Supporting Information

Yoshida et al. 10.1073/pnas.1415525112

SI Results

Exon Array Analysis. As discussed in the main text, scatter plots showed that the exons affected by RECTAS were a very minor fraction of the entire exon set examined (Fig. S4*B*). The exons with statistically significant changes in relative expression numbered only 250, which accounted for only 0.10% of the exons examined, and the Pearson's correlation coefficient between pre- and posttreatments with RECTAS was 0.9975. We also evaluated the effects of RECTAS on gene expression levels. Even when the threshold was not very strict ($P < 0.01$ without multiple test corrections and a fold change of >1.3 or <1/1.3), only 55 genes (0.16% of the total), including *IKBKAP*, met the criteria (Fig. S4*C*). The very high Pearson's correlation coefficient (0.9978) also implies that the expression of only a limited number of genes was affected by RECTAS.

Sequence Analysis of the Exons Responsive to RECTAS. As discussed in the main text, we found that ESSs were enriched in the inclusion-type exons. The average ESS sites per 100 bases was 20.1 for the control set and 25.0 for the inclusion-type exon set, with the difference being statistically significant ($P = 0.035$ with the Mann–Whitney *U* test). The inclusion-type exons had 46.0 ESSs on average, whereas the control exons had 33.0 ESSs on average (Fig. 4*B*). We did not find clear differences in the number of ESSs for the exclusion-type exon set; the average number of ESS sites was 30.5, and the average number of ESSs per 100 bases was 20.2 (Fig. 4*B*). There were almost no differences in exonic splicing enhancers (ESEs) in the three datasets; the average values of ESEs per 100 bases were 17.2, 17.5, and 17.8 for the control, exclusion-type, and inclusion-type exon sets, respectively (Fig. S4*D*).

tRNA Modification Analysis. The analyses of tRNA modification by liquid chromatography/mass spectrometry (LC/MS) are highly complicated because the mass of the RNase T_1 fragments from isolated tRNA with or without modifications is specific but limited to very small changes. Moreover, modifications of individual tRNAs in mammals are poorly understood. In this study, we focused on 5-methyluridine derivatives (xm^5U) because several studies have suggested the involvement of IKAP/ELP1 in the formation of the modification. We choose Val, Arg, and Gly from seven types of tRNAs containing xm^5U for analysis for the following reasons. Technically, reciprocal circulating chromatography (RCC) isolates not only the UNN anticodon tRNA but also the isoacceptor CNN anticodon tRNA when the sequence of the isoacceptor tRNA is highly homologous to the UNN anticodon tRNA. In LC/MS analysis, unmodified UNN anticodon fragments cannot be distinguished from contaminated CNN anticodon fragments due to the close mass proximity. In Val, Arg, and Gly tRNAs, we were able to isolate UNN anticodon tRNA without contamination by isoacceptor tRNAs. However, isoacceptor $tRNA^{Thr}$(CGU) was found to have been slightly contaminated in the $tRNA^{Thr}$(UGU) preparation. For this reason, only the mass chromatograms of $tRNA^{Thr}$(UGU/CGU) are shown because we were not able to calculate strictly the frequency of the modification (Fig. S5*E*).

The presence of mcm^5s^2U and $mchm^5U$ at wobble positions has been reported in bovine $tRNA^{Arg}$(UCU) and $tRNA^{Gly}$(UCC), respectively (1, 2). For $tRNA^{Val}$(UAC), no such modification has been reported in mammals, although it has been suggested that this tRNA contains ncm^5U at the wobble position (3, 4). None of these modifications, however, have been demonstrated in human tRNAs. In addition to this evidence, we confirmed that modifications are involved in RNase T_1 fragments containing wobble uridines by collision-induced dissociation (CID).

The frequency of ncm^5U in $tRNA^{Val}$(UAC) was reduced to 64% and 29% in FD patient nos. 42 and 50, respectively (Fig. 5*B*). In $tRNA^{Arg}$(UCU), the modification status of mcm^5s^2U was reduced to 74% and 70% in FD patient nos. 42 and 50, respectively (Fig. 5*C*). In addition to unmodified wobble uridine, a small amount of mcm^5U was detected in this tRNA. The frequency of $mchm^5U$ in $tRNA^{Gly}$(UCC) was also reduced to 79% and 68% in FD patient nos. 42 and 50, respectively (Fig. 5*D*). The chm^5U and mcm^5U were detected in this tRNA.

Additionally, we analyzed $tRNA^{Thr}$(UGU), a modification that has not been found in mammals, although it has been reported that $tRNA^{Thr}$(UGU) contains ncm^5U in yeast. We found that the wobble uridine of $tRNA^{Thr}$(UGU) is mostly modified with a group whose mass is equal to ncm^5U. CID analysis of the anticodon loop indicates that the modification is likely to be ncm^5 (Fig. S5*F*). In the analysis of $tRNA^{Thr}$(UGU), isoacceptor $tRNA^{Thr}$(CGU) was found to be a slight contaminant. For this reason, the LC/MS analysis was not able to distinguish an RNase T_1 fragment, UCUUGp, without m^3 at C32 from an RNase T_1 fragment of UCUCGp derived from the contaminated isoacceptor because of the close mass proximity. Therefore, as demonstrated in Fig. S5*E*, we showed the mass chromatograms of the RNase T_1 fragments modified with (*Left*) or without (*Right*) m^3 at position 32 (C32), separately. The frequency of this modification in RNase T_1 fragments with m^3C32 was reduced in the cells of patients with FD (Fig. S5*E*, *Left*). On the other hand, a fragment modified with ncm^5U at the wobble position without m^3 at C32 was not detected, suggesting that the formation of ncm^5U requires m^3C32 (Fig. S5*E*, *Right*). These results suggested that IKAP is also involved in the formation of the modification in $tRNA^{Thr}$(UGU).

SI Materials and Methods

Cells from Patients. Fibroblast cell lines from FD patient nos. 50 and 42, as well as the heterozygous FD carrier cell line, were obtained from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository: the cell line of patient no. 50 is GM00850, the cell line of patient no. 42 is GM02342, and the heterozygous cell line is GM04664.

Cell Culture. SH-SY5Y and HeLa cells were cultured in RPMI medium and DMEM (Nacalai Tesque), respectively, supplemented with 10% (vol/vol) FBS at 37 °C in 5% (vol/vol) CO_2. FD carrier cells and cells from patients with FD were cultured in DMEM supplemented with 15% (vol/vol) FBS. SH-SY5Y and HeLa cells were transfected with FuGeneHD (Promega).

