PNAS
www.pnas.org

Supporting Information for

Noncanonical immune response to the inhibition of DNA methylation by Staufen1 via stabilization of endogenous retrovirus RNAs

Yongsuk Ku, Joo-Hwan Park, Ryeongeun Cho, Yongki Lee, Hyoung-Min Park, MinA Kim, Kyunghoon Hur, Soo Young Byun, Jun Liu, Young-suk Lee, David Shum, Dong-Yeop Shin, Youngil Koh, Je-Yoel Cho, Sung-Soo Yoon, Junshik Hong, and Yoosik Kim

Corresponding authors: Yoosik Kim and Junshik Hong
Email: ysyoosik@kaist.ac.kr (Y.K).
hongjblood@snu.ac.kr (J. H).

This PDF file includes:

Supporting Information Materials and Methods

Cell Lines. Wild-type and Staufen1 (Stau1) knockout (KO) HCT116 cells were cultured in RPMI media (Welgene) supplemented with 10% Fetalgro bovine growth serum (RMbio). KG-1 cells were grown in RPMI-1640 (Welgene) supplemented with 10% fetal bovine serum (Thermo Fisher). All cells were incubated at 37 °C in a humidified atmosphere of 5 % CO_2. All cell lines used in this study were male. All cell lines were authenticated using short tandem repeat (STR) profiling (PowerPlex 1.2; Promega), and results were compared with reference STR profiles available through the ATCC.

Chemical Treatment. Cells were treated with 500nM of decitabine for 24 h and incubated with fresh media for 4 additional days before analysis. For siRNA transfection, Lipofectamine 3000 (Thermo Fisher Scientific) was used following the manufacturer's instruction. For KG-1 cells, electroporation was done in buffer R using Neon (Thermo Fisher Scientific). Electroporation was conducted in a single 20-ms pulse of 1,700 V condition by following the manufacturer's instruction. Information on siRNA sequences is provided in SI Appendix, Table S5.

Formaldehyde Cross-linking and Immunoprecipitation (fCLIP). Cells were crosslinked using 0.75% paraformaldehyde (PFA) for 10 min at room temperature and quenched with 250 mM glycine for 10 min. Before immunoprecipitation, Immobilized Protein A Plus beads (Thermo Fisher Scientific) were rinsed with the wash buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM EDTA, 0.1% NP-40, 0.1% SDS, 0.1% Triton X-100, 0.1% Sodium deoxycholate) and incubated with target antibody for 3 h at 4°C.

Crosslinked cells were lysed in the fCLIP lysis buffer (20 mM Tris-HCl, pH, 7.5, 15 mM NaCl, 10 mM EDTA, 0.5% NP-40, 0.1% Triton X-100, 0.1% SDS, 0.1% Sodium deoxycholate) on ice for 10 min and sonicated for complete lysis. NaCl concentration was adjusted to 150 mM, and cell debris was removed through centrifugation. The lysate was immunoprecipitated by incubating with antibody-conjugated beads for 3 h at 4 °C. Samples were washed 4 times with the wash buffer and eluted with the elution buffer (400 mM Tris-HCl, pH 7.5, 200 mM NaCl, 40 mM EDTA, 4% SDS, 12 M urea). Protein from the eluate was removed using proteinase K (Sigma-Aldrich) for overnight at 65°C. Eluted RNA was purified using acid-phenol chloroform extraction. Purified RNA was either reverse transcribed and analyzed using qPCR or processed further to prepare a high-throughput sequencing library. The sequencing library was prepared and analyzed using NovaSeq by Theragen.

RNA-FISH Probe Preparation. RNA probes were synthesized from genomic DNA with reverse primers containing T7 promoter sequences (5′-TAATACGACTCACTATAGGG-3′). RNA was transcribed from the PCR products using MEGAscript T7 kit (Thermo Fisher Scientific) with DIG RNA labeling mix (Sigma-Aldrich). Following RNA transcription, Turbo DNase (Thermo Fisher Scientific) was added to remove the template DNA. RNA was purified using acid-phenol chloroform and hydrolyzed to ~200 bp using the hydrolysis buffer (40 mM $NaHCO_3$ and 60 mM Na_2CO_3, pH 10). After incubation, stop buffer (0.2 M NaOAc, pH 6) was added to halt the hydrolysis reaction, and 200 μL of hybridization

buffer (50% formamide, 10% dextran sulfate, 0.1% SDS, 300 ng/mL salmon sperm DNA, 2× saline sodium citrate (SSC), and 2 mM vanadyl ribonucleoside complex (VRC)) was added. Sequences for the primers used to generate RNA-FISH probes are provided in SI Appendix, Table S6.

RNA Extraction and RT-qPCR. Total RNA was extracted using TRIsure (Bioline) following the manufacturer's instruction. Purified nucleic acid was treated with DNase I (TaKaRa) to remove the genomic DNA, and RNA was reverse transcribed using RevertAid reverse transcriptase (Thermo Fisher Scientific). cDNA was analyzed using QuantStudio real-time PCR (Thermo Fisher Scientific) with SensiFAST SYBR Lo-Rox Kit (Bioline). Primers used in this study are provided in SI Appendix, Table S7.

Generation of the Stau1 KO Cells. Single guide RNA for targeting human Stau1 exons was designed with a web tool, CHOPCHOP, and subcloned into a pSpCas9(BB)-2A-GFP-px458 plasmid with Cas9 protein. The KO cell line was generated following the procedure from Ran et al. (2). Briefly, a PCR product was designed with forward and reverse primers having a single guide RNA. The PCR product was inserted into the plasmid containing Cas9 protein. The plasmid was transfected to HCT116 cells using Turbofect (Thermo Fisher Scientific) following the manufacturer's instruction. After 24 h, cells with green fluorescence signals were sorted using BD FACSAria II, and single cells were seeded on a 96-well plate. The individual cell was cultured for 3 to 4 weeks, and the expression of Stau1 was examined via western blotting to identify the KO clones.

Western Blot. Cells were collected using a scraper and washed once with cold PBS. Cells were lysed using the RIPA buffer supplemented with protease and phosphatase inhibitors (10 mM Tris-Cl at pH 8.0, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 140 mM NaCl, protease inhibitor, phosphatase inhibitor I and II, 1 mM DTT). Lysates were sonicated, and cell debris was removed through centrifugation. 30~40 µg of total protein was separated on a 10% SDS-PAGE gel and transferred to a PVDF membrane using the Amersham semidry transfer system. The following primary antibodies were used in this study: PKR, MDA5, eIF2α, and peIF2α antibodies were purchased from Cell Signaling Technology; Flag antibody was purchased from Sigma Aldrich; pPKR and Stau1 antibodies were purchased from Abcam; GAPDH and Lamin A/C antibodies were purchased from Santa Cruz Biotechnology.

