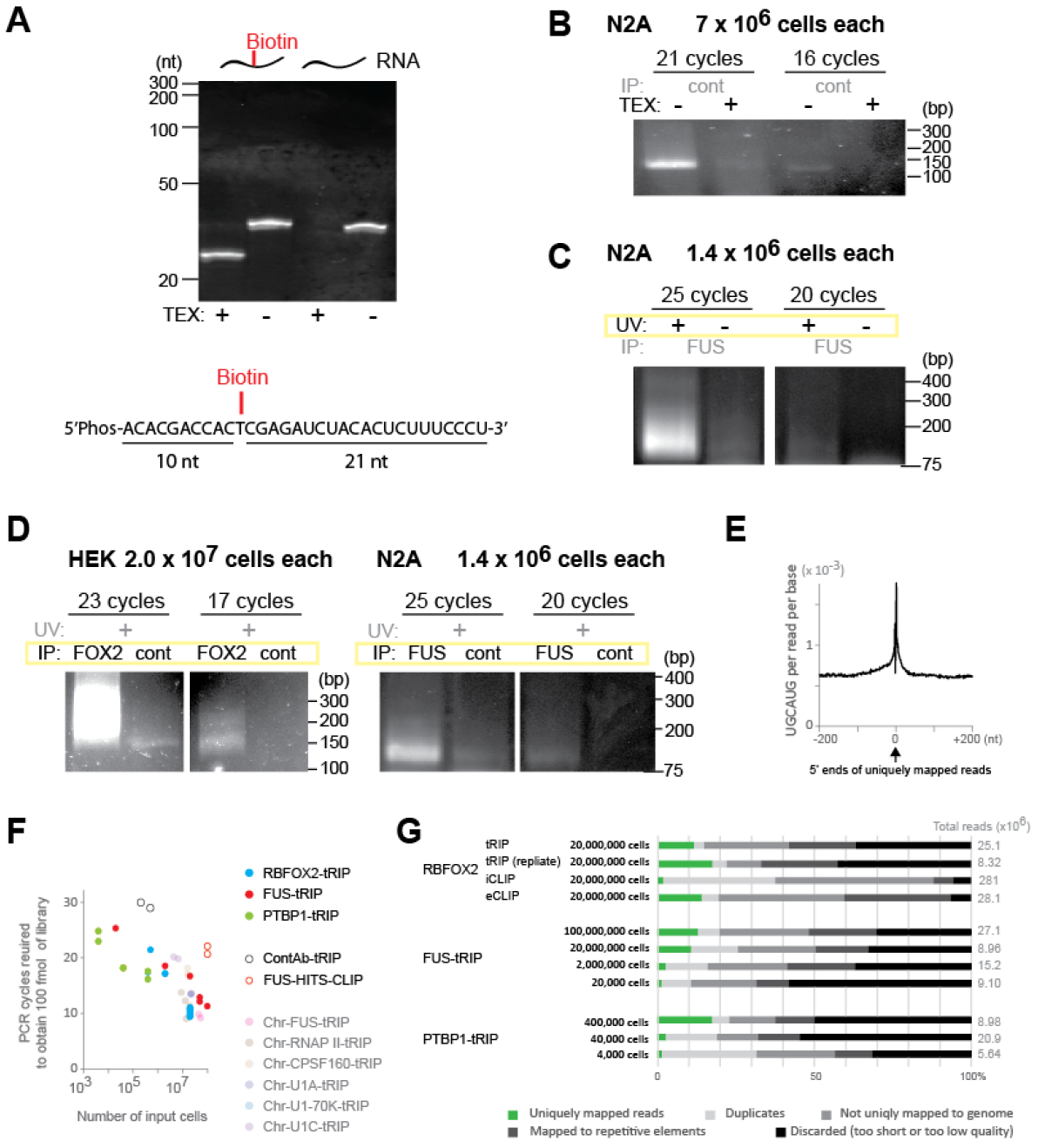
Table of contents



Appendix Figure S1.

A. *In vitro* prevention of TEX-dependent digestion of an RNA/DNA hybrid probe by attachment of biotin in the middle of the probe. The 32 nt single stranded RNA/DNA hybrid probe (2.5 μg) with or without biotin at the middle T nucleotide (bottom scheme) was incubated with 0.5 unit of TEX (+) or not (-) in 1 × TEX reaction buffer A (Epicentre) for 1 hr at 30°C. Then the samples were mixed with loading buffer, incubated for 3 min at 80°C, and analyzed on the polyacrylamide gel. The sequence of the probe and the position of the biotinylated nucleotide are shown at the bottom.