Splicing Substrate Construction. To construct pCDC-IKBKAP Ex20WT and pCDC-IKBKAP Ex20FD, the WT or mutant exon 20 of the human *IKBKAP* gene with 150 flanking nucleotides from both the upstream and downstream introns was amplified by PCR and inserted between the SacI and StyI sites of pCDC (5). IKBKAP Ex20-21WT and IKBKAP Ex20-21FD were constructed by PCR of the IKBKAP splicing reporter vectors. The first part containing 48 nt of exon 20 and the second part containing 30 nt of exon 21 were amplified by PCR. These two fragments were subcloned into the HindIII, BamH1 site of pcDNA3.1(+)/myc-His A (Invitrogen) to make a substrate of *IKBKAP* pre-mRNA containing 76 nt of the upstream exon, 219 nt of the shortened intron 20, and 64 nt of the downstream exon. The resulting

length of the mRNA is equivalent to the length of pCDC (5). IKBKAP int-Ex20-int WT and IKBKAP int-Ex20-int FD were constructed in a similar way. Exon 20 (73 nt) with a flanking int sequence (127 nt upstream and 79 nt downstream) was amplified by PCR. The primers for plasmid construction are listed in Table S1.

Analysis of Transfected Cells. SH-SY5Y and HeLa cells were transfected with FuGeneHD according to the manufacturer's instructions. All plasmids used for transfection were prepared using a Midi prep kit (MACHEREY-NAGEL GmbH & Co.). Cells were grown in a monolayer in 12-well plates and then transfected with 0.5 μg per well of a reporter plasmid or cotransfected with 0.5 μg each of reporter plasmid and either plasmid expressing RNA binding protein. After a 24-h or 48-h incubation, cells were analyzed as follows. For semiquantitative RT-PCR analysis, cells were recovered with TRIzol (Invitrogen) (*SI Materials and Methods*, *Semiquantitative RT-PCR*). For microscopic analysis, cells were washed with PBS and then fixed with 4% (vol/vol) paraformaldehyde (Nacalai Tesque) for 10 min. The fixed cells were stained for 30 min with 5 μg/mL Hoechst 33342 (Sigma-Aldrich) and 1% Triton-X in PBS, and then washed with PBS once to remove excess Hoechst. After staining the nucleus, cells were kept in PBS and visualized using an Olympus IX81 microscope with a DP72 digital camera (Olympus).

Semiquantitative RT-PCR. Total RNAs were prepared from cultured cells with TRIzol according to the manufacturer's instructions. If necessary, the RNAs were treated with RNase-free DNase (RQ1; Promega) according to the manufacturer's manual. First-strand cDNA was synthesized using RT (Prime Star; Takara Bio, Inc.) with random hexamers and amplified by PCR using an appropriate set of primers. Semiquantitative RT-PCR was performed with Ex Taq polymerase (Takara Bio, Inc.). Cycle conditions were as follows: 94 °C for 2 min; followed by 28 cycles (reporter), 38 cycles (*IKBKAP*), or 23 cycles (*GAPDH*) of denaturation at 94 °C for 10 s; annealing at 50 °C (*IKBKAP*) or 58 °C (*GAPDH*) for 30 s; and elongation at 72 °C for 30 s, with a final incubation at 72 °C for 5 min in a PCR thermal cycler (BIOMETRA). PCR products were separated by electrophoresis and stained with ethidium bromide. Images were obtained with a gel imaging system (ChemiDoc; Bio-Rad Laboratories). The primers for semiquantitative RT-PCR are listed in Table S1.

Small Chemical Compounds. A total of 620 compounds from the Prestwick Chemical Library (Prestwick Chemicals), and 18 compounds (Namiki Shoji Co., Ltd.) provided by the Medical Research Support Center, Graduate School of Medicine, Kyoto University, were screened. Part of the library was newly synthesized by following the procedures described in *SI Materials and Methods* (*Synthesis of Small Chemicals*). Kinetin powder was purchased from Nacalai Tesque.

Fluorescence Quantification in Cell-Based Screening of Small Chemical Compounds. The visualized images of the expression of *IKBKAP-FD* reporter in HeLa cells were analyzed, and the GFP/RFP ratio was calculated using the compartment analysis algorithm (Thermo Fisher Scientific). Because E19/20/21-GFP proteins were predominantly in the nucleus, whereas E19/21-RFP proteins were mainly in the cytoplasm, we were able to take advantage of the compartment analysis algorithm. The compartment analysis algorithm was used to define the primary object, to apply a nucleic mask and a cytoplasmic mask, and to quantify GFP and RFP intensity in the masks, respectively. The primary object was defined as the nucleus by Hoechst staining. The nucleic mask was set as inside an area one pixel smaller than the primary object. The cytoplasmic mask was defined as the area one to five pixels outside of the primary object. The score was calculated as the mean of each value of the GFP/RFP ratio of an object.

In Vitro Splicing and Complex Formation Assay. The templates, CDC-IKBKAP Ex20WT and CDC-IKBKAP Ex20FD, were linearized with SmaI. In vitro transcription and purification of the transcribed RNAs were performed as described previously (5). In vitro splicing was also conducted as described previously (6), except that HeLa cell nuclear extracts (CIL BIOTECH) were preincubated with DMSO (0.05%) or RECTAS (20 μM) (at 37 °C for 15 min) and with pre-mRNA (at 37 °C for an additional 10 min) before the splicing reaction. The splicing reaction was incubated at 30 °C. RNA was analyzed by 6% (vol/vol) polyacrylamide denaturing PAGE. The gels were dried under vacuum and visualized by a phosphorimager. Spliceosomal complexes were analyzed by mixing 5 μg of heparin with a 12.5-μL splicing reaction at each time point and incubated at room temperature for 10 min. The complexes were analyzed in a loading buffer with a final concentration of 50 mM Tris·glycine, 4% (vol/vol) glycerol, 0.25% xylene cyanol, and bromophenol blue on a 2% (wt/vol) agarose (Seakem GTG agarose) gel run in 50 mM Tris·glycine at 50 V for 4 h at room temperature. The E-complex assay was performed as previously described (7), with 37 °C preincubation for 10 min. The E-complex was formed by a 30 °C incubation, and heparin (1.6 μg per lane) was added and incubated for a further 10 min where indicated.

Single-Dose Oral Administration. RECTAS or kinetin at a dose of 50 mg or 100 mg suspended in 0.5% carboxymethylcellulose was administered orally to male Jcl:ICR 7-wk-old mice (Charles River Laboratories). These mice were anesthetized at each time point with pentobarbital (Mylan), and blood was sampled, allowed to clot, and centrifuged for serum preparation. Mice were perfused with saline, and brains were removed. Brain homogenates were prepared using Polytron (Kinematica) in saline. Levels of RECTAS or kinetin in brain supernatants and serum were analyzed by LC/MS using an Agilent 6420 Q-TOF mass spectrometer with an Agilent 1290 nano-flow HPLC system (Agilent Technologies) and a ZORBAX HILIC Plus (Kinetin) or ZORBAX Eclipse Plus C18 (RECTAS) column (Agilent Technologies).