Immunocytochemistry. Cells were washed once with PBS and fixed with 4% PFA for 10 min at room temperature. Fixed cells were permeabilized in 0.1% Triton X-100 and blocked in 1% BSA for 1 h. Cells were incubated in the primary antibody diluted in 1% BSA for 2 h. Cells were washed 4 times with 0.1% (v/v) Tween-20 in PBS and incubated with Alex Fluor conjugated secondary antibodies. Cells were imaged with a Zeiss LSM 760 confocal microscope with a C-Apochromat 63x objective (NA = 1.40). Following primary antibodies were used in this study: PKR and pPKR antibodies were purchased from Santa Cruz Biotechnology; eIF2α and peIF2α antibodies were from Cell Signaling Technology; Stau1 antibody was from Abcam.

Sulforhodamine B (SRB) Assay. Cells were fixed with 10% trichloroacetic acid solution and washed once with PBS. Cells were dried at room temperature and stained with 0.053% SRB solution (Chem Cruz). Stained cells were washed with 1% acetic acid and dried thoroughly. Cells were solubilized with 10 mM Tris (pH 10.5) and analyzed by Varioskan LUX multimode microplate reader (Thermo Fisher Scientific)

MTT. For KG-1 cells, cell viability was evaluated with MTT assay. Cells in a 96-well plate were incubated with 10 μl of 5 mg/ml MTT solution (Thermo Fisher Scientific) for 4 h. Crystals were dissolved in DMSO, and the absorbance at 590 nm was measured using a Varioskan LUX multimode microplate reader (Thermo Fisher Scientific).

Investigations for Outcomes of the DNMTi Treatment. Expressions of dsRBPs were estimated by RT-qPCR and analyzed for their association with clinical characteristics and treatment outcomes. Objective treatment response to DNMTi treatment was defined as achievement of complete remission (CR), CR with incomplete hematologic recovery (CRi), or morphologic leukemia-free status according to the 2017 European LeukemiaNet (ELN) criteria (1). As for higher-risk myelodysplastic syndrome (HR-MDS), the 2017 ELN criteria was also used as this study focused on the alteration of the natural history of disease rather than hematological improvement for MDS.

Means of dsRBP RNA expression were compared by an independent two-sample t-test. Kaplan-Meier plot was used to analyze progression-free survival (PFS) from the day of DNMTi treatment initiation to the day of disease progression or death and allogeneic hematopoietic stem cell transplantation (HSCT)-censored overall survival (OS) from the

day of DNMTi treatment initiation to the day of allogeneic HSCT or death. The prognostic effects of variables were investigated using the Cox proportional regression model.

Data Analysis of the J2 fCLIP-seq Data. Initial transcript files were quality checked by FastaQC. Reads were aligned with the human genome (hg38) using Hisat2 (ver.2.1.0) with the cufflink option (3). Non-coding RNA was analyzed by counting raw transcript reads using the featurecount software (4). Each transcript was matched by a manually made ncRNA gtf reference file, which was extracted from the ncRNA database. Raw counts were normalized and analyzed with the DESeq(ver.1.36.0) software (5), which is capable of handling non-replicated RNA-seq data.

Differentially Expressed Gene Analysis. For differential analysis, four sets (siLuc_DAC, siLuc_DMSO, siStau1_DAC, siStau1_DMSO) were grouped and compared using both DESeq and cuffdiff (3, 5). DESeq blind method and fit-only for the dispersion of no replicate samples were used for the analysis. After the differential analysis, each RNA was sorted into one of four annotated categories; DNA, LINE, SINE, and ERV. In each category, DEG data was ordered by most significantly differentially expressed genes, most strongly upregulated, and most strongly downregulated genes for further analysis. Normalized count data of replicate RNA-seq data were also processed identically. Each DEG data was ordered by most significant, most upregulated, and most downregulated. Normalized FPKM RNA-seq data was sorted for the most significant DEG and was compared with the previous count data for validation.

Gene Ontology Analysis of Differentially Expressed RNAs. Each most upregulated and downregulated RNAs was assembled into a gene list for gene ontology (GO) analysis. KEGG pathway and GO analysis were done using the ClueGo software (Cytoscape,v.3.7.1) (6).

TINCR Sequence Motif Analysis. The TINCR motifs were searched in the dsRNA-seq data and ERV classes by defining the sense (HCWNCYNCHNNNYCH) and antisense (WGRNNNDGNRGNWGR) motif largely based on the previously reported TINCR box motif (7). ERV classes and sequences were downloaded from the Dfam database under the classification Retroviridae and taxon Homo sapiens, which resulted in 517 ERV reference sequences (8). These 517 ERVs are classified into four distinct transposable element classifications, namely ERV1, ERVL, ERVK, and ERVL-MaLR.

Data Analysis of the RNA-seq Data. Transcript files were quality checked and aligned identically. Mapped reads were assembled by two distinct methods. Initial transcript raw counts were assembled using the hg38 gtf file by the featurecount software (4). Count data with replicates were normalized and analyzed with the DESeq software (5). The same transcript raw counts were assembled using the cufflink software (ver.2.2.1) (3). Each file was converted to abundance files by cuffquant (ver.2.2.1). Abundance files were normalized by FPKM and compared by the cuffdiff (ver.2.2.1) and cuffnorm (ver.2.2.1).

Statistical Analysis. For statistical analyses, an unpaired one-tailed Student's t-test was used. P-value <0.05 was considered to be statistically significant. All figures show mean ± s.e.m.

Supporting Information References

1. Dohner H*, et al.* (2017) Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* 129(4):424-447.
2. Ran FA*, et al.* (2013) Genome engineering using the CRISPR-Cas9 system. *Nat Protoc* 8(11):2281-2308.
3. Trapnell C*, et al.* (2012) Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat Protoc* 7(3):562-578.
4. Liao Y, Smyth GK, & Shi W (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30(7):923-930.
5. Gentleman RC*, et al.* (2004) Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol* 5(10):R80.
6. Bindea G*, et al.* (2009) ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 25(8):1091-1093.
7. Kretz M*, et al.* (2013) Control of somatic tissue differentiation by the long non-coding RNA TINCR. *Nature* 493(7431):231-235.
8. Storer J, Hubley R, Rosen J, Wheeler TJ, & Smit AF (2021) The Dfam community resource of transposable element families, sequence models, and genome annotations. *Mob DNA* 12(1):2.