B. Removal of free linkers by TEX treatment. tRIP libraries are constructed from 7×10^6 N2A cells using mouse control IgG (cont) with TEX treatment (+) or not (-). The tRIP libraries are PCR-amplified with indicated numbers of cycles, and analyzed on a 4% NuSieve agarose gel. Note that the band arising from free linkers (126 bp) is eliminated by TEX treatment.

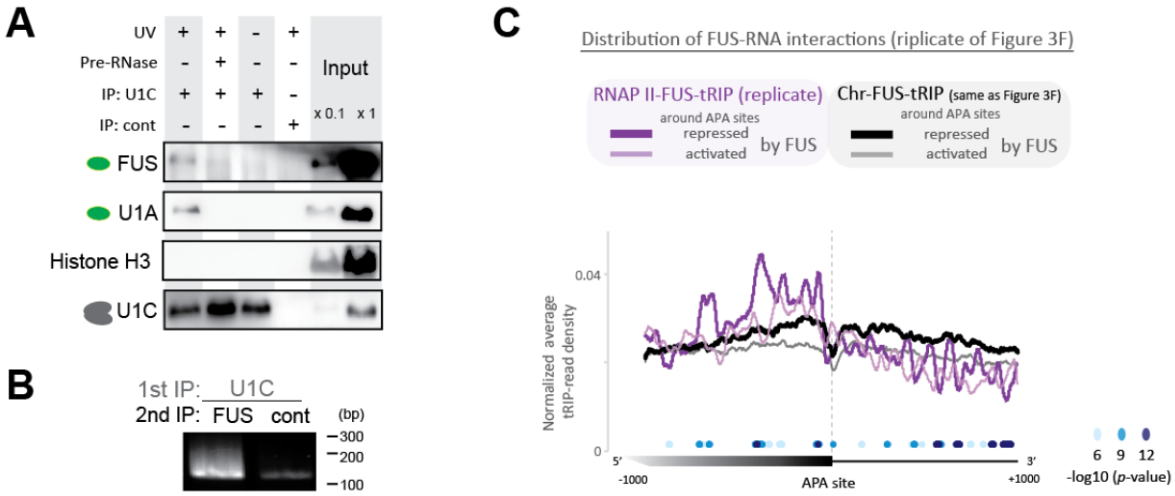
C. UV-crosslinking-dependent generation of tRIP libraries. tRIPs of FUS are performed using N2A cells with UV-crosslinking (+) or not (-). The tRIP libraries are PCR-amplified with indicated numbers of cycles, and analyzed on 4% NuSieve agarose gels.

D. Immunoprecipitation-dependent generation of tRIP libraries. Indicated numbers of HEK and N2A cells are UV-crosslinked, and tRIP experiments are performed using anti-RBFOX2 (FOX2), anti-FUS (FUS), or control antibody (cont). The tRIP libraries are PCR-amplified with indicated numbers of cycles, and analyzed on 4% NuSieve agarose gels.

E. The enrichment of RBFOX2 motif (UGCAUG) at each position around the 5' end of RBFOX2-tRIP reads.

F. The number of PCR cycles required to obtain 100 fmol of the indicated tRIP library, extrapolated from the final amount of tRIP library and the number of PCR cycles, assuming doubling at each cycle.

G. Mapping efficiencies of reads of tRIP-seqs, RBFOX2-iCLIP (GSM2055496), and RBFOX2-eCLIP (GSM2055434). The tRIP-, iCLIP-, and eCLIP-reads were mapped to the genome using the same pipeline.