Immunoblot. Total proteins were extracted from the cells by scraping with CelLytic MT Cell Lysis Reagent (Sigma-Aldrich) containing a protease inhibitor mixture (Roche). After 10 min of centrifugation at 17,800 × *g* at 4 °C, the supernatant was collected and protein concentrations were measured using a Bio-Rad Protein Assay Kit (Bio-Rad). Proteins were separated via 5–20% gradient SDS/PAGE and then transferred onto a polyvinylidene fluoride membrane (Millipore) by electroblotting. The membranes were blocked with 5% (wt/vol) skim milk in PBS and probed with a mouse anti-IKAP antibody (Abcam) at 1:1,000 or a mouse anti-GAPDH antibody (Ambion) at 1:4,700 overnight at 4 °C, followed by incubation with HRP-conjugated secondary antibody [i.e., goat anti-mouse IgG HRP (Abcam)] at 1:5,000. Immunoblots were visualized by an ECL reaction with the ImmunoStar Kit (Wako Chemicals USA) or Chemi-Lumi One (Nacalai Tesque) and were detected with ChemiDoc.

Expression Analysis. We performed expression analysis with Affymetrix GeneChip Human Exon 1.0 ST arrays. Cells from FD patient no. 42 were plated on a six-well plate at 1×10^5 cells per well in 2 mL of complete medium. After 3 d of culture, cells were treated with 0.1% DMSO or 2 μM RECTAS in flesh medium at 37 °C and 5% CO_2 for an additional 6 h. After treatment, total RNA was extracted using TRIzol reagent, and the RNA quality was evaluated with an Agilent 2100 Bioanalyzer RNA NanoChip system (Agilent Biotechnologies). For the microarray experiment, biotin-labeled RNA was prepared using 100 μg of total RNA. The first cDNA, cRNA, and second cDNA were synthesized using an Ambion WT Expression Kit as described by the manufacturer (Ambion). The second cDNA was then fragmented using uracil

DNA glycosylase and apurinic/apyrimidic endonuclease-1, and biotin-labeled with terminal deoxynucleotidyl transferase using a GeneChip WT Terminal Labeling Kit (Affymetrix). Hybridization was performed using 5 μg of the biotinylated target, which was incubated with the GeneChip Human Exon 1.0 ST array at 45 °C for 16 h. After hybridization, nonspecifically bound material was removed by washing, and specifically bound target was detected using a GeneChip Hybridization, Wash and Stain Kit, and a GeneChip Fluidics Station 450 (both from Affymetrix). The arrays were scanned using a GeneChip Scanner 3000 7G (Affymetrix), and raw data were extracted from the scanned images and analyzed with the Affymetrix Power Tools software package. Expression values for each array probe were calculated by the RMA method using Affymetrix Expression console software. Bioinformatic analyses for detecting altered splicing events were performed as described previously (8). If an exon had >1.2- or <0.833-fold change in expression between the control and RECTAS treatments, and if the differences among the fold changes of the other exons in a gene were >2.5 SD, we defined it as altered exon splicing. In this analysis, we calculated relative expression values based on fold changes normalized with gene expression values to cancel out the effects of gene expression changes. We drew a heat map with these normalized expression values for exons for which alteration of splicing regulation had been defined. The heat map was drawn with the MultiExperiment Viewer (MeV) data analysis tool (9).

We also analyzed expression profiles of the genes with the dataset. We calculated the expression values of a gene as the mean of the expression values of the exons constituting the gene. We set the threshold for detecting up- or down-regulated genes under RECTAS treatments as fold changes that were >1.3 or <0.77, respectively, and P values of t tests as <0.01. These calculations were performed using Perl scripts and the R statistical package (www.r-project.org).

Sequence Analysis. We used the hg18 human genome for sequence analyses, according to exon array design. For construction of the control exon set, we listed 4,192 exons from genes that included RECTAS-responding exons, but did not respond to RECTAS per se, as an initial pool of control exons. We randomly chose 100 exons from this pool and used them as the control exon set.

The SpliceAid program was redeveloped with Perl for computing on a local environment. Information on the *cis*-element was obtained from the SpliceAid webpage. According to description on the webpage, *cis*-elements on exon regions and with positive scores were treated as ESEs and those *cis*-elements with negative scores were treated as ESSs. We used the statistical environment R to draw box plots. In the box plots, positions of the maximum and minimum values within the ranges from the first quarter + 1.5 * interquartile range (IQR) to the third quarter − 1.5 * IQR, whereas IQR was defined as the third − first quarters, are shown as horizontal lines outside of the boxes and joined to the boxes with vertical lines.

For the MEME study, we found motifs with lengths of 6, 8, and 10. With each parameter, we listed 50 motifs. We had three datasets (inclusion-type, exclusion-type, and control exon sets) from which we obtained 450 motif candidates. Existence of the motifs in the exon sequences was tested with the "mast" program in the MEME program set, with the parameter "−mt 1e-3."

RNA Preparation for tRNA Modification Analysis. For tRNA modification analysis in carrier cells and cells of patient nos. 42 and 50, total RNAs were recovered from cells cultured for 2 d after confluency (cultured for 7 d in total). The time course in carrier cells and cells of patient no. 42 with or without RECTAS treatment is described in the main text. Total RNAs were prepared from cultured cells with TRIzol and precipitated with 2-propanol according to the manufacturer's instructions. The RNA pellet was dried and stored. For RCC isolation of tRNA, the RNA pellets were dissolved in water and purified by anion exchange chromatography. Briefly, the RNA samples were applied to 1 mL of DEAE Sepharose FF (GE Healthcare) in 5-mL disposable columns (Bio-Rad) equilibrated with 5 mL of buffer A [10 mM Hepes-KOH (pH 7.5), 200 mM NaCl, and 2 mM DTT]. The RNA samples on DEAE Sepharose were washed with buffer A and then eluted with buffer B [10 mM Hepes-KOH (pH 7.5), 1 M NaCl, and 2 mM DTT].