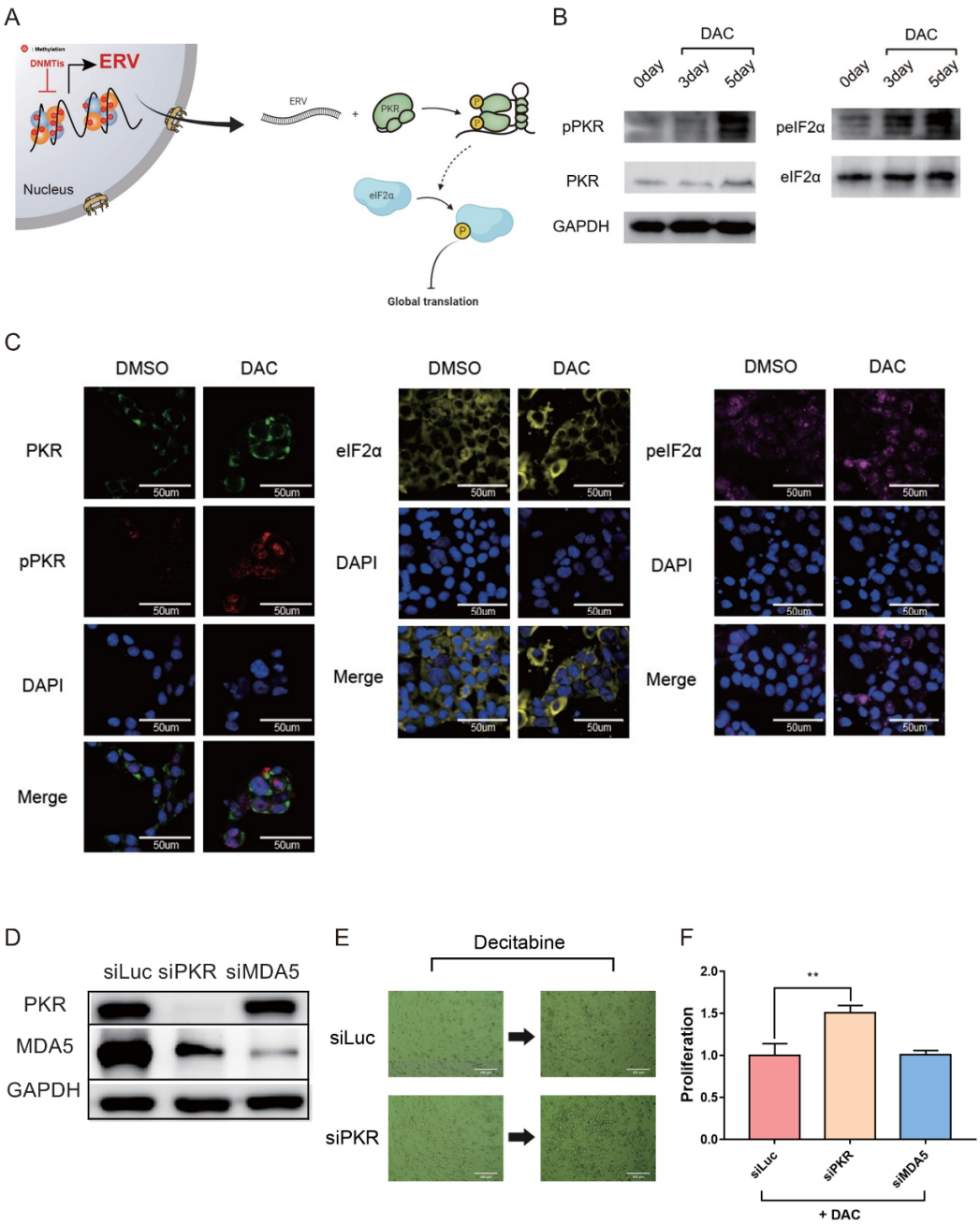

Fig. S1. Decitabine treatment activates PKR signaling. (A) A schematic for the activation of PKR signaling by decitabine. (B, C) Western blotting (B) and immunocytochemistry (C) analyses showed increased phosphorylation of PKR and its downstream substrate eIF2α five days after the decitabine treatment. (D) The knockdown of PKR and MDA5 was confirmed using western blotting. (E, F) The knockdown of PKR rescued cell death from the decitabine treatment. Bright-field images (E) and SRB assay (F) clearly showed increased cell proliferation in PKR-deficient cells compared to that of the control cells five days after transient exposure to low dose decitabine.