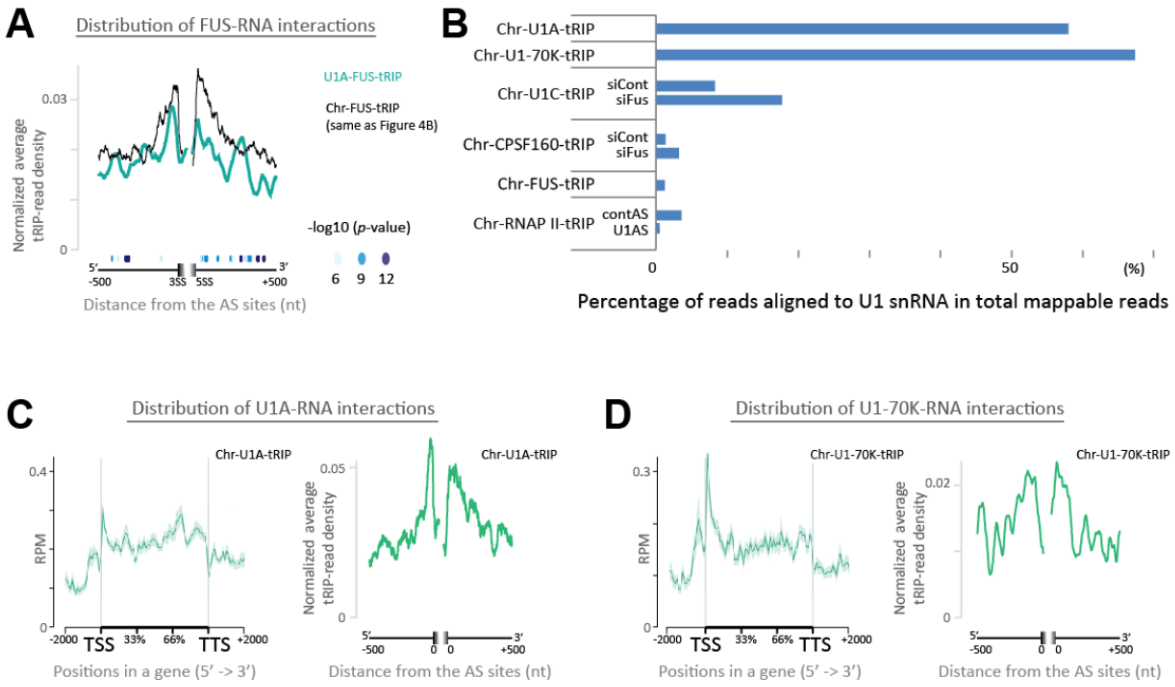


Appendix Figure S2.

A. U1 snRNP-RBP-RNA complex was immunoprecipitated by anti-U1C antibody or control antibody (cont), and subjected to immunoblotting. The input lanes contained 0.4% (×1) or 0.04% (×0.1) of lysates used in the immunoprecipitation experiments.

B. Generation of tRIP libraries of U1 snRNP-RBP-RNA complexes. U1C-specific antibody was used for the 1st immunoprecipitation, instead of RNAPII-specific antibody in the tRIP of RNAPII-RBP-RNA complexes (Fig 2C). The panels show PCR-amplification of tRIP libraries generated from U1 snRNP-RBP-RNA complex.

C. Read distributions of a biological replicate of RNAP II-FUS-tRIP shown in Fig 2E (purple lines) around APA sites repressed (bold lines) or activated (thin lines) by FUS. Black lines show read distributions of Chr-FUS-tRIP (same as Fig 2E). The *p*-values for the differences between RNAP II-FUS-tRIP and Chr-FUS-tRIP around FUS-repressed APA sites are indicated by circles.