Human $tRNA^{Val}$(UAC), $tRNA^{Arg}$(UCU), and $tRNA^{Gly}$(UCC) were homogeneously isolated by the RCC method using an RCC device, following the previously described method with a minor modification (10). The 5′-terminal amino-modified DNA probes were covalently immobilized on NHS-activated Sepharose 4 Fast Flow (GE Healthcare) according to the manufacturer's instructions. The DNA resins were packed into the special tips that were attached to multichannel head of the RCC device. DNA probes for RCC are listed in Table S1. A tRNA fraction enriched by DEAE chromatography (55–200 μg) that was dissolved in 2 mL of 6× NME buffer [1.2 M NaCl, 30 mM MES·NaOH (pH 6.0), 15 mM EDTA, and 1 mM DTT] was passed through and captured by affinity tip columns by pipetting the RNA mixture 40 times at 66 °C. Unbound RNAs were washed by pipetting in 700 μL of 0.1× NME buffer [20 mM NaCl, 0.5 mM MES·NaOH (pH 6.0), 0.25 mM EDTA, and 0.5 mM DTT] in series, followed by eight washes with 600 μL of 0.1× NME buffer at 40 °C. RNAs were eluted from the column with three washes of 400 μL or 600 μL of 0.1× NME buffer at 68 °C. The eluted RNAs were precipitated with ethanol. The total yield from each tip column was calculated by measuring UV absorbance at 260 nm.

LC/MS Analysis of tRNAs. One picomole of isolated tRNA was digested with 50 U RNase T_1 (Epicentre) in 20 mM NH_4OAc (pH 5.3) at 37 °C for 60 min. After digestion, an equal volume of 0.1 M triethylamine acetate (pH 7.0) was added to the reaction solution. The digest (0.5 pmol) was analyzed using a linear iontrap–orbitrap hybrid mass spectrometer (LTQ Orbitrap XL; Thermo Fisher Scientific) equipped with a custom-made nanospray ion source, a splitless nano-HPLC system (DiNa; KYA Technologies), a C18 trap cartridge (HiQ sil C18HS-3; KYA Technologies), and a C18 capillary column (KYA Technologies).

Northern Blotting. The total RNAs used for Northern blotting were the same as used in the tRNA modification analysis by RCC and LC/MS. Four micrograms of the total RNA was electrophoresed on a 10% (vol/vol) polyacrylamide gel containing 7 M urea and blotted onto a nylon membrane (Hybond N+; Amersham Biosciences) with 0.5× TBE. The membrane was air-dried, and the blotted RNA was fixed onto the membrane by UV irradiation (254 nm, 120 mJ/cm^2). Northern blotting was conducted using a standard protocol (11). Oligonucleotide probes were end-labeled with [γ-^{32}P]ATP. Radioactivity was visualized by exposing the membrane to an imaging plate and analyzing with a bioimaging analyzer (Typhoon; GE Healthcare). The sequences of oligonucleotide probes were identical for RCC isolation.

Cell Viability Assay. Cells were pretreated with DMSO or RECTAS before plating into a 96-well plate for ∼4–5 d. The pretreated cells were seeded into 96-well plates at a density of 0.3×10^4 cells per well in complete medium, and ∼2–3 h later, DMSO (0.02%) or RECTAS (10 μM) was added. Cell viability was measured at the indicated time points using the Cell Titer Glo Assay Kit according to manufacturer's instructions (Promega).

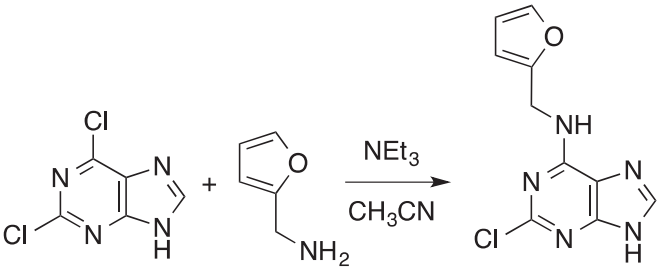
Synthesis of Small Chemicals.

General remarks. All reactions were performed in dried glassware in an atmosphere of argon, unless otherwise noted. The melting

point was measured on a YANACO MP-J3 instrument or an Opti Melt MPA100 (both from Stanford Research Systems) and is uncorrected. ^{1}H NMR spectra were obtained with a Bruker AVANCE 500 spectrometer at 500 MHz. ^{19}F NMR spectra were obtained with a Bruker AVANCE 400 spectrometer at 376 MHz. DMSO-d_6 (catalog no. DLM-10; CIL BIOTECH) was used as a solvent for obtaining NMR spectra. Chemical shifts (δ) are given in parts per million down-field from the solvent peak (δ 2.49 for ^{1}H NMR) as an internal reference or as α,α,α-trifluorotoluene (δ-63.0 ppm for ^{19}F NMR in $CDCl_3$) as an external standard with coupling constants given in hertz. High-resolution mass spectra were measured on a Bruker micrOTOF mass spectrometer under negative electrospray ionization conditions.

2-Furylmethylamine (catalog no. F0091) and 6-chloro-2-fluoropurine (catalog no. C2221) were purchased from Tokyo Chemical Industry Co., Ltd. 2,6-Dichloropurine (catalog co. 322-35482), acetonitrile (catalog no. 014-00386), and triethylamine (catalog no. 202-02646) were purchased from Wako Pure Chemical Industries, Ltd. All other chemical reagents used were of commercial grade and were used as received.

2-Chloro-6-(2-furylmethyl)purine (RECTAS).



To a mixture of 2,6-dichloropurine (189 mg, 1.00 mmol) and 2-furylmethylamine (97.0 mg, 1.00 mmol) dissolved in acetonitrile (20 mL) was added triethylamine (0.150 mL, 1.08 mmol) at room temperature. After stirring for 3 h at 60 °C, the resulting precipitate was collected by filtration, washed with water and diethyl ether, and dried in vacuo to give 2-chloro-6-(2-furylmethyl)purine (19.8 mg, 0.0795 mmol, 8.0%) as a colorless solid. The spectra data of the product were identical to those spectral data reported in the literature (12).

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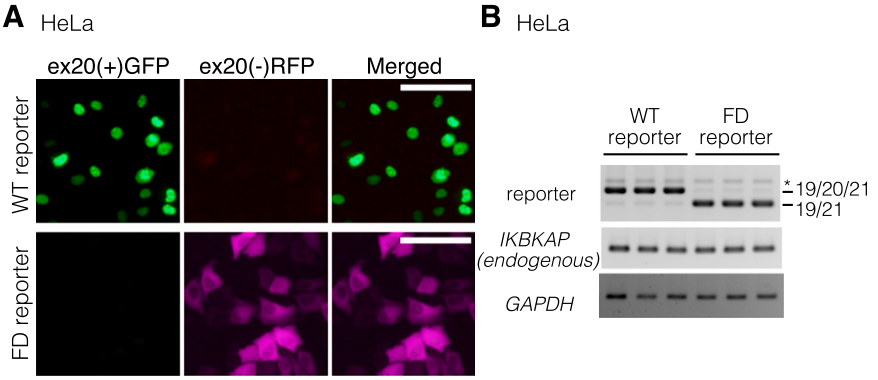

Fig. S1. Visualization of disease-specific splicing using the SPREADD system. (*A*) Microscopic analysis of HeLa cells expressing the *IKBKAP-WT* reporter (*Upper*) and the *IKBKAP-FD* reporter (*Lower*). The results are presented as in Fig. 1C. (Scale bar: 100 μm.) (*B*) RT-PCR analysis of mRNAs derived from HeLa cells expressing the *IKBKAP-WT* reporter or the *IKBKAP-FD* reporter. The results are presented as in Fig. 1*D*. An asterisk indicates the PCR product corresponding to a hybrid of 19/20/21 and 19/21.