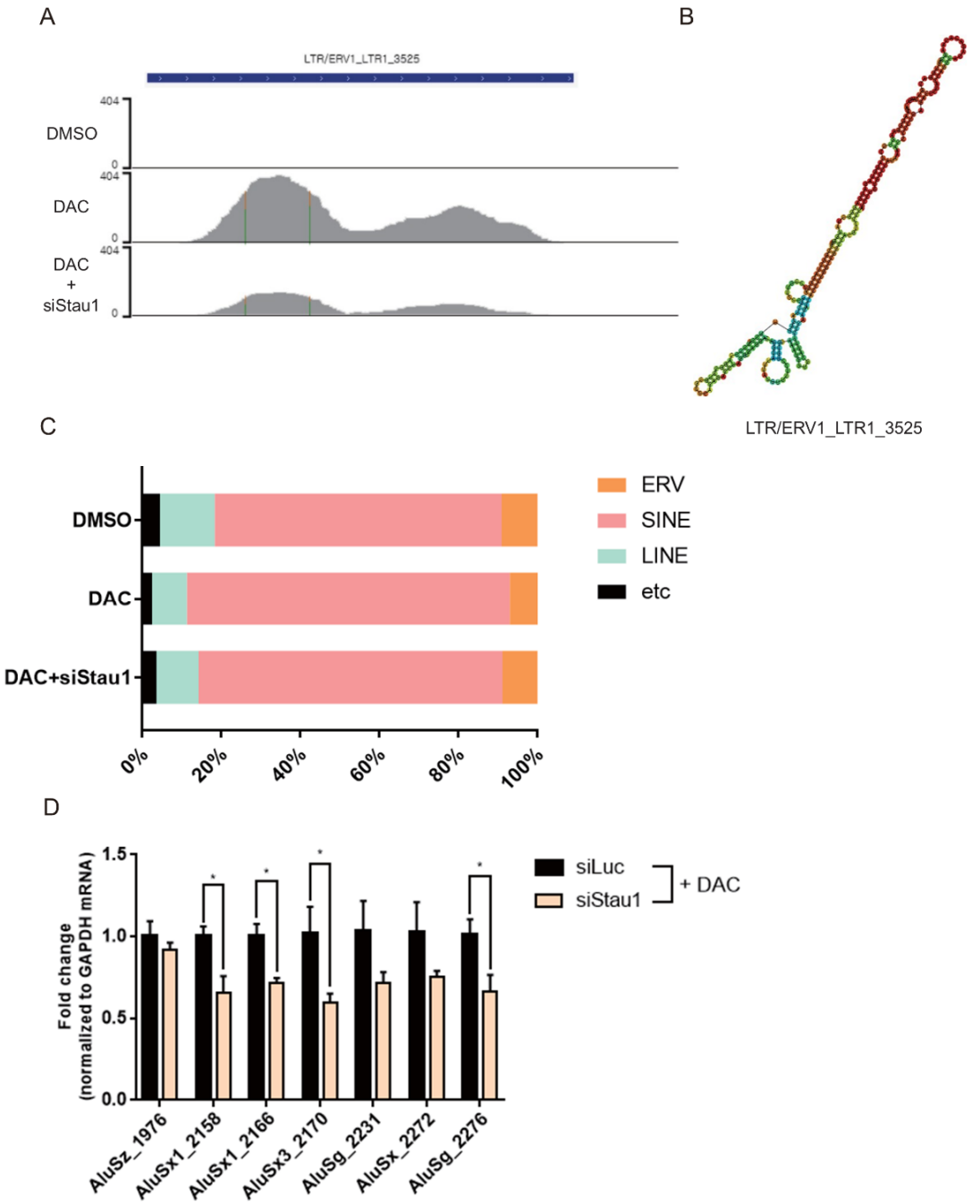

Fig. S2. Decitabine treatment affects dsRNA expression globally. (A) An example of J2 fCLIP dsRNA-seq read accumulation pattern mapped to an ERV locus in DMSO control, decitabine treated, and Stau1-deficient cells treated with decitabine. ERV1_LTR1_3525 locus is shown. (B) RNAFold prediction of the secondary structure of the ERV1_LTR1_3525 RNA. (C) Distribution of sequencing reads mapped to retrotransposable elements that can form dsRNAs. (D) Knockdown of Stau1 resulted in decreased expression of most Alu RNAs examined. *n= 3* and error bars denote s.e.m. *p-value < 0.05; **p-value < 0.01 ***p-value < 0.001 ****p-value < 0.0001

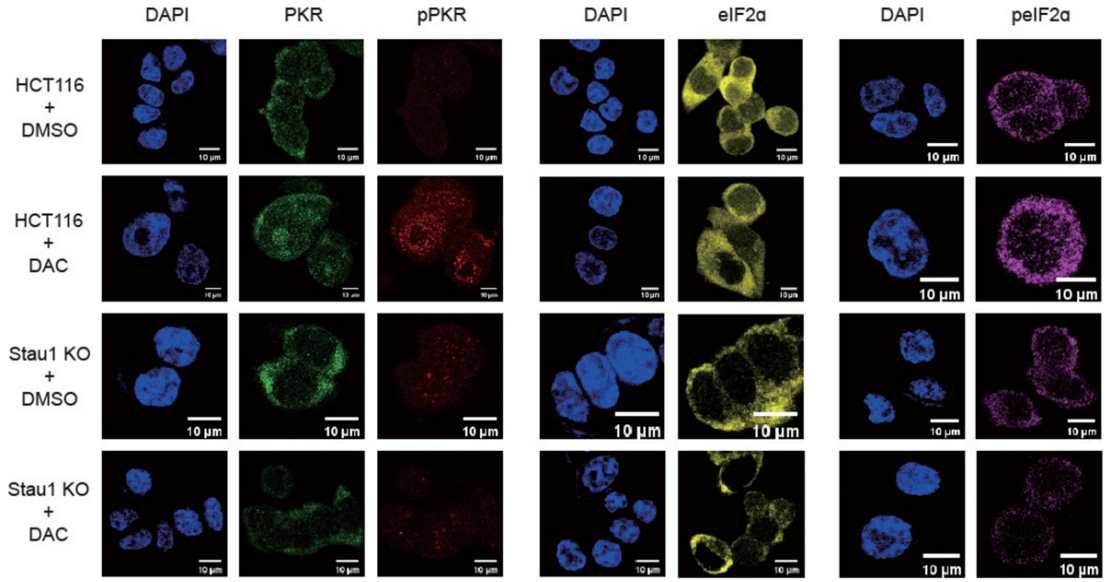

Fig. S3. Stau1 affects PKR activation by decitabine. Immunocytochemistry analysis revealed that decitabine treatment did not induce PKR and eIF2α phosphorylation in Stau1 KO cells.