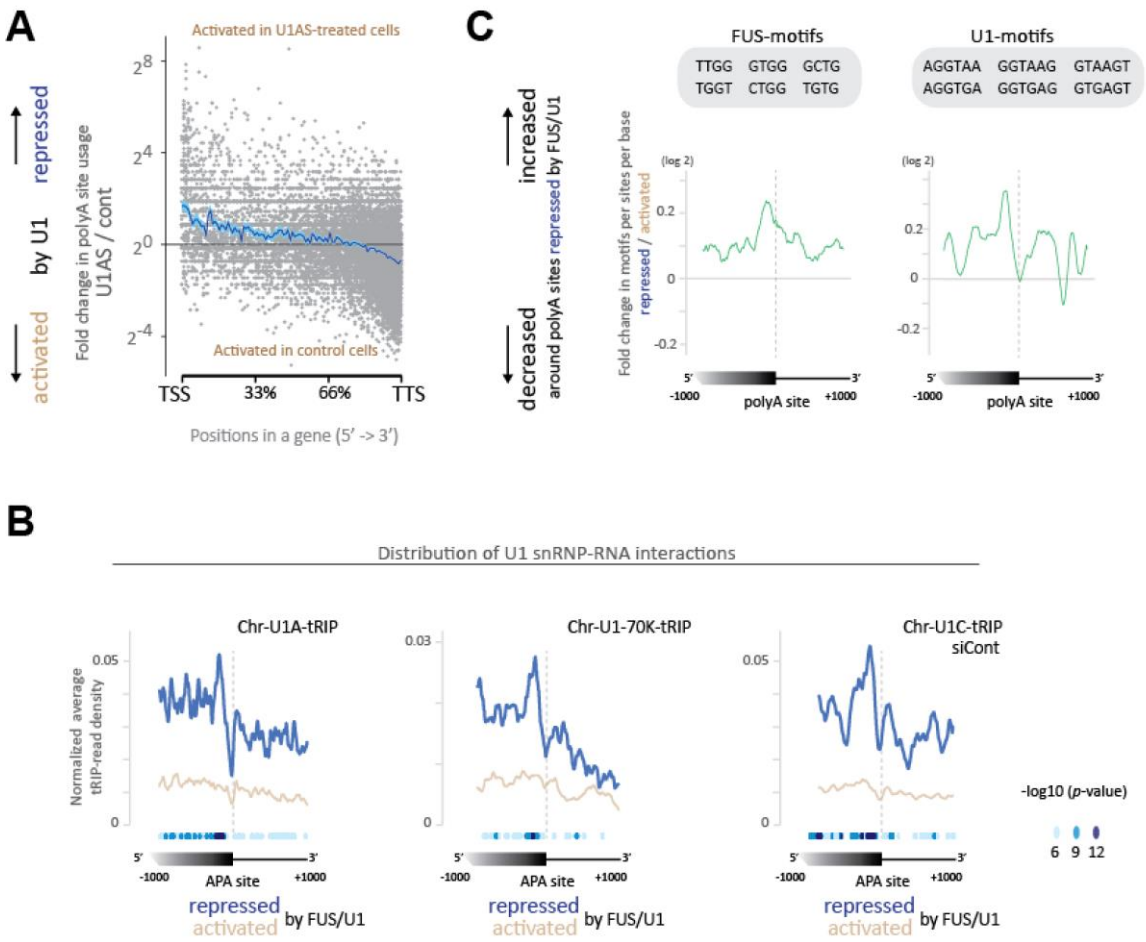


Appendix Figure S3.

A. Read distributions of U1A-FUS-tRIP (blue lines) around AS sites. Black lines show read distributions of Chr-FUS-tRIP (same as Fig 3A). The *p*-values for the differences between U1A-FUS-tRIP and Chr-FUS-tRIP are indicated by circles.

B. Ratios of reads aligned to U1 snRNA in total mappable reads of Chr-U1A-tRIP, Chr-U1-70K-tRIP, Chr-U1C-tRIP, Chr-CPSF160-tRIP, Chr-FUS-tRIP, and Chr-RNAP II-tRIP. Note that large fractions of U1A and U1-70K bind to U1 snRNA, whereas U1C bind to U1 snRNA to a less extent. This is likely because parallel binding of U1C to the 5' splice sites decreases the number of Chr-U1C-tRIP reads mapped to U1 snRNA.

C and D. Read distributions of Chr-U1A-tRIP (C) and Chr-U1-70K-tRIP (D). The left graphs show read distributions of tRIP-seqs mapped to the relative positions of all mouse coding genes. The ngs.plot tool [1] was used to plot average RPM on a gene structure. The standard error of mean RPM is shown as a semi-transparent shade around the average curve. The right graphs show read distributions of tRIP-seqs around AS sites.