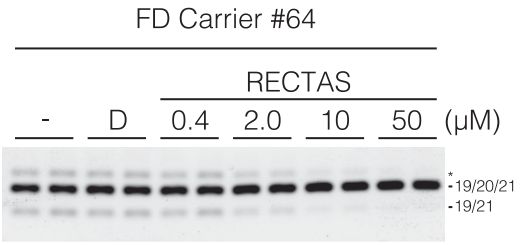

Fig. S2. Promotion of exon 20 inclusion with endogenous *IKBKAP* pre-mRNAs in carrier cells treated with RECTAS. RT-PCR analyses of endogenous *IKBKAP* mRNAs in carrier fibroblasts treated with the indicated concentrations of RECTAS were conducted. D, DMSO control; -, medium change only.

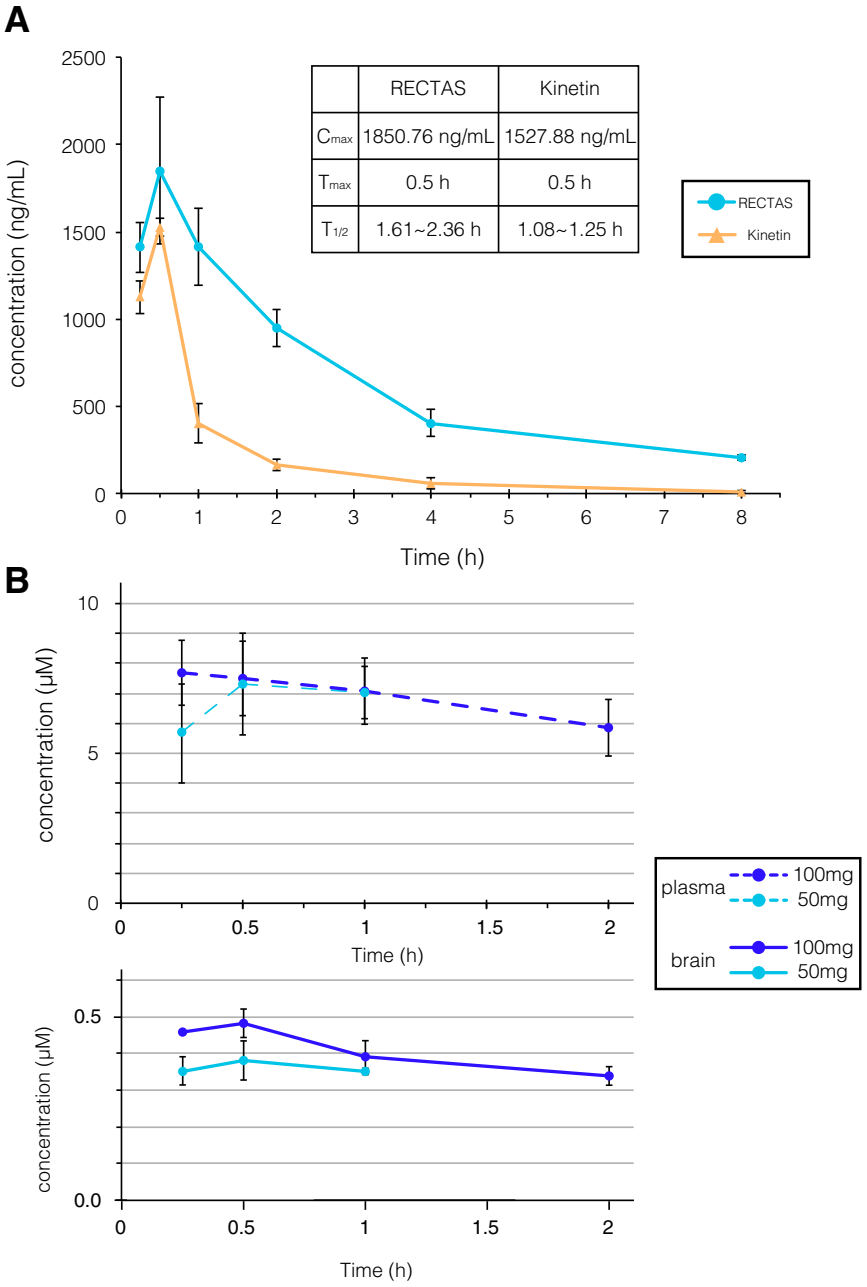

Fig. S3. Pharmacokinetic profile of RECTAS after oral administration to mice. (*A*) Mean concentration in plasma vs. time profile of RECTAS and kinetin in imprinting control region (ICR) mice after oral administration of a single 50-mg/kg dose of RECTAS or kinetin. Each time point is the mean ± SEM ($n = 3$). The first collection time was 15 min after the administration of compounds. (*B*) Mean concentration in the plasma (*Top*) and brain (*Bottom*) vs. time profile of RECTAS in ICR mice after oral administration of a single 50- or 100-mg/kg dose of RECTAS. Each time point is the mean ± SEM ($n = 3$). The first collection time was 15 min after the administration of compounds.