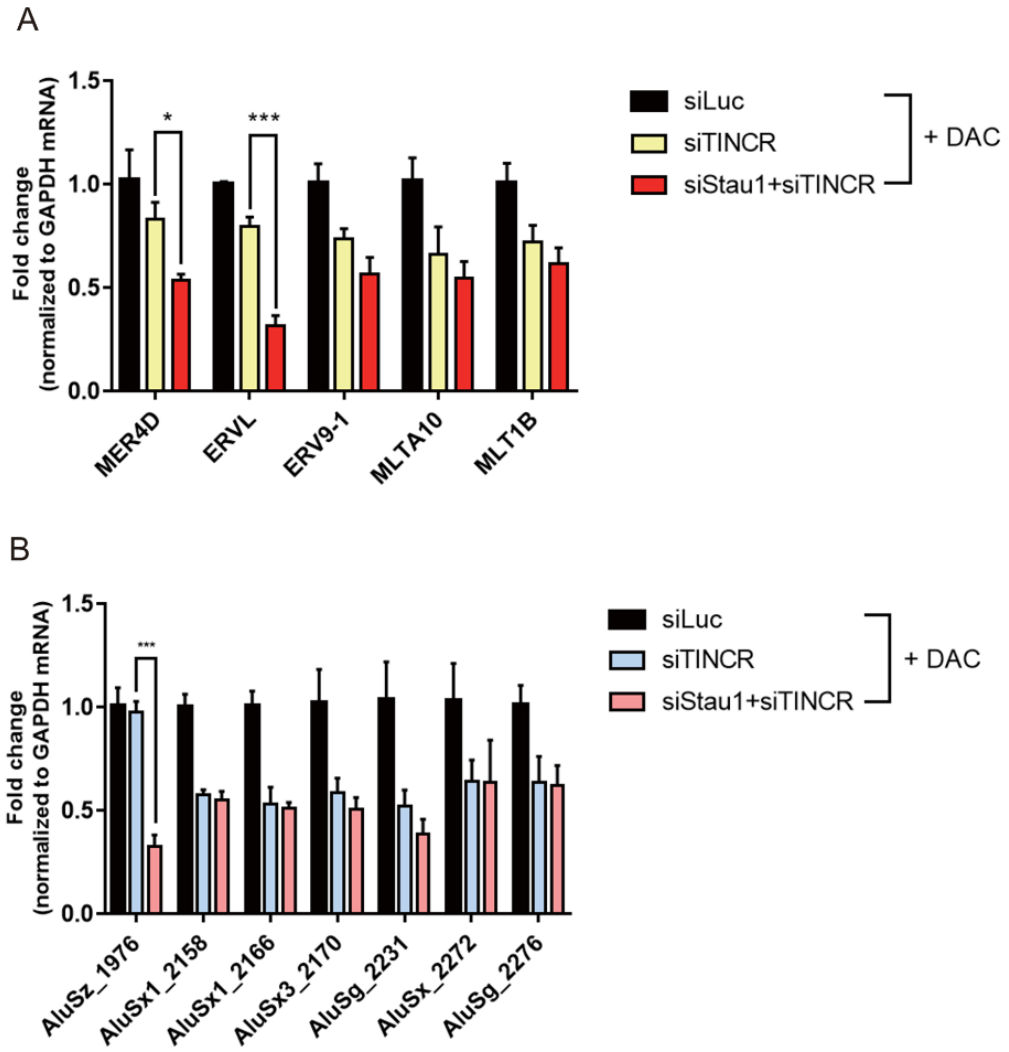

Fig. S4. Stau1-dependent and -independent regulation of dsRNAs by TINCR. (A, B) Knockdown of TINCR resulted in a decreased expression of most ERV (A) and Alu (B) RNAs. Notably, simultaneous knockdown of Stau1 and TINCR resulted in a significant decrease for some ERV and AluSz_1976 RNAs, indicating that there may exist Stau1-independent regulation of these RNAs by TINCR. *n= 3* and error bars denote s.e.m. *p-value < 0.05; **p-value < 0.01 ***p-value < 0.001 ****p-value < 0.0001

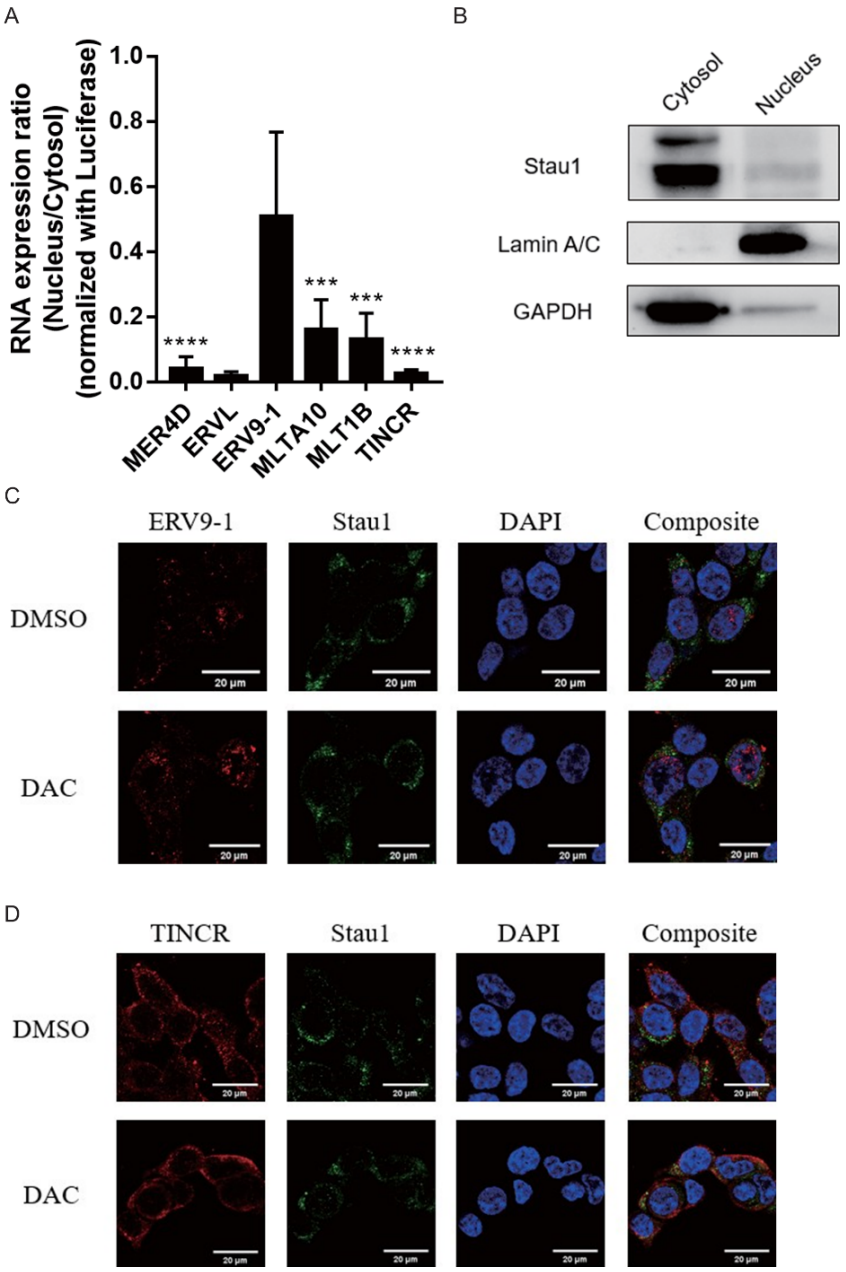

Fig. S5. ERV, Stau1, and TINCR interact in the cytosol. (A) The nuclear to cytosolic expression ratio of ERV and TINCR RNAs. RNA expressions from free cytosolic and nuclear fractions were analyzed using RT-qPCR and normalized using Luciferase mRNA spike-in. *n= 3* and error bars denote s.e.m. (B) Western blotting of cytosolic and nuclear compartments showed Stau1 is expressed mostly in the cytosol. Lamin A/C was used as a nuclear marker while GAPDH was used as a cytosolic marker. (C, D) Co-staining of ERV9-1 (C) or TINCR (D) RNA with Stau1 protein by combining RNA-FISH and immunocytochemistry confirmed the subcellular fractionation data that ERV, TINCR, and Stau1 were mostly cytosolic. *p-value < 0.05; **p-value < 0.01 ***p-value < 0.001 ****p-value < 0.0001.

