNA controls (ACTB, GAPDH, ELAVL4) and Y RNAs. Error bars represent SEM. Changes in cytoplasmic RNA levels were not significant (ns; p<0.05; two-tailed t-test). (C) UV stress does not induce changes in protein distribution. Western Blot and its quantification showing cytoplasmic and nuclear protein levels of nELAVL, RO60, as well as cytoplasmic (HSP90 and GAPDH) and nuclear (RNA PolII and Histone H3) markers. Bar graphs depict the percentage of cytoplasmic protein levels (cytoplasmic protein levels divided by the sum of cytoplasmic and nuclear protein levels). Error bars represent SEM. Changes in cytoplasmic proteins levels were not significant (ns; p<0.05; two-tailed t test).

DOI: 10.7554/eLife.10421.024
Figure 7—figure supplement 4. Up to 5% of nELAVL CLIP map to Y RNAs in AD_Y subjects (AD subjects with increased nELAVL/Y RNA association) and UV stressed cells when mapped with Bowtie 2 (allowing multiple alignments and reporting one). Columns represent the percentage of nELAVL tags that mapped to Y RNAs with Bowtie 2.
DOI: 10.7554/eLife.10421.025
A

\[ \text{\% of changing peaks} \]

\[
\begin{array}{c|c|c}
& \text{increase} & \text{decrease} \\
\hline
\text{AD}_Y & 100 & 0 \\
\text{AD}_nY & 80 & 20 \\
\text{UV} & 60 & 40 \\
\hline
\end{array}
\]

n: 8076 1305 290

B

\[ \Delta I (\text{mock - mock}_{-UV}) \]

\[ R = 0.01 \]

nELAVL-dependent UV changes

C

\[ \text{Fraction Exon Inclusion} \]

\[ \text{nELAVL} \]

\[ \text{RNAi} \]

\[ \text{RNAi & UV} \]

D

\[ \text{nELAVL peak binding fold change [log2]} \]

\[ \text{AD}_Y \]

\[ \text{AD}_nY \]

E

\[ \text{CBFA2T2} \]

CLIP brain

<table>
<thead>
<tr>
<th>RNAseq</th>
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<tbody>
<tr>
<td>mock</td>
</tr>
<tr>
<td>\text{nELAVL}</td>
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<tr>
<td>\text{mock}_{-UV}</td>
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1kb

100nt
Figure 8. nELAVL/Y RNA correlates with loss of nELAVL-mediated splicing. (A) Samples with high nELAVL/Y RNA association show decreased nELAVL binding on mRNA targets. Columns represent significantly changing nELAVL binding sites. Shown are changes in AD subjects with and without Y RNA association (AD_Y and AD_nY) and changes upon UV treatment. The number of nELAVL binding sites (n) within each category is indicated. (B) Identification of nELAVL-dependent UV-induced splicing changes. Comparison of the differential inclusion rate of expressed cassette exons upon UV stress between mock and nELAVL RNAi treated IMR-32 cells (n = 9397). Significant UV-induced splicing changes that do not change upon UV stress in nELAVL RNA treated cells are boxed in dark blue (FDR<0.05 and ΔI>0.1; n = 260). (C) Many exons that are alternatively spliced upon nELAVL RNAi treatment also change during UV stress in an nELAVL-dependent manner. Shown is the inclusion rate of expressed cassette exons in IMR-32 cells that were subjected to mock or nELAVL RNAi (n = 9397). nELAVL RNAi induced splicing changes are colored in light blue (n = 553), and are additionally boxed in dark blue if they are UV-induced in an nELAVL-dependent manner (n = 68). The plot is related to Figure 4A but contains additional cassette exons expressed in UV stressed cells. (D) nELAVL binding adjacent to exons that are alternatively spliced upon nELAVL RNAi and UV treatment decreases only in AD subjects with an increased Y RNA association. Displayed is the change in nELAVL peak binding. nELAVL peak binding changes were not significant except for CBFA2T2 (boxed in pink). * FDR<0.05 (derived from edgeR). (E) UCSC Genome Browser images depicting an overview and an enlarged view of a cassette exon within the CBFA2T2 gene that is alternatively spliced in nELAVL RNAi and UV-treated IMR-32 cells. The nELAVL binding track in human brain and RNAseq tracks in mock and nELAVL RNAi treated non-stressed and UV-stressed IMR-32 cells are shown.