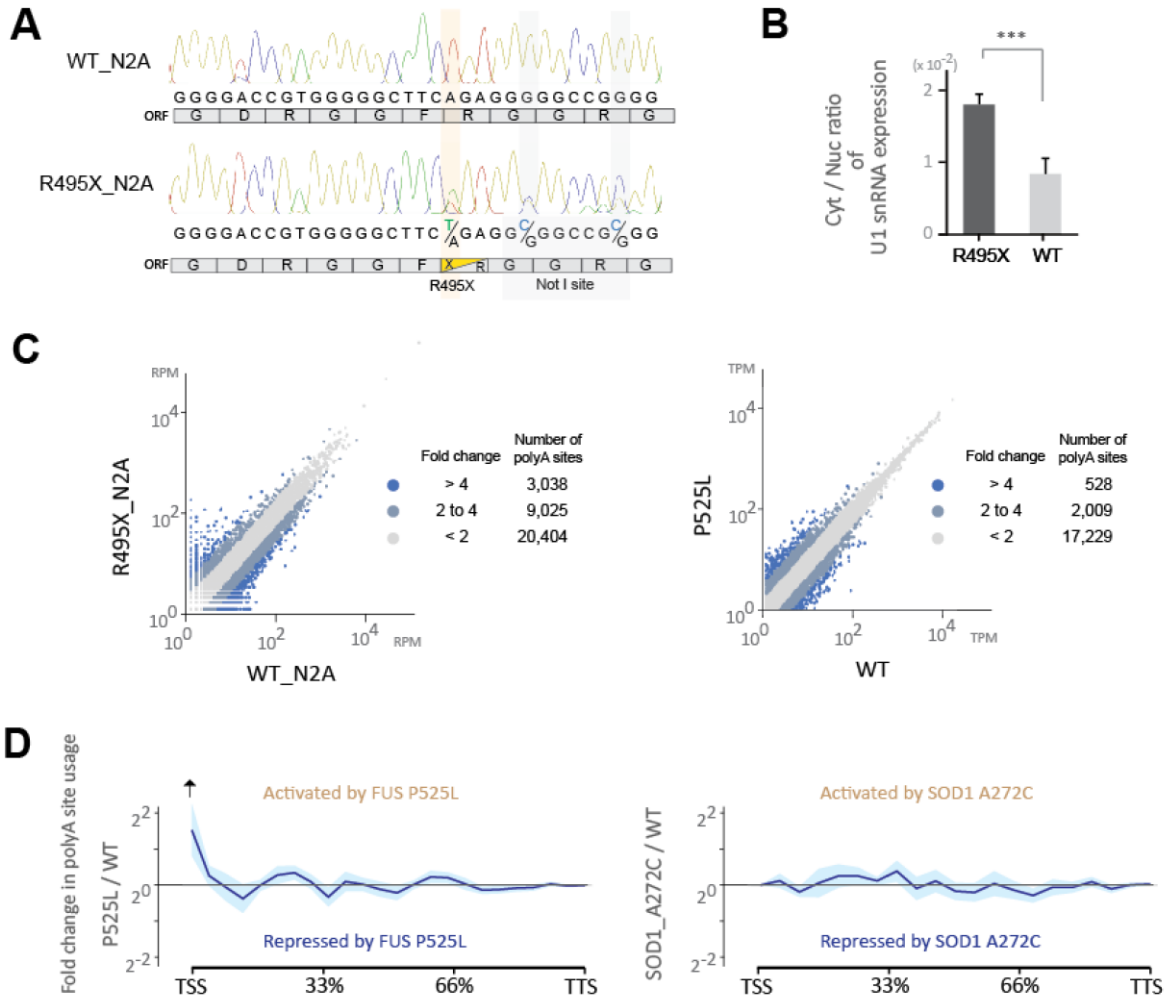


Appendix Figure S4.

A. Change in polyA site usage by U1-inhibition. Each point indicates fold change of RPM of a polyA site by U1-inhibition (U1AS/cont) on the relative positions of all mouse coding genes. The average is shown in blue line. The standard error of the mean is shown in semi-transparent blue shade.

B. Read distributions of Chr-U1A-tRIP, Chr-U1-70K-tRIP and Chr-U1C-tRIP around APA sites repressed or activated by FUS/U1. The *p*-values for the differences between repressed and activated APA sites are indicated by circles.

C. Enrichments of FUS and U1 snRNP-recognition motifs upstream to the FUS/U1 snRNP-repressed polyA sites (the first quadrant in Fig 4A). The analyzed motifs are shown at the top. FUS-motifs are according to our previous report [2]. U1-motifs are parts of the consensus sequence of the 5' splice site, AGGTRAGT. The frequency of repressed APA sites was divided by that of activated sites at each nucleotide position, and is plotted on the graph.



Appendix Figure S5.

A. Sanger sequencing analysis showing the introduction of the heteroallelic mutation corresponding to the human FUS mutation, R495X, into mouse N2A cells (R495X_N2A). The sequence of the parental N2A cells (WT_N2A) is shown above. Two synonymous mutations were also introduced to avoid repeated cutting by the CRISPR-Cas9 and to add a restriction enzyme recognition site for Not I.