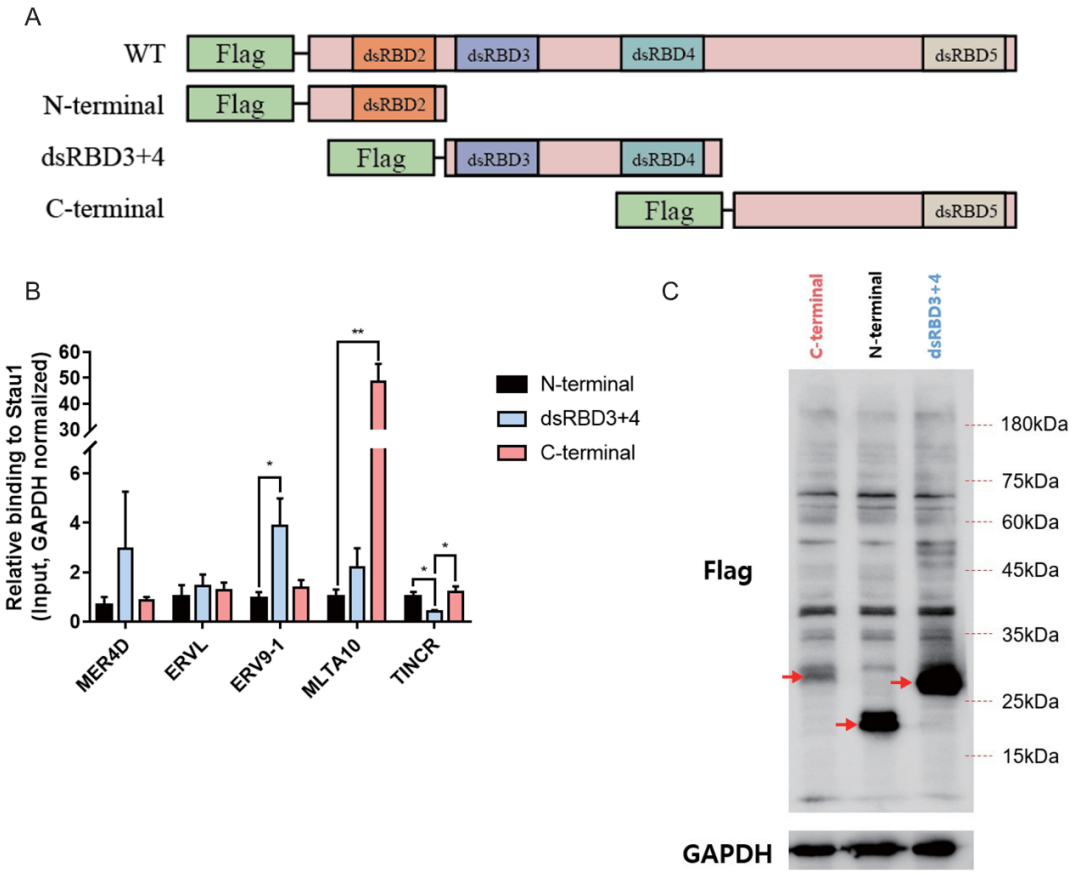

Fig. S6. TINCR and ERV RNAs bind to different regions on Stau1 protein. (A) Schematic of Stau1 protein fragments used in this analysis. (B) We expressing different Stau1 fragments and performed an fCLIP experiment to analyze their interaction with ERV and TINCR RNAs. GAPDH normalized RNA enrichment relative to the N-terminal fragment is shown. *n= 3* and error bars denote s.e.m. (C) Expression of the fragments was confirmed using western blotting. The red arrows denote the expected fragments. *p-value < 0.05; **p-value < 0.01 ***p-value < 0.001 ****p-value < 0.0001.

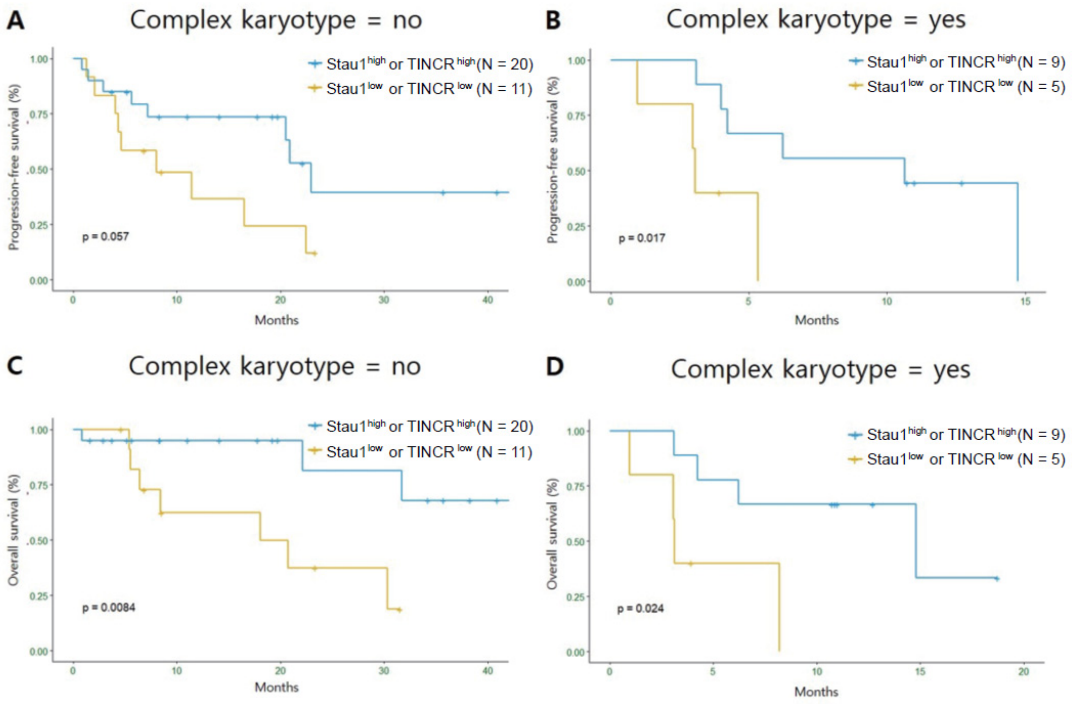

Fig. S7. Analysis of MDS and AML patient samples reveal the clinical significance of Stau1 and TINCR. (A-D) Patients with low Stau1 and TINCR expressions exhibited poor PFS and OS regardless of complex karyotype.