DOI: 10.7554/eLife.10421.026
Figure 8—figure supplement 1. UV does not affect nELAVL RNA or protein levels. (A) nELAVL protein levels decrease upon nELAVL RNAi treatment in non-stressed and UV-stressed IMR-32 cells but are not affected by UV stress. Western Blot and its quantification showing protein levels of nELAVL and the housekeeping genes HSP90 and Histone H3 in mock and nELAVL RNAi treated non-stressed and UV-stressed IMR-32 cells. Protein expression was normalized to the housekeeping gene Histone H3 and to mock RNAi treated cell. Error bars represent SEM. p-values were calculated with a two-tailed t-test (ns: not significant; *p<0.01). (B) nELAVL mRNA levels decreased upon nELAVL RNAi treatment in non-stressed and UV-stressed IMR-32 cells but were not affected by UV stress. Shown is the mRNA abundance assessed by RNAseq in response to nELAVL RNAi and UV stress. FDR values were derived from edgeR (ns: not significant; * FDR<1e^{-3}; ** FDR<1e^{-4}; *** FDR<1e^{-20}).

DOI: 10.7554/eLife.10421.027
Figure 8—figure supplement 2. Analysis of splicing changes in nELAVL RNAi and UV treated IMR-32 cells and AD subjects with and without Y RNA association (AD_Y and AD_nY). Shown is the differential inclusion rate of cassette exons that change similarly upon nELAVL RNAi and UV treatment and that were adjacent (+/- 2.5 kb) to intronic nELAVL binding sites in human brain. Splicing changes in AD subjects were not significant except for UTX (boxed in pink). * FDR<0.05 (GLM likelihood ratio test).

DOI: 10.7554/eLife.10421.028
Figure 9. Y RNA overexpression is linked to nELAVL sequestration from mRNA targets. (A) Validation of Y RNA overexpression. Shown are RNA expression fold changes of Y3wt or Y3mut infected IMR-32 cells compared to non-infected IMR-32 cells assessed by qPCR. Y RNAs expression increased while control mRNAs (ACTB, GAPDH, ELAVL4) were not affected. Error bars represent SEM. p values were calculated with a two-tailed t-test (ns: not significant; * p<0.05). (B) The expression of endogenous Y3-like Y RNAs increases upon Y3wt but not Y3mut infection. Box plots represent the distribution of endogenous Y3-like and non-Y3-like Y RNA expression fold changes upon Y3wt or Y3mut infection. Y3-like Y RNAs show a slight increase in abundance upon Y3wt compared to non-Y3-like Y RNAs (p = 0.057; one-tailed t-test). In contrast, the mRNA abundance of Y3-like Y RNAs does not change upon Y3mut infection, when compared to non-Y3-like Y RNAs (p = 0.602; one-tailed t-test). (C) Identification of Y3 dependent splicing changes. Shown is the exon inclusion fraction of cassette exons that are expressed in IMR-32 cells subjected to Y3wt or Y3mut infection (n = 10,189). Exons changing significantly between Y3wt and Y3mut infection (FDR<0.05 and ΔI>0.1) are colored in light blue (n = 191). (D) Exons that are alternatively spliced upon Y3wt infection are enriched for nELAVL bound exons. Bar graph representing total expressed exons (n = 10,189), exons that change in either Y3wt (n = 240; blue points in the left panel of Figure 9—figure supplement 4) or Y3mut (n = 151; blue points in the right panel of Figure 9C) infected cells compared to non-infected cells, and exons that change in Y3wt compared to Y3mut infected cells (n = 191; blue points in Figure 9C). Exons that are alternatively spliced upon Y3wt infection compared to either non-infected (p = 0.037; hypergeometric test) or Y3mut infected cells (p = 0.069; hypergeometric test) are enriched for nELAVL bound exons.