B. Distribution of U1 snRNA in R495X_N2A (R495X) and WT_N2A (WT) cells. Cytoplasmic and nuclear lysates were harvested as described elsewhere [3], and total RNA was extracted from the lysates with TRIZOL (ThermoFisher) according to the manufacture's instruction. Real-time RT-PCR was performed to quantify U1 snRNA and 5S RNA using the following primers, U1/F; 5'-GATACCATGATCATGAAGGTGGTT-3', U1/R; 5'-CACAAATTTGCATCGAGTTTCC-3', 5S/F; 5'-CGGCCATACCACCCTGAAC-3', and 5S/R;5'- GCGGTCTCCCATCCAAGTAC-3'. Expression level of U1 snRNA was normalized to that of 5S RNA, and nuclear/cytoplasm ratio of

the normalized U1 snRNA-expression level was calculated. Mean and SD are shown. ***$p < 0.001$ by Student's *t*-test.

C. Scatter plots showing correlation of polyA site usage between cells bearing the indicated FUS mutation (R495X or P525L) and wild-type control cells (WT). The RPM or transcripts per million (TPM) of a polyA site in FUS-mutated cells was plotted against that in control cells.

D. Change in polyA site usage by the P525L FUS mutation. Fold change of polyA site usage by the FUS P525L mutation (left panel, FUS-P525L/WT) or the SOD1 A272C mutation (right panel, SOD1-A272C/WT) on the relative positions of all mouse coding genes is plotted. To avoid division by zero, which occurs in the calculation of the sites not detected in control cells, 0.1 was added to all TPM values. The average is shown in blue line. The standard error of the mean is shown in semi-transparent blue shade.

Appendix Table S1: Comparison of tRIP protocol with CLIP protocols

		tRIP		eCLIP [5]		HITS-CLIP [6]		iCLIP [7]	
Application									
RNA-protein interaction		✓		✓		✓		✓	
RNA-modification		✓							
Protocol									
UV crosslinking	Day1	Depending on the analysis	Day1	✓	Day1	✓	Day1	✓	
Cell lysis		✓		✓		✓		✓	
RNase treatment		on beads		Total cell lysates		Total cell lysates		Total cell lysates	
IP		✓		✓		✓		✓	
Dephosphorylation of RNA				✓		✓		✓	
5' end labeling				✓		✓		✓	
3' linker ligation		✓		✓	Day2	✓	Day2	✓	
SDS-PAGE	Day2	Deadenylase- and TEX-treatments		✓		✓		✓	
Transfer to membranes				✓		✓		✓	
Cut membrane			Day2	✓		✓		✓	
Proteinase K treatment		✓		✓		✓		✓	
Purification of RNA		Column purification		Phenol /chloroform Column purification		Phenol /chloroform ETOH precipitation		Phenol /chloroform ETOH precipitation	
Reverse transcription		✓	Day3	✓	Day4	✓	Day3	✓	
Purification of cDNA/RNA				Silane-beads pufirication	Day3	Phenol /chloroform		Phenol /chloroform	ETOH precipitation
Modification of 5' end		PolyA tailing		5' linker ligation		5' linker ligation	Day5	Circularization of cDNA	ETOH precipitation
Purification of cDNA/RNA			Day4	Silane-beads pufirication		ETOH precipitation	Day4	Gel purification	ETOH precipitation
PCR amplification and purification		✓		✓	Day4	✓	Day6	✓	
Hands-on time		2 days		4 days		4 days		6 days	

An oblique line indicates that there is no corresponding experimental step.

Appendix Table S2. Gene Ontology analysis using the GOrilla database.

GO ID	GOTERM_BP5	*p*-value
GO:0023061	signal release	4.28E-07
GO:0099643	signal release from synapse*	1.21E-06
GO:0032502	developmental process	1.26E-06
GO:0007269	neurotransmitter secretion*	2.59E-06
GO:0032940	secretion by cell	8.58E-06
GO:0046903	secretion	8.88E-06
GO:0006836	neurotransmitter transport*	1.50E-05
GO:0032501	multicellular organismal process	1.76E-05
GO:0017156	calcium ion regulated exocytosis	3.73E-05
GO:0048856	anatomical structure development	5.74E-05

Two thousand ninety-four genes, which harbor polyA sites affected more than 4-fold in R495X_N2A cells, are analyzed using the "Process" dataset with GOrilla [4]. For background, 8,924 genes, which harbor polyA sites detected in both polyA-seq of R495X_N2A and that of WT_N2A (RPM > 1), are used.