Supporting Information Tables

Table S1. Patient baseline demographics and disease characteristics

Characteristic	Total N (%)	MDS N (%)	AML N (%)
Total number of patients	46	23	23
Gender			
Male	32 (69.6)	15 (65.2)	17 (73.9)
Female	14 (30.4)	8 (34.8)	6 (26.1)
Median age, years (range)	70 (35-89)	67 (35-84)	73 (65-89)
Disease			
MDS	23 (50.0)		
AML	23 (50.0)		
De novo AML	18 (39.1)		
Secondary AML	5 (10.9)		
ECOG performance status			
1	19 (41.3)	9 (39.1)	10 (43.5)
2	18 (39.1)	8 (34.8)	10 (43.5)
3	9 (19.6)	6 (26.1)	3 (13.0)
Hypomethylating agent			
Azacitidine	11 (23.9)	10 (43.5)	1 (4.3)
Decitabine	35 (76.1)	13 (56.5)	22 (95.7)
MDS IPSS risk			
Intermediate-2		17 (73.9)	
High		6 (26.1)	
AML European LeukemiaNet risk			
Favorable			3 (13.0)
Intermediate			15 (65.2)
Adverse			5 (21.7)
Cell counts			
Hemoglobin (range)	8.3 (2.5-11.5)	7.6 (2.5-11.4)	8.6 (5.6-11.5)
White blood cell (range)	3045 (550-143590)	2950 (1160-21000)	3460 (550-143590)
Absolute neutrophil count (range)	811 (3-7520)	600 (3-5859)	1200 (23-7520)
Platelet (range)	57.5 (4-564)	60 (4-564)	54 (7-146)
BM blast (range)	18.1 (0.8-97.4)	8.8 (0.8-15.7)	5 (20.4-97.4)
Karyotype			
Complex	14 (30.4)	10 (43.5)	4 (17.4)

Monosomal	11 (23.9)	9 (39.1)	2 (8.7)
RUNX1 mutation			
Yes	8	6	2
No	23	10	13
ASXL1 mutation			
Yes	7	5	2
No	24	11	13
TP53 mutation			
Yes	10	8	2
No	26	8	18
Allogeneic hematopoietic stem cell transplantation			
Yes	9 (19.6)	9 (39.1)	0 (0)
No	37 (80.4)	14 (60.9)	23 (100)
Objective response			
Yes	13 (28.3)	11 (45.8)	2 (8.7)
No	33 (71.7)	13 (54.2)	21 (91.3)
Death			
Yes	18 (39.1)	5 (21.7)	13 (56.5)
No	28 (60.9)	18 (78.3)	10 (43.5)

MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; ECOG, Eastern Cooperative Group; IPSS, International Prostate Symptom Score

Table S2. Treatment response according to dsRNA-binding protein expression and in the analyzed patients

dsRNA binding proteins		Objective response	
		No	**Yes**
Stau1	Low (< median)	19	4
	High (≥ median)	14	9
	P = 0.189*		
TINCR	Low	20	3
	High	13	10
	P =0.047*		
Stau1 and TINCR	$Stau1^{low}/TINCR^{low}$	16	1
	Others	17	12
	P =0.016*		
Stau1 and TINCR	$Stau1^{high}/TINCR^{high}$	23	6
	Others	10	7
	P = 0.181*		

*by Fisher's exact test

Table S3. Variables associated with progression free survival in patients with higher-risk myelodysplastic syndrome or acute myeloid leukemia

	Univariate analysis		Multivariate analysis	
Variables	HR (95% CI)	p^*	HR (95% CI)	p^*
Stau1^{low}/TINCR^{low}	23.0 (1.07-4.92)	0.033	2.72 (1.23-6.01)	0.014
Age (<70 vs ≥70)	0.74 (0.33-1.64)	0.458		
Sex (Male vs Female)	1.79 (0.77-4.15)	0.176		
Disease (MDS vs AML)	0.85 (0.38-1.91)	0.697		
ECOG PS (0-1 vs 2-3)	2.70 (1.14-6.42)	0.025	2.41 (1.00-5.81)	0.050
HMA (AZA vs DAC)	0.96 (0.38-2.41)	0.932		
Complex karyotype	2.60 (1.11-6.10)	0.029	2.95 (1.21-7.23)	0.018
Monosomal	2.25 (0.94-5.42)	0.070		
RUNX1 mutation	1.02 (0.37-2.84)	0.969		
ASXL1 mutation	1.05 (0.35-3.16)	0.930		
TP53 mutation	1.15 (0.44-3.01)	0.775		

HR, hazard ratio; CI, confidence interval; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; ECOG PS, Eastern Cooperative Group Performance Status; AZA, Azacitidine; DAC, Decitabine

*P values were derived from the Cox proportional regression model including all variables in the table.

Table S4. Variables associated with allogeneic hematopoietic stem cell transplantation-censored overall survival in patients with higher-risk myelodysplastic syndrome or acute myeloid leukemia

	Univariate analysis		Multivariate analysis	
Variables	HR (95% CI)	P^*	HR (95% CI)	P^*
$Stau1^{low}/TINCR^{low}$	3.70 (1.36-10.04)	0.010	4.97 (1.73-14.27)	0.003
Age (<70 vs ≥70)	1.63 (0.53-5.00)	0.393		
Sex (Male vs Female)	1.93 (0.70-5.33)	0.203		
Disease (MDS vs AML)	1.76 (0.62-5.05)	0.289		
ECOG PS (0-1 vs 2-3)	4.36 (1.26-15.08)	0.020	3.15 (0.88-11.26)	0.077
HMA (AZA vs DAC)	1.14 (0.37-3.48)	0.815		
Complex karyotype	4.45 (1.50-13.21)	0.007	5.82 (1.80-18.87)	0.003
Monosomal	3.01 (1.03-8.81)	0.045		
RUNX1 mutation	0.50 (0.11-2.25)	0.364		
ASXL1 mutation	0.81 (0.18-3.66)	0.779		
TP53 mutation	2.16 (0.68-6.87)	0.192		

HR, hazard ratio; CI, confidence interval; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; ECOG PS, Eastern Cooperative Group Performance Status; AZA, Azacitidine; DAC, Decitabine

*P values were derived from the Cox proportional regression model including all variables in the table.