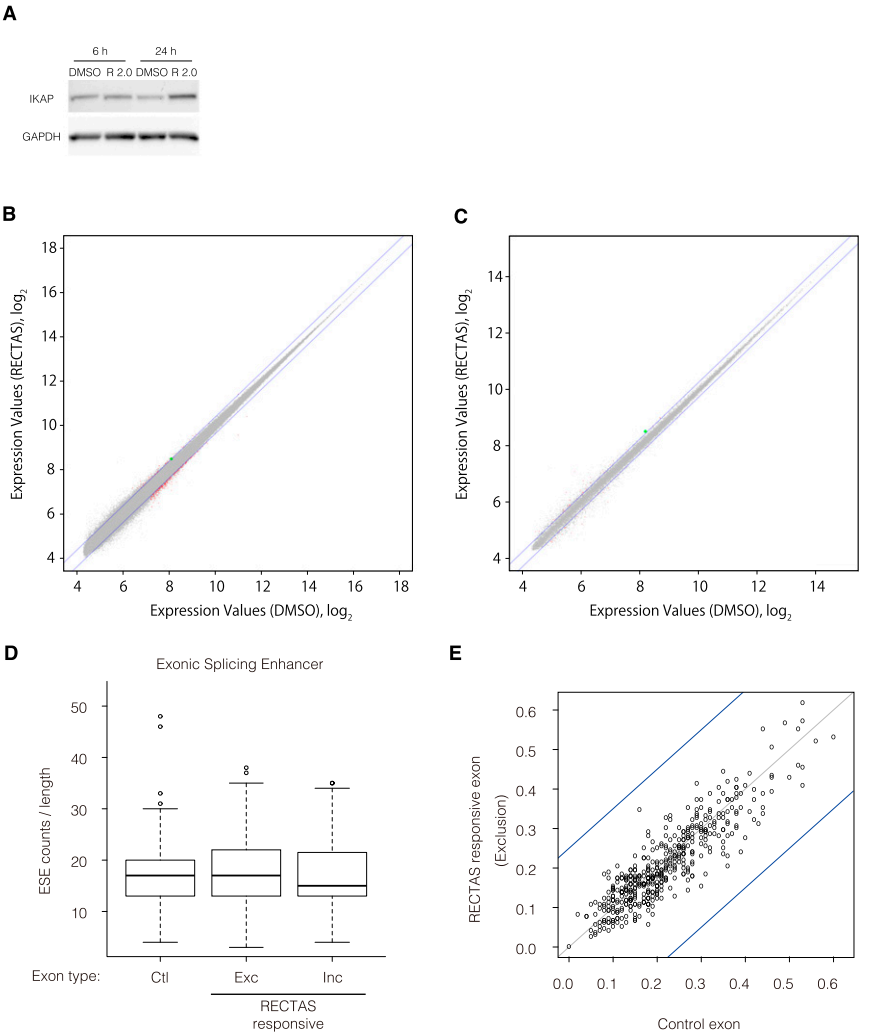

Fig. S4. Splicing events and gene expression influenced by treatment with RECTAS. (*A*, *Top*) IKAP expression in cells of FD patient no. 42 treated with DMSO or 2 μM RECTAS for 6 or 24 h. (*A*, *Bottom*) GAPDH was used as a control. (*B*) Scatter plots show the expression values (log_2) of each exon in the indicated pairs of treatment (RECTAS vs. DMSO). Spots with values that changed by more than the threshold are highlighted in red ($P < 0.01$). (*C*) Scatter plots show the expression values (log_2) of each gene in the indicated pairs of treatment (RECTAS vs. DMSO). Spots with values that changed greater than the threshold are highlighted in red ($P < 0.01$). The *IKBKAP* spot is highlighted in green. (*D*) Tukey box plots show count comparisons of ESE per 100 bases of exon sequences. The plots are as described in Fig. 4*B*. Ctl, control exons; Exc, exclusion-type exons; Inc, inclusion-type exons. (*E*) Comparisons of fractions of exons with sequence motifs between exclusion-type exons and control exons. The plots are as described in Fig. 4*C*.