GO terms with the ten best *p*-values are indicated.

*GO terms with neuron-specific functionalities.

Appendix Table S3: The amount of materials used for construction of each tRIP library

Library	Cells	Fraction	Cell number (million cells)	DNase I (μl)	**Benzonase (unit)	1st antibody (μg)	2nd antibody (μg)	Protein G beads (μl)	RNase III (unit)
RBFOX2	HEK293T	Whole	20	30	10	10	-	100	2
FUS	N2A	Whole	100	60	0.025	10	-	100	1
			20	60	0	10	-	100	0.4
			2	10	0	2	-	10	0.2
			0.02	0.5	0	0.2	-	4	0.002
ContAb	N2A	Whole	0.2	10	0	0.5	-	5	0.025
PTBP1	C2C12	Whole	0.4	5	0.25	2	-	20	0.2
			0.04	0.5	0.025	0.2	-	2	0.01
			0.004	0.05	0.0025	0.4	-	4	0.002
Chr-FUS	N2A	Chromatin	45	60	0.25	10	-	100	4
Chr-CPSF160	N2A	Chromatin	16	20	0	5	-	50	4
Chr-U1-70K	N2A	Chromatin	22	30	0	2	-	20	1**
Chr-U1C	N2A	Chromatin	23	60	0	10	-	100	2**
U1C-FUS	↓	↓	↓	↓	↓	↓	0.4	4	↓
Chr-U1A	N2A	Chromatin	23	60	0	10	-	100	2**
U1A-FUS	↓	↓	↓	↓	↓	↓	0.4	4	↓
Chr-RNAP II	N2A	Chromatin	18	30	0	5	-	50	1
RNAP II-FUS	↓	↓	↓	↓	↓	↓	0.5	5	↓
RNAP II-U1C	↓	↓	↓	↓	↓	↓	0.4	4	↓
RNAP II-CPSF160	↓	↓	↓	↓	↓	↓	0.4	4	↓

* Total cell lysates were treated with Benzonase (Merck Millipore) for 5 min at 37°C to promote the fragmentation of RNA, following the DNase I treatment, when large amounts of RNA-protein complexes were harvested. The treatment yielded RNA fragments in ~500-to-thousands-nt length.

**Incubation at 37°C for 20 min.

Appendix Table S4. Accession numbers in the DDBJ sequence read archive and figure numbers of the high-throughput sequencing analysis

	Analysis	Cell number (million cells)	Treatment	Cell fraction	1st antibody	2nd antibody	Accession number	Figure number
tRIP-seq	RBFOX2	20	-	Whole	RBFOX2	-	DRA008138	Figs 1BDF, S1EG
	RBFOX2 (replicate)	20	-	Whole	RBFOX2	-	DRA005742	Fig S1G
	FUS	100	-	Whole	FUS	-	DRA008251	Fig 1CEGH
	FUS	20, 2, 0.02	-	Whole	FUS	-	DRA008141	Fig 1CGH
	ContAb	0.2	-	Whole	Control	-	DRA008252	Fig 1C
	PTBP1	0.4, 0.04, 0.004	-	Whole	PTBP1	-	DRA005743	Figs EV2CD, S1G
	m6A	0.4, 0.04, 0.004		Whole	m6A	-	DRA005746	Figs EV2ABCE
	Chr-FUS	45	-	Chromatin	FUS	-	DRA008141	Figs 2DE, 3AB, S2C, S3A
	Chr-CPSF160	16	-	Chromatin	CPSF160	-	DRA008137	Fig EV3C
	Chr-RNAP II	18	siCont, siFus	Chromatin	RNAPII	-	DRA008140	Fig EV3AB
	Chr-U1-70K	22	-	Chromatin	U1-70K	-	DRA008142	Figs S3D, S4B
	Chr-U1A	23	-	Chromatin	U1A	-	DRA008142	Figs S3C, S4B
	Chr-U1C	23	siCont, siFus	Chromatin	U1C	-	DRA008142	Figs 3C, S4B
	RNAP II-CPSF160	18	siCont, siFus	Chromatin	RNAPII	CPSF160	DRA008137	Figs 2F, EV3B
	RNAP II-FUS	18	-	Chromatin	RNAPII	FUS	DRA005757	Figs 2DE, 3B, EV3B, S2C
	RNAP II-FUS	18	contAS, U1AS	Chromatin	RNAPII	FUS	DRA008140	Fig 4CD
	RNAP II-U1C	18	siCont, siFus	Chromatin	RNAPII	U1C	DRA008142	Figs 3D, 4CD
	U1A-FUS	23	-	Chromatin	U1A	FUS	DRA008142	Figs 4E, S3A
	U1C-FUS	23	-	Chromatin	U1C	FUS	DRA008142	Figs 2D, 3B, 4E
PolyA-seq		1.4	siCont+ContAS, siCont+U1AS, siFus+contAS, siFus+U1AS	Whole	-	-	DRA008136	Fig 4AB
PolyA-seq		2	R495X, WT	Whole			DRA009662	Fig 6CD