Table S5. siRNA sequences

Genes	Sense (5' – 3')	Antisense (5' – 3')
siLuc	CUU ACG CUG AGU ACU UCG A	UCG AAG UAC UCA GCG UAA G
siStau1_A	GCU AAA AGC ACC AGA GAA AUU	AAU UUC UCU GGU GCU UUU AGC
siStau1_B	GUU UGA GAU UGC ACU UAA A	UUU AAG UGC AAU CUC AAA C
siStau1_C	CGA GAG ACA CGG UGG GUA AUU	AAU UAC CCA CCG UGU CUC UCG
siTINCR_A	UAU UCC UUC AGC CAG UAC CCA GGU CUU	AAG ACC UGG GUA CUG GCU GAA GGA AUA
siTINCR_B	UUU CCA AGG UGG CAC AGU GCU UUC CUU	AAG GAA AGC ACU GUG CCA CCU UGG AAA
siPKR_A	GCA GGG AGU AGU ACU UAA A	UUU AAG UAC UAC UCC CUG C
siPKR_B	GCA UGG GCC AGA AGG AUU U	AAA UCC UUC UGG CCC AUG C
siPKR_C	GCA GAU ACA UCA GAG AUA A	UUA UCU CUG AUG UAU CUG C
siPKR_D	CCU GAG ACC AGU GAU GAU U	AAU CAU CAC UGG UCU CAG G
siPACT	AGG AAU GCU GCU GAG AAA U	AUU UCU CAG CAG CAU UCC U
siDHX9	CCG UAA AUG AAC GUA UGC U	AGC AUA CGU UCA UUU ACG G
siMDA5	GUA CAA UGA GGC CCU ACA A	UUG UAG GGC CUC AUU GUA C
siADAR	CAC CAA GGG AAG UUG ACU A	UAG UCA ACU UCC CUU GGU G
siTRBP	CAC GUC AGC UAC CUG GAU A	UAU CCA GGU AGC UGA CGU G

Table S6. RNA-FISH probe primer sequences

Gene	Forward (5' – 3')	Reverse (5' – 3')
ERVL	CAC GAG GGG CTA AGG AAG AG	TAA TAC GAC TCA CTA TAG GGG CAG CAG CTC ACA CTG ATG T
ERV9-1	CCA GTT AGG AAG CCT TGT GC	TAA TAC GAC TCA CTA TAG GGC CTT CGT ATC CCA TTC TCC A
TINCR	GAA GCG CTA CCA CAT CAA GG	TAA TAC GAC TCA CTA TAG GAG GCC TGA GAA GGA GCT AG

Table S7. RT-qPCR primer sequences

Gene	Forward (5' – 3')	Reverse (5' – 3')
GAPDH	CTC CTC CAC CTT TGA CGC TG	TCC TCT TGT GCT CTT GCT GG
Stau1	CAC CTC CGT GTT TGG TCT TT	GGT CAC GCT GAG TAG GAA GC
PKR	GAG GGG AAT GAT GTG ATT GG	CTG GGC TGT CAC TTC TAG CC
ADAR1	GTC CCG AGG AAG TGC AAG AC	AGC AGG AAA CTA CTG GGG GA
DHX9	TTG GCA GTA CAC GGT ATG GA	ATA GCC TCC ACC AAC ACC TG
MDA5	CAA ATG GCG AGA TAA TCT GCA A	TTT GAA AGC CAC TAC ATA ATT CTT TAT TTT
PACT	GCC CAC TTT CAC CTT CAG AG	CTT GGA AGG GTC AGG CAT TA
DHX9	TTG GCA GTA CAC GGT ATG GA	ATA GCC TCC ACC AAC ACC TG
MLT1A0	TCT CAC AAT CCT GGA GGC TG	GAC CAA GAA GCA AGC CCT CA
MLT1B	TGC CTG TCT CCA AAC ACA GT	TAC GGG CTG AGC TTG AGT TG
ERVL	ATA TCC TGC CTG GAT GGG GT	GAG CTT CTT AGT CCT CCT GTG T
MLT1C627	TGT GTC CTC CCC CTT CTC TT	GCC TGT GGA TGT GCC CTT AT
MER4D	CCC TAA AGA GGC AGG ACA CC	TCA AGC AAT CGT CAA CCA GA
MLT2B4	CTG CTC CCC ACA GTG TCT C	CCA GGT TCA AAC TGT TCC AG
ERV_MER34	GAA TTC AGT GCC ACT AAG CAG AC	TCG GTA TAT CCA AGA CAT GAT CC
ERV_Fb1	ATA TCC CTC ACC ACG ATC CTA ATA	CCC TCT GTA GTG CAA AGA CTG ATA
ERV_9-1	TCT TGG AGT CCT CAC TCA AAC TC	ACT GCT GCA ACT ACC CTT AAA CA
ERV_F	CAG GAA ACT AAC TTT CAG CCA GA	TAA AGA GGG CAT GGA GTA ATT GA
ERV_2432	TCC CCA ATG TTG GAG GTA AG	GGA GAG GTG CCA TAC ACG TT
ERV_3525	CCC ACA GAC CTA GGT GAG GA	ACG TTG TTC CAC CTG TCG AT
ERV_58570	TTA AGC GGA AGC GAC TCA TT	CCT CTG ACC ATT TGC CTT GT
AluSx3_2170	CCT GTG GTC CCA GCT ACT TG	TGT CAC ATG GAA CGC TCT GT
AluSg_2276	CCT GAC CTC ATG TTC CAC CT	GTA GAG ACG GGG TTT CAC CA
AluSz_1976	GCC CAG CCT TGC ATT TAA TA	TGT GCC ACC ATA CCA AGC TA
AluSx_2272	TGT AGT GAG CCG AAA TCA CG	CCA GGA GCT AAA GGT TGC AG
AluSx1_2166	ATC TTG GCT CAC TGC AAC CT	TGG ATC ACA TGA GGT CAG GA

AluSg_2231	GGA GAA TCG CTT GAA CCT GA	TGG GCA ACA GAG TGA GAC TG
AluSx1_2158	CAC GGT GGC TCA CAT CTG TA	GGG ATT ATA AGT GCC CAC CA