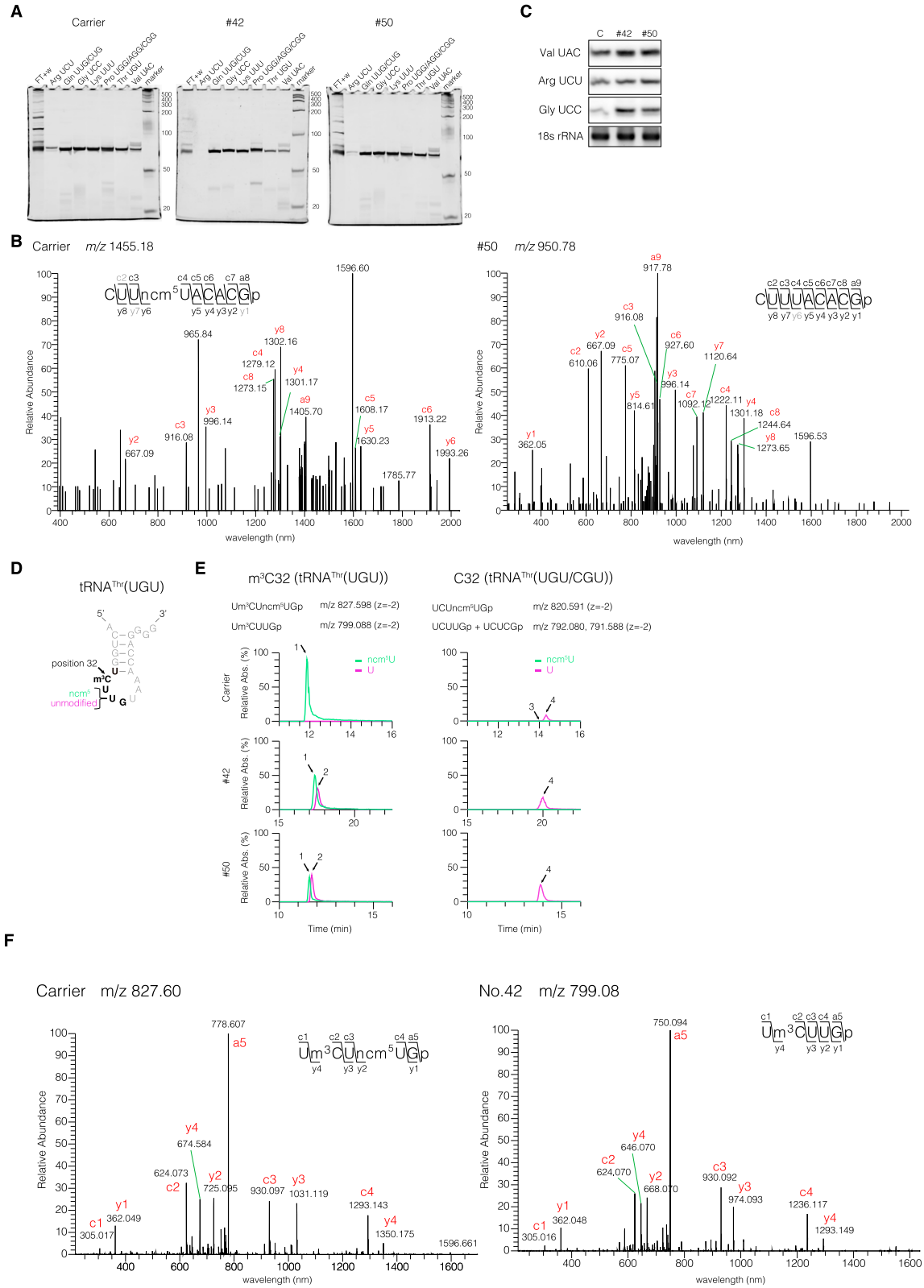

Fig. S5. Isolation of tRNAs by RCC and MS analysis of an anticodon-containing fragment in tRNA^{Val}(UAC). (*A*) PAGE (10%, 7 M urea) of tRNAs from cells derived from patients with FD or a carrier, isolated by RCC. RNAs were visualized by SYBR Gold staining. (*B*) CID spectrum of the anticodon fragment from RNase T_1 digests of the tRNA^{Val}(UAC). The doubly charged (1,455.18 *m/z*) or quadruply charged (950.78 *m/z*) ions were used as precursor ions for CID. The letters in gray indicate that the product ion was detected in other CID spectra using different charged ions as precursors. The nomenclature used for product ions is defined in the literature (13). The fragmentation pattern suggests the sequence C-U-U-ncm^{5}U-A-C-A-C-Gp or C-U-U-U-A-C-A-C-Gp as indicated in the carrier or FD patient #50, respectively. Because the presence of mcm^{5}s^{2}U and mchm^{5}U at the wobble position has been reported in bovine tRNA^{Arg}(UCU) and tRNA^{Gly}

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(UCC), respectively (1, 2), and it was suggested that tRNA^{Val}(UAC) contains ncm^{5}U at the wobble position (3, 4), we assumed that these human tRNAs also contained the modifications described above. (*C*) Northern blot analysis of tRNAs Val(UAC), Arg(UCU), and Gly(UCC) in carrier cells (C) and cells of FD patients #42 and #50; 18s rRNA was used as a loading control. (*D*) Secondary structure with the sequences at the anticodon stem–loop region of tRNA^{Thr}(UGU) is shown. The wobble uridine residue of the tRNA is indicated with possible modifications. The reported modifications m^{3}C near the anticodon are also indicated. The sequences indicated in bold letters are the RNase T$_1$ fragments harboring the anticodon. (*E*) Mass chromatograms of the RNase T$_1$ fragments of tRNA^{Thr}(UGU) are shown as in Fig. 5 *B–D*. The mass chromatograms of the RNase T$_1$ fragments modified with (*Left*) or without (*Right*) m^3 at position 32 (C32) are shown separately. (*F*) CID spectrum of the anticodon fragment from RNase T$_1$ digests of the tRNA^{Thr} (UGU). The doubly charged (827.60 *m/z*) or doubly charged (799.08 *m/z*) ions were used as precursor ions for CID. The letters in gray indicate that the product ion was detected in other CID spectra using different charged ions as precursors. The nomenclature used for product ions is as in Fig. S5*B*. The fragmentation pattern suggests the sequence C-m^{3}C-U-ncm^{5}U-Gp or U-m^{3}C-U-U-Gp as indicated in the carrier or in FD patient no. 42, respectively. Because the presence of ncm^{5}U at the wobble position in tRNA^{Thr}(UGU) has been reported in yeast (14), we assume that the wobble uridine is also modified with ncm^{5}U.