Appendix Table S5. Antibodies employed in the study

Mouse monoclonal anti-CPSF160 (G-10)	Santa Cruz Biotechnology	Cat# sc-166281, RRID:AB_2084362
Mouse monoclonal anti-FUS (4H11)	Santa Cruz Biotechnology	Cat# sc-47711, RRID:AB_2105208
Rabbit polyclonal anti-FUS	Abcam	Cat# ab84078 RRID: AB_2105201
Rabbit polyclonal anti-N6-methyladenosine (m6A)	Abcam	Cat# ab151230, RRID:AB_2753144
Goat polyclonal anti-PTBP1 (N-20)	Santa Cruz Biotechnology	Cat# sc-16547, RRID:AB_2253470
Rabbit polyclonal anti-RBFOX2	Bethyl Laboratories	Cat# A300-864A, RRID:AB_609476
Rabbit polyclonal anti-RNAP II (N-20)	Santa Cruz Biotechnology	Cat# sc-899, RRID:AB_632359
Mouse monoclonal anti-Rpb1 CTD (4H8)	Cell Signaling Technology	Cat# 2629, RRID:AB_2167468
Rabbit polyclonal anti-SNRPA (U1A)	Thermo Fisher Scientific	Cat# PA5-27474, RRID:AB_2544950
Rat monoclonal anti-U1 snRNP C (U1C) (4H12)	Bio Academia	Cat# 70-400, RRID:AB_10550948
Mouse monoclonal anti-U1-70k (H111)	Synaptic Systems	Cat# 203 011, RRID:AB_887903
Normal mouse IgG antibody	Santa Cruz Biotechnology	Cat# sc-2025, RRID:AB_737182
Normal rabbit IgG antibody	Santa Cruz Biotechnology	Cat# sc-2027, RRID:AB_737197
Mouse monoclonal anti-CPSF160 (G-10)	Santa Cruz Biotechnology	Cat# sc-166281, RRID:AB_2084362
Mouse monoclonal anti-FUS (4H11)	Santa Cruz Biotechnology	Cat# sc-47711, RRID:AB_2105208
Rabbit polyclonal anti-FUS	Abcam	Cat# ab84078 RRID: AB_2105201
Rabbit polyclonal anti-N6-methyladenosine (m6A)	Abcam	Cat# ab151230, RRID:AB_2753144
Goat polyclonal anti-PTBP1 (N-20)	Santa Cruz Biotechnology	Cat# sc-16547, RRID:AB_2253470
Rabbit polyclonal anti-RBFOX2	Bethyl Laboratories	Cat# A300-864A, RRID:AB_609476
Rabbit polyclonal anti-RNAP II (N-20)	Santa Cruz Biotechnology	Cat# sc-899, RRID:AB_632359
Mouse monoclonal anti-Rpb1 CTD (4H8)	Cell Signaling Technology	Cat# 2629, RRID:AB_2167468
Rabbit polyclonal anti-SNRPA (U1A)	Thermo Fisher Scientific	Cat# PA5-27474, RRID:AB_2544950
Rat monoclonal anti-U1 snRNP C (U1C) (4H12)	Bio Academia	Cat# 70-400, RRID:AB_10550948
Mouse monoclonal anti-U1-70k (H111)	Synaptic Systems	Cat# 203 011, RRID:AB_887903
Normal mouse IgG antibody	Santa Cruz Biotechnology	Cat# sc-2025, RRID:AB_737182
Normal rabbit IgG antibody	Santa Cruz Biotechnology	Cat# sc-2027, RRID:AB_737197

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