DOI: 10.7554/eLife.10421.029
Figure 9—figure supplement 1. Y3 overexpression does not induce changes in protein distribution. Western Blot and its quantification showing cytoplasmic and nuclear protein levels of nELAVL, RO60, as well as cytoplasmic (HSP90 and GAPDH) and nuclear (RNA PolII and Histone H3) markers. Bar graphs depict the percentage of cytoplasmic protein levels (cytoplasmic protein levels divided by the sum of cytoplasmic and nuclear protein levels). Error bars represent SEM. Changes in cytoplasmic proteins levels were not significant (ns; p<0.05; two-tailed t-test).

DOI: 10.7554/eLife.10421.030
Figure 9—figure supplement 2. Validation of Y RNA overexpression. Bar graphs represent log2 number of reads of Y3wt and Y3mut in non-infected, Y3wt and Y3mut infected IMR-32 cells. Read numbers were assessed by searching raw fastq files for Y3wt and Y3mut sequences, respectively. Searched sequences were either 40 (left panel) or 68 (right panel) nucleotides in length. Both sequence lengths encompassed the sequence mutated in Y3mut. While the 40nt Y3wt sequence is present in numerous Y3 RNA copies, the 68nt Y3wt sequence should only be present in the infected Y3wt RNA and the endogenous canonical hY3 RNA. Error bars represent SEM. p values were calculated with a two-tailed t-test (ns: not significant; * p<0.01). DOI: 10.7554/eLife.10421.031
Figure 9—figure supplement 3. Y3 overexpression does not lead to nELAVL 3'UTR target sequestration. (A) Correlation of mRNA abundance changes upon Y3wt and Y3mut infection compared to non-infected cell. The mRNA abundance fold change (Y3 infected/non-infected) of Y3mut infected cells was plotted against the mRNA abundance fold change of Y3wt infected cells. Transcripts that change significantly (FDR<0.05) upon either Y3wt infection (n = 502) or Y3mut infection (n = 1920) are colored in light blue. Transcripts that change in both infections and are therefore likely to be virus dependent are colored in dark blue (n = 349). Shown are only transcripts that are expressed (n = 12,659) and the R value is indicated. (B) The mRNA abundance change (Y3mut/Y3wt infection) of transcripts was plotted against average mRNA abundance. Significantly changing transcripts (FDR<0.05; n = 435) are colored in blue, and are not enriched for nELAVL 3'UTR targets.
DOI: 10.7554/eLife.10421.032
Figure 9—figure supplement 4. Identification of Y3wt and Y3mut dependent splicing changes. Shown is the exon inclusion fraction of cassette exons that are expressed in IMR-32 cells subjected to Y3wt or Y3mut infection (n = 10,189). Exons changing significantly (FDR<0.05 and ΔI > 0.1) upon Y3wt (left panel; n = 240) or Y3mut (right panel; n = 151) infection are colored in light blue. DOI: 10.7554/eLife.10421.033
Figure 9—figure supplement 5. Exons that are alternatively spliced upon Y3wt infection are enriched for nELAVL RNAi dependent exons. Bar graph representing total expressed exons (n = 10,189), exons that change in either Y3wt (n = 240; blue points in the left panel of Figure 9—figure supplement 4) or Y3mut (n = 151; blue points in the right panel of Figure 9—figure supplement 4) infected cells compared to non-infected cells, and exons that change in Y3wt compared to Y3mut infected cells (n = 191; blue points in Figure 9C). Exons that are alternatively spliced upon Y3wt infection compared to either non-infected (p = 0.011; hypergeometric test) or Y3mut infected cells (p = 0.099; hypergeometric test) are enriched for nELAVL RNAi dependent exons.

DOI: 10.7554/eLife.10421.034