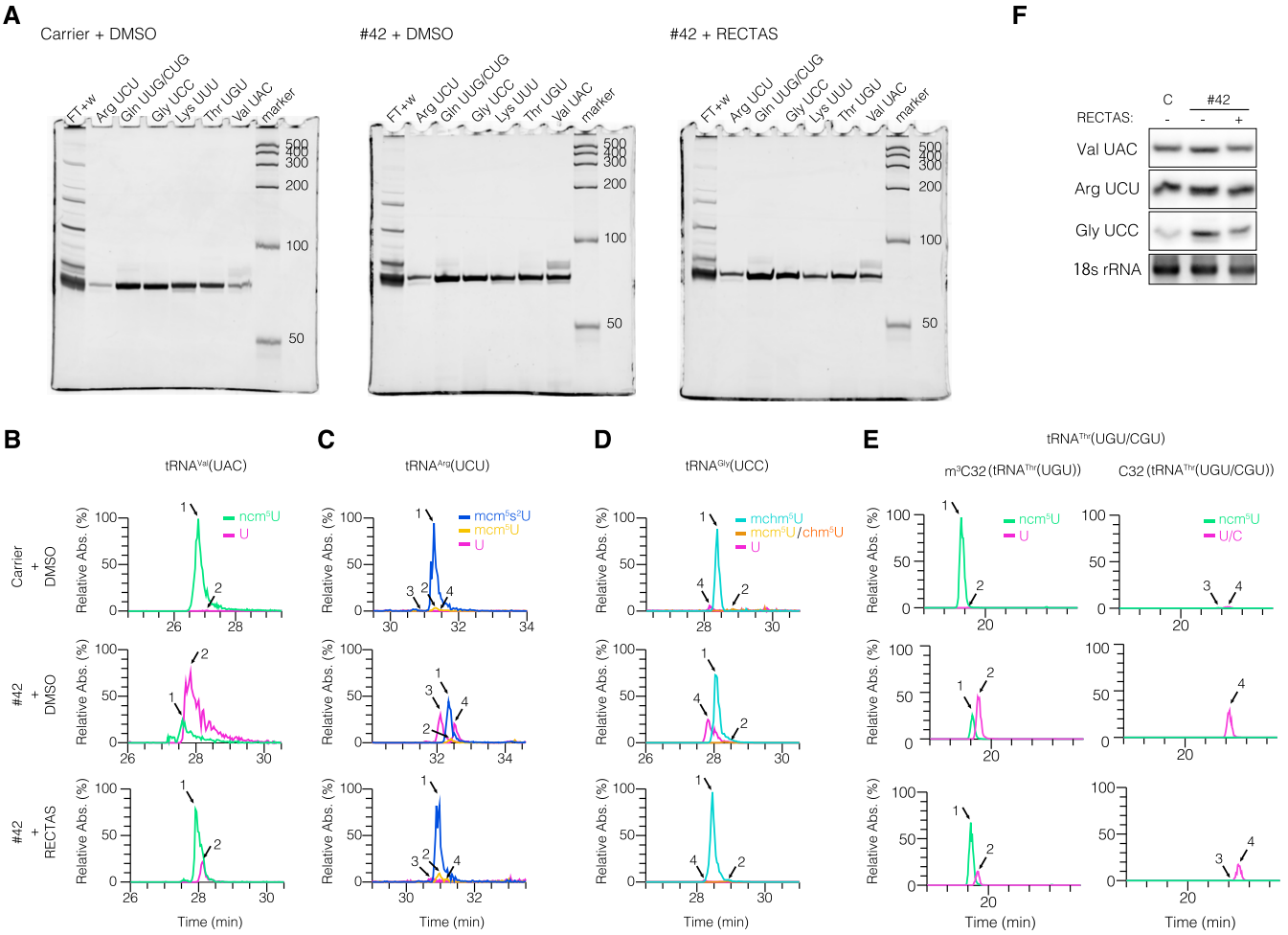

Fig. S6. Recovery of uridine modification of tRNAs by RECTAS in cells of patients with FD. (*A*) PAGE (10%, 7 M urea) of tRNAs from DMSO- or RECTAS-treated cells derived from patients with FD or a carrier, isolated by RCC. RNAs were visualized by SYBR Gold staining. (*B–E*) LC/MS analyses of the RNase T$_1$ fragments of tRNAs obtained from DMSO-treated Carrier cells (*Top*) and FD patient #42 cells treated with either 0.02% DMSO (*Middle*) or 2 μM RECTAS (*Bottom*). The mass chromatograms detecting RNase T$_1$ fragments harboring an unmodified or modified uridine residue for tRNA^{Val}(UAC) (*B*), tRNA^{Arg}(UCU) (*C*), tRNA^{Gly}(UCC) (*D*), and tRNA^{Thr}(UGU) (*E*) are shown as in Fig. 5 *B–D* and Fig. S5*E*. (*F*) Northern blot analysis of tRNAs Val(UAC), Arg(UCU), and Gly(UCC) in carrier (C) and FP patient #42 cells treated with DMSO or RECTAS. 18s rRNA was used as the loading control.

Table S1. Oligonucleotide sequences used in this study for plasmid construction, semiquantitative PCR, and the probes for RCC and Northern blotting

For the cloning of a genomic *IKBKAP* gene fragment of exon 19–21
- FW: 5′-ATACAAAAGTTGTTACAGGCCGGCCTGAGCAGCAAT-3′
- RV: 5′-GAAAGCTGGGTATGCATTCAAATGCCTCTTTAAACA-3′

For the introduction of FD mutation
- FW: 5′-TGGTTGGACAAGTAAGCGCCATTGTACTGTTTG-3′
- RV: 5′-CAAACAGTACAATGGCGCTTACTTGTCCAACCA-3′

For the introduction of single base deletion in exon 20 to adjust ORFs
- FW: 5′-GGTTTTAGCTCAGATCGGAAGTGGTTGGAC-3′
- RV: 5′-GTCCAACCACTTCCGATCTGAGCTAAAACC-3′

For semiquantitative PCR
- For endogenous *IKBKAP*
  - FW: 5′-GGATTGTCACTGTTGTGC-3′
  - RV: 5′-CAAGTTAATATGATTCACAGAATCT-3′
- For SPREADD reporters
  - FW: 5′-AGCATGGCCTTTGCAGGGCTGGC-3′
  - RV: 5′-TGACGTCCTCGGAGGAGGCGA-3′
- For endogenous *GAPDH*
  - FW: 5′-CCATCACCATCTTCCAGGAGCGAG-3′
  - RV: 5′-GTGATGGCATGGACTGTGGTCATG-3′

For amplification of the first part of *IKBKAP* Ex20-21WT/FD construction
- FW: 5′-GGGAAGCTTTCATCATCGAGCCCTGGTTTTAG-3′ (HindIII site italicized)
- RV: 5′-TTAGGTACCCGAAATTGTAATAAAATACTTATTG-3′ (KpnI site italicized)

For amplification of the second part of *IKBKAP* Ex20-21WT/FD construction
- FW: 5′-GTGGTACCTGATTTTTTTATTCTGTAATTTC-3′ (KpnI site italicized)
- RV: 5′-GATGGATCCGGGGACCACTTTGTAC-3′ (BamHI site italicized)

For construction of *IKBKAP* int-Ex20-int WT and *IKBKAP* int-Ex20-int FD
- FW: 5′-AACAAGCTTCTGCTAAAAGTGTGCTG-3′ (HindIII site italicized)
- RV: 5′-TTAGGTACCCGAAATTGTAATAAAATACTTATTG-3′ (KpnI site italicized)

For each tRNA purification by RCC and Northern blot probe
- $tRNA^{Val}$(UAC): 5′-TGGTTCCACTGGGGCTCGAACCCAGGACCTTCTGC-3′
- $tRNA^{Arg}$(UCU): 5′-GAAGTCCAATGCGCTATCCATTGCGCCACAGAGCC-3′
- $tRNA^{Gly}$(UCC): 5′-GAAGGCAGCTATGCTCACCACTATACCACCAACGC-3′
- $tRNA^{Thr}$(UGU): 5′-GGCTCCATAGCTCAGGGGTTAGAGCACTGGTCTTG-3′

FW, forward, RV, reverse.

Table S2. List of RNase T_1 fragments detected in the mass chromatograms shown in Figs. 5 and 6 and Figs. S5 and S6

tRNA	Sequence of T_1 fragments	*m/z*	Charge state	Depicted color	Peak no.
$tRNA^{val}$ (UAC)	CUUncm^{5}UACACGp	726.833	(−4)	Green	1
	CUUUACACGp	712.583	(−4)	Magenta	2
$tRNA^{Arg}$ (UCU)	Am^{3}CUmcm^{5}s^{2}UCUt^{6}AAUUCAACGp	717.517	(−7)	Blue	1
	Am^{3}CUmcm^{5}UCUt^{6}AAUUCAACGp	715.235	(−7)	Yellow	2
	Am^{3}CUUCUt^{6}AAUUCAACGp	704.946	(−7)	Magenta	3
	ACUUCUt^{6}AAUUCAACGp	702.944	(−7)	Magenta	4
$tRNA^{Gly}$ (UCC)	CCUmchm^{5}UCCAAGp	734.341	(−4)	Light blue	1
	CCUmchm^{5}UCCAAGp + CCUmcm^{5}UCCAAG*	730.837, 730.342	(−4)	Orange	2
	CCmUUCCAAGp	715.841	(−4)	Magenta	3
	CCUUCCAAGp	712.337	(−4)	Magenta	4
$tRNA^{Thr}$ (UGU)	Um^{3}CUncm^{5}UGp	827.598	(−2)	Green	1
	Um^{3}CUUGp	799.088	(−2)	Magenta	2
	UCUncm^{5}UGp	820.591	(−2)	Green	1
	UCUUGp + UCUCGp*	792.08, 791.588	(−2)	Magenta	2

The nucleotide sequences of RNase T_1 fragments from each tRNA are shown with the *m/z*, charge state, and depicted color in Figs. 5 and 6. The wobble uridines are described in bold letters.
*Fragments marked could not been separated due to the adjacent *m/z* in LC/MS-